

FIFTH EDITION

Small Animal
CLINICAL DIAGNOSIS
by **LABORATORY METHODS**

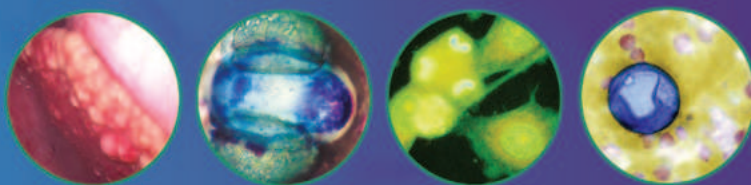


Michael D. Willard
Harold Tvedten

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Preface

Our intent has been to help veterinarians and veterinary students appropriately select and accurately interpret laboratory tests in as simple, practical, and rapid a manner as possible. The popularity of the first and then subsequent editions has surprised and pleased us. Simple is good. With age one forgets the small details and remembers only the major principles that get one through the day.

As with the first four editions, this fifth edition of *Small Animal Clinical Diagnosis by Laboratory Methods* is intended to present organized methods of answering commonly asked questions that reflect the problems frequently encountered with interpretation of laboratory test results. Most authors have updated their chapters as opposed to writing new ones with the idea that they could focus on making them as timely as possible. New authors have been added in some areas. We have attempted to make this text as current as possible, but this goal borders on impossible in a profession that is continually advancing. References and pathophysiology have been kept to a minimum because this text is designed to be user-friendly to both the busy clinician in the middle of a hectic day, as well as the student who is learning the art/science of problem solving.

Michael D. Willard and Harold Tvedten

*Dedicated to my wife, Gladys,
without whom I could not have done this,
nor would I have cared to do it.*

Michael D. Willard

*Dedicated to the thousands of veterinary students
and veterinarians, hundreds of medical technicians,
and tens of clinical pathology residents with whom I have
shared tens of thousands of discussions on diagnosis
with laboratory methods over the last 40 years. It is for them
and with them that it is fun to come to work each day.
Speakers at veterinary meetings need not fear my questions,
for they are only questions I ask myself each day.*

Harold Tvedten

General Laboratory Concepts

Harold Tvedten and Jennifer S. Thomas

TEST SELECTION AND ASKING THE RIGHT QUESTION

Veterinarians have many choices regarding laboratory testing. Important factors include availability of reference laboratory testing, reliability and ease of in-clinic testing, cost-effectiveness, accuracy, and turnaround time. One must determine what tests to perform in-clinic and what tests to send out to a veterinary reference laboratory or to a local human laboratory. Recent improvements in the automation and ease of use of analyzers designed for in-clinic use are changing what is acceptable. Correct choices vary with the needs and patient population of each veterinary clinic. No one answer fits all situations.

To get a specific and meaningful answer from laboratory testing, the diagnostician must ask a specific and meaningful question and understand whether a particular laboratory test is likely to yield a useful answer. As an example, compare the likely outcome of asking the following questions: "Is the animal anemic?" "What is wrong with the animal?" A microhematocrit procedure (in addition to knowledge of the animal's hydration status) will usually answer the first specific question, but a serum chemistry profile, complete blood count (CBC), urinalysis, and fecal examination may or may not answer the second vague, nonspecific question. A clinician should ask, "What will a high, low, or normal test result specifically mean in terms of making a correct diagnosis, providing accurate prognostic information, or choosing an appropriate therapeutic plan?" If the answer is meaningful (i.e., it will change some action taken by the clinician), the test is worth the cost. Normal laboratory results may eliminate certain diseases (i.e., have high negative predictive value [NPV]) and can be as valuable as abnormal results.

NOTE: To choose the appropriate test that will provide a specific diagnostic answer, a very specific question must be asked.

SIMPLE STATISTICS AND PRACTICAL INTERPRETATIONS

A reasonable level of skepticism about laboratory results should be maintained. *Clinicians should not believe all numbers.* All laboratory data should be interpreted in the

context of the history, physical examination, and other diagnostic findings in a patient. Unexpected results are common and should stimulate the clinician to reevaluate the provisional diagnosis and look for additional diseases or consider possible causes for erroneous laboratory results. Trends over several days are often more informative than test results on a single day. Typically, not all test results that "should be" abnormal in a disease situation are abnormal in each affected patient.

NOTE: A reasonable level of skepticism about laboratory results should be maintained.

When interpreting laboratory tests, it is important to keep in mind that reference intervals include the results expected in 95% of normal animals. Thus 5% of results in normal animals (i.e., 1 of 20) are expected to be outside the reference intervals. If a profile of 20 tests is performed, only 36% of normal animals would have all 20 results within the 95% confidence interval reference values. Diagnosticians must expect some false-positive and false-negative test results. No tests are 100% sensitive and 100% specific for a disease.

NOTE: Only slightly more than one third of normal animals are likely to have "normal" results in all tests of a 20-test profile. The clinician should not over interpret small changes from reference intervals.

Abnormal results in normal animals are often only slightly above or below the reference interval. The magnitude of a change helps determine one's confidence that a disease is present. Large alterations usually allow greater confidence that the animal is abnormal, because they are less likely the result of statistical chance. With many tests, increasing magnitude of deviation from normal also reflects a more severe disease and worsening prognosis.

Laboratory methods vary in their ability to provide the same result when a sample is repeatedly analyzed (i.e., analytical precision). The coefficient of variation (CV) is often used to indicate the precision of an assay. Assays with a low CV have a high degree of precision; small changes in results can be attributed to changes in the patient and not random variation in the assay itself.

Assays with a high CV have poorer precision; small changes in results may be due to variation in the assay and not indicative of disease in the patient. For example, because of the great imprecision of a manual leukocyte, platelet, or erythrocyte count, results can vary 10% to 20% only because of technique; therefore mild changes from one day to the next may reflect only imprecision in the procedure rather than actual changes in the patient.

Evaluating populations of apparently healthy animals with screening tests is much different from testing individual sick animals. The predictive value of a test is strongly affected by the prevalence of disease in a population.³ For example, if a disease occurs in 1 of 1000 animals and a test is 95% specific and sensitive for the disease, *what is the chance that an animal with a positive test result actually has the disease (i.e., positive predictive value [PPV])?* Most students, residents, and clinicians answered this question incorrectly; the average response was 56% with a range of 0.095% to 99%. If the test is 95% sensitive, 95% of all animals with the disease should be detected. Therefore the one animal in 1000 that has the disease should be positive. If the test has a specificity of 95%, then 5% of the 999 animals in 1000 that do not have the disease, or about 50, will have a false-positive test result. The PPV (i.e., the number of true-positive tests/total number of positive test results) of this test is only about 2%, because only 1 of those 51 animals with a positive test result will have the disease. There are mainly false-positive results to interpret and explain to the animal owners.

Screening tests with a high sensitivity are often useful to rule out a disease. In the example above with a test that has a sensitivity of 95%, 5% of animals with the disease will not be detected and will be false negatives. Using the above situation, if 1 in 1000 animals has the disease, then 0.05 animals will have a false-negative result. If the specificity is 95%, then 949 of 999 animals in 1000 that do not have the disease are true negatives. Thus the NPV (number of true-negative tests/total number of negative test results; 949/949.05) is greater than 99.9%.

NOTE: Evaluating test results from populations of apparently healthy animals is much different from evaluating results in individual sick animals.

If a test is performed only when the disease is likely instead of screening all animals (including those with no clinical signs) for a disease, then the frequency of diseased animals in the test population is higher. Testing for disease in sick patients is exemplified by heartworm testing. Consider an example in which a test for heartworm disease is 99% sensitive and 90% specific and is used in 100 outside dogs in a heartworm-endemic area.⁵ If the incidence of disease is 50%, then one should identify 49.5 of the 50 ill dogs and obtain 5 false-positive results in the 50 dogs without heartworms. Thus the PPV in this situation is 49.5/54.5 or 91%. There are still false positives to interpret, but greatly fewer.

Receiver operating characteristic (ROC) curves (Figure 1-1) are used to determine the effectiveness of a test in diagnosis. ROC curves plot the true-positive rate (as

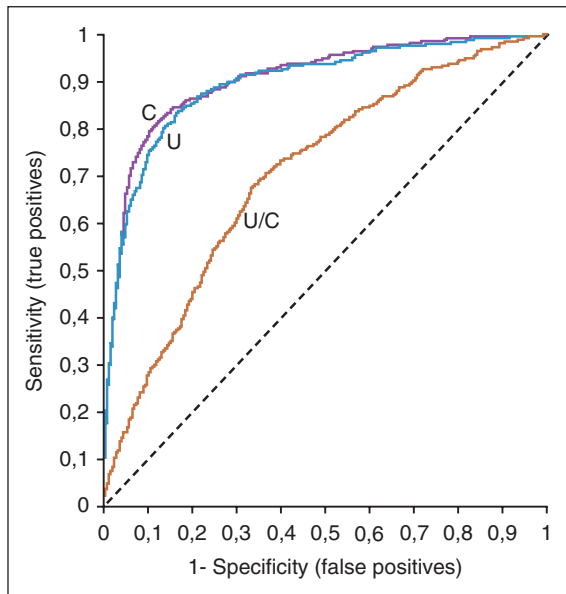


FIGURE 1-1. A ROC curve is a way to show the effectiveness of a test. Increased serum urea concentration, creatinine concentration, and urea/creatinine ratios were compared in diagnosis of 417 dogs with renal failure, 1463 normal dogs, and 2418 sick dogs without renal disease.² The area under the ROC curves show that serum creatinine and urea concentrations were very similar in diagnostic accuracy but the urea/creatinine ratio was obviously worse than either of them.

indicated by the diagnostic sensitivity of an assay) against the false-positive rate (1 – the diagnostic specificity of an assay) calculated at various concentrations over the range of the test's results. A good test has a great increase in the true-positive rate along the y axis for a minimal increase in the false-positive rate along the x axis. The 45-degree line in Figure 1-1 would indicate an ineffective test, which would have an equal increase in false positives and in true positives. Whether a positive result on such a test was a true positive or a false positive would be random chance, like tossing a coin. Figure 1-1 illustrates that serum creatinine and urea (measured as blood urea nitrogen [BUN]) are very good tests of renal failure in dogs and very similar in effectiveness.² The urea/creatinine ratio is noticeably worse than either creatinine or urea, as illustrated by being closer to the 45-degree angle line (and having less area under the curve).

ROC curves are also useful in selecting upper and lower decision thresholds that can be used to decide when a diagnosis can be ruled in or ruled out. Note that decision (or diagnostic) thresholds are different than reference intervals. Animals with a test result below the lower decision threshold limit are unlikely to have the disease being tested for; animals with a test result above the higher decision threshold limit are likely to have the disease.

Diagnostic thresholds for renal failure are suggested where the creatinine or urea ROC curves in Figure 1-1 rapidly change their upward angle and begin to turn and

plateau to the right. Lower to the left along the curve is a higher concentration threshold with greater specificity and lower sensitivity. More to the upper right is a lower threshold with greater sensitivity and lower specificity. At the bend in the curve, the test has optimal sensitivity (increase in true positives) with minimal loss of specificity (increase in false positives).

REFERENCE VALUES

Reference values (i.e., reference ranges, reference intervals, “normal” ranges) are used to determine if a test result appears normal or abnormal. A laboratory result is meaningless without knowing what values normal animals in that situation should have. It is not unusual for a veterinarian to request that a test be performed in a species for which the laboratory has no reference values, nor is it unusual to find that the laboratory has not validated the test for accuracy in disease diagnosis in species not commonly tested. Reference intervals may be presented as a range or a mean (or median) plus and minus 2 standard deviations. Reference intervals should optimally also have 95% confidence intervals around the upper and lower values to help show that the limits can be “fuzzy.” Too often veterinarians use an upper or lower value as an exact breakpoint between normal and abnormal. For example, if a serum sodium reference interval is 146 to 156 mmol/L, a common error is to consider 146 mmol/L normal but 145 mmol/L as indicating hyponatremia despite the fact that imprecision in the method or rounding off of values may mean that these values are essentially the same. Another example is that less than 60,000 reticulocytes/ μ L is often incorrectly given as a breakpoint between regenerative and nonregenerative anemia. The 60,000 should be considered an approximate, rule-of-thumb, mean reference value.

One uses the mean or range of reference values in different situations. Upper and lower reference values are best for individual patients without a previous evaluation. The best reference values are a patient’s own values (if available) before an illness, because individual animals or members of special groups (e.g., sight hounds, puppies) may have unique characteristics. When comparing groups of animals (e.g., a research project), one should use a mean or median value for the groups for interpretation of changes (e.g., packed cell volume [PCV] 45%) instead of published reference values (e.g., PCV 37% to 54%).

Specific reference values should be used for different methods and instruments. One laboratory’s reference values for canine reticulocytes for the ADVIA 120 instrument (Siemens Healthcare Diagnostics) is 11,000 to 111,000/ μ L (see Appendix II). The XT-2000iV analyzer (Sysmex Corporation) reports higher numbers of reticulocytes than the ADVIA 2120 and should have a different set of canine reference values (19,400–150,100/ μ L). A current problem with available automated reticulocyte results on most hematologic samples is that many nonanemic dogs appear to have a regenerative erythropoietic response because they have reticulocyte counts higher than currently available reference values. This may occur because, even with properly established reference values, there may be variations in how some samples were collected (excited

dog versus calm dog), or changes in instrument software may change the sensitivity of detection of reticulocytes.

Mean values are used to detect trends that depart from the norm. For example, if a dog has a low carbon dioxide partial pressure (PCO_2) (indicating a respiratory alkalosis trend) and a low bicarbonate (HCO_3^-) concentration (indicating a metabolic acidosis trend), the diagnostician uses pH to indicate which is the disease change and which is likely compensation. A pH that is within the reference range can still be discriminating by how it deviates from the mean for pH. For example, a low-normal pH indicates an acidifying tendency and that the disease process is more likely metabolic acidosis with respiratory compensation, rather than respiratory alkalosis with metabolic compensation. Means are used with increases in enzyme activity that should be reported as an x -fold increase (e.g., a tenfold increase over the mean). It is more common to use the fold increase over the upper reference value, because mean values are often not available.

Reference values are often suboptimal. New reference intervals should, theoretically, be established whenever a laboratory changes instruments, methods, or even types of reagents. The expense is considerable and often prohibitory considering the number of species involved; the variety of breeds; the effect of age, sex, and other factors; and the number of “normal” animals for a reference population optimally needed for each category. An ideal reference population should include 120 individuals for parametric and 200 individuals for nonparametric distributed values. A robust method for determining reference intervals is recommended when only 20 to 40 individuals are available.⁹

Unfortunately, use of readily available animals for reference populations is often found to be inappropriate because of later findings of subclinical disease or deviations from “the typical adult dog or cat” because of factors such as breed, age, sex. Results from any reference population should be evaluated for animals with values that deviate from the main group to see if those animals came from one kennel (e.g., breed-related deviations such as those in greyhounds, nutritional or toxic disorder in the kennel population) or for any other explanation for why those animals should be removed from the reference population.

An alternative method to generate reference intervals when new techniques, reagents, or instruments are added to laboratories is to perform at least 20 to 60 duplicate analyses with both the new and the previous or “standard” procedure. Regression analysis is used to predict the new procedure’s reference values from the previous reference values, assuming the previous values were properly established from an appropriate reference population. It is very important to include a wide range of low and high results in the group of duplicate samples.

Reference values in hematology or clinical chemistry books and articles will vary from a clinic’s own instruments and methods but are useful to identify factors that typically cause deviations from “the typical adult dog or cat” due to breed, age, sex, and the like. The number of tests analyzed in most clinics for some species (e.g., pet birds, wildlife, zoo animals) might be too low to justify establishing reference values. Literature values are often used for many tests if a laboratory does not have its own.

One example is the International Species Inventory System (ISIS) Physiologic Data Reference Values for zoo animals. Many of the species are uncommon, and only a few may be present in a state or country. The ISIS values were derived from normal animals at 65 institutions so that a reasonably sized database could be established.

Selected or “groomed” hospital patient data may be used to reevaluate reference values for one or more parameters that come under question. For example, a reagent company may change the formulation of reagents for a test (e.g., calcium) and suddenly many patients appear to have abnormally high or low calcium concentrations. Patient values are not from animals proven to be normal but are a readily available source of recently obtained, inexpensive, and locally produced data. These data represent the laboratory’s current patient population. In the above example, if values for calcium concentration in the laboratory’s current patient population (minus patients having a disease affecting calcium) are compared to previous reference values, then new reference values derived from patient results can be a temporary adjustment. One would expect to find a shift in the quality control (QC) results that chronologically matches obtaining new reagents.

INTERNATIONAL SYSTEM OF UNITS

The International System of Units (Système International d’Unités [SI units]) has standardized the reporting of data for improved comparison of results throughout most the world, with the exception of the United States, Brazil, and a few other countries. Units used for serum enzyme activity were particularly inconsistent in the past, when many enzyme procedures had results reported in units named after the author of the procedure. Now enzyme activity is reported as international units per liter (IU/L) in the United States or ukat/L in many other countries. Note that IU/L for enzyme activity is not an SI unit! The SI unit for enzyme activity is ukat/L. U.S. laboratories still use “traditional” units such as mg/dl. Appendix II includes common conversion factors to convert a result from one unit of measure (e.g., mg/dl) to another unit of measure (e.g., mmol/L). Unfortunately, laboratories in the same hospital may report results using different units of measure, causing confusion for clinicians when interpreting those results.

SOURCES OF LABORATORY ERROR

Laboratory error is common and needs to be detected early to avoid misdiagnosis. Laboratories should be asked to recheck results that do not make sense in the context of the animal’s history, physical examination, or other diagnostic findings, such as marked hyperkalemia or hypoglycemia in a clinically normal animal. A variety of artifacts may cause the measured concentration or activity of an analyte or multiple analytes in a panel to be falsely increased or decreased. Spurious results make it difficult to accurately interpret laboratory results; artifacts may cause abnormal results in a healthy animal or mask abnormal results in a sick animal. Depending on the

BOX 1-1. COMMON CAUSES FOR PREANALYTICAL LABORATORY ERRORS

- Incomplete sample labeling
- Improper venipuncture techniques
- Sample contamination when collecting via catheter
- Wrong anticoagulant
- Delayed mixing of blood with anticoagulant
- Inadequate mixing of blood just before aspiration into the instrument
- Delayed removal of serum or plasma from cells
- Delayed sample analysis
- Improper sample storage
- Inadequate warming of refrigerated samples before analysis
- Inadequate patient preparation (e.g., not fasted)
- Interfering substances in sample

cause, artifacts may make it impossible to determine the real concentration or activity of an analyte. Anytime one spurious result is found in a panel of tests, all results should be closely evaluated to determine whether they have also been affected.

Preanalytical Errors

When an artifact is suspected, it is useful to determine if the spurious findings resulted from a preanalytical or analytical error.¹² Preanalytical problems occur before the laboratory analyzes the sample and are the most common cause for laboratory errors.^{1,11} Common types of preanalytical errors are listed in **Box 1-1**. Most preanalytical errors are the result of sample collection or handling problems that can be avoided. Whenever possible, new samples should be collected if these types of preanalytical errors are suspected. Treatment with drugs often causes artifacts in laboratory testing. For example, chloride concentration usually cannot be accurately measured in patients receiving potassium bromide because the most commonly available assays cannot distinguish bromide from chloride. Certain drugs are insoluble in urine (e.g., sulfa drugs), causing crystalluria.

Some sources of preanalytical errors (e.g., hyperbilirubinemia, lipemia, or *in vivo* hemolysis) are physiologic or pathologic in the patient and may not be easily controlled. The severity of effect of these interferants depends on the analyte measured, the species involved, and the analytical method used.

Hemolysis

Hemolysis is recognized by reddish discoloration of plasma or serum. It can cause significant artifacts on a CBC, hemostasis profile, and chemistry panel. Hemolysis occasionally occurs *in vivo*; it more commonly results from *in vitro* erythrocyte damage associated with improper sample handling or collection. Samples that are hemolyzed due to handling or collection errors are best

discarded. *In vitro* hemolysis can be minimized with the following: (1) use of sharp needles to collect blood; (2) employment of proper venipuncture technique, including clean entry into a vessel, limiting vessel stasis, and avoidance of excess negative pressure during blood collection; (3) gentle mixing and handling of tubes promptly after collection; (4) proper centrifugation techniques to separate plasma or serum from cells; (5) prompt removal of serum or plasma from cells; and (6) prevention of overheating or freezing.

Hemolysis may falsely increase the measured serum concentration of substances that are present in higher concentrations in the erythrocyte cytoplasm than in plasma (e.g., lactate dehydrogenase [LDH], aspartate aminotransferase [AST], creatine kinase [CK]).^{1,14} These changes vary according to species or breed and are independent of the analyzer or methodology used. Hemolysis releases free hemoglobin that may interfere with spectrophotometric assays that measure substances at wavelengths similar to the absorbance range of hemoglobin. Finally, hemolysis releases erythrocyte contents that may positively or negatively interfere with the chemical reactions used to measure analytes. The errors associated with these last two hemolysis effects vary significantly, depending on the specific analyzer and methodology used. Therefore referral laboratories or analyzer manufacturers should provide information about the effects of hemolysis on their specific analytical system. Veterinarians should not choose laboratories that report out results on hemolyzed, lipemic, or icteric samples over laboratories that refuse to do so. Reporting out a result may not indicate that a laboratory has better methods unaffected by hemolysis, lipemia, or icterus, but merely that they report questionable results with a disclaimer at the bottom of the report.

Lipemia

Lipemia causes serum or plasma to appear milky white and turbid when triglyceride concentrations exceed 300 to 400 mg/dl. Lipemia alters the light-scattering property of blood,¹ causing alterations of a variety of hematologic and chemical results, depending on the methodology and analyzer used. Fat in lipemic serum displaces an equal volume of the aqueous part of serum. Because analytes such as sodium are dissolved in the water portion of serum, and not in the lipid portion, this may cause a false decrease in sodium (pseudohyponatremia) and other substances but not a physiologic hyponatremia, because it is the concentration of electrolytes in the aqueous plasma in contact with cell membranes that have a biologic effect. Whether this volume-related problem occurs depends on how an instrument aspirates and dilutes a sample or on the method of analysis. Lipemia increases erythrocyte fragility and often causes hemolysis.

Recent feeding is the most common cause of lipemia. Fasting 12 hours before blood collection can usually prevent postprandial lipemia. If lipemic samples are unavoidable (e.g., patient with a lipid metabolism disorder), then the serum or plasma can be cleared by ultracentrifugation or by the addition of a polymer that binds lipids for removal by centrifugation (e.g., LipoClear). Ultracentrifugation is the standard by which other lipid-clearing methods are judged and is used by many referral

laboratories; however, ultracentrifugation is not available in most practices. When using clearing polymers, it is important to first determine whether the procedure will falsely alter the concentration or activity of measured analytes for the analyzer system used.

Analytical Errors

Analytical problems occur during actual performance of a laboratory assay. Operator errors include improper sample aliquoting, incorrect analyzer use, improper reagent handling, and unauthorized modification of a procedure. Reagent problems include using out-of-date or improperly stored reagents. Analyzer malfunctions may result from improper maintenance, aging of instruments, or purchase of a lower quality instrument. In general, analytical errors are minimized by proper training of technicians and strict adherence to a quality assurance program, including documented standard operating procedure for testing protocols, equipment maintenance, and QC.

If a laboratory error is suspected, the first step is usually to repeat the analysis using the same sample. If the suspect results do not repeat, operator error or a random error is likely. If the suspect results repeat on the second analysis, a new blood sample should be collected and the analysis repeated. If the suspect results do not repeat on the new sample, then a preanalytical error is likely. If the suspect results repeat, then either there is an analytical problem or the results accurately reflect the patient's status. Test reagents and analyzer function should be evaluated. If they are functioning properly, then the patient should be reassessed for an alternative diagnosis. If laboratory error is still suspected, then the sample should be sent to a referral laboratory for analysis.

SAMPLE COLLECTION: RIGHT AND WRONG

Proper sample collection and handling techniques are required to avoid preanalytical laboratory errors and obtain reliable results. Tubes should be labeled to assure that results are reported for the correct patient. Clean venipuncture is needed to avoid hemolysis, tissue contamination, or inappropriate clotting. Samples should be collected from a blood vessel that can rapidly provide the required amount of blood without causing vessel collapse. If blood is collected from a catheter, it is important to discard an appropriate amount of fluid from the catheter to assure that the patient sample is not contaminated. Collection tubes containing anticoagulant should be filled first and promptly mixed (see Chapter 5 for sample collection for platelet counting and hemostasis testing). Improper filling (usually incomplete) of the tubes alters the ratio of anticoagulant to blood and significantly affects hemostasis testing and even some CBC, cytologic, or chemical assays. Collection of blood into the wrong anticoagulant may interfere with some chemical assays⁸ (see Box 1-2 for recommendations for collection tubes). Serum tubes with clot activator can damage cell morphology in cytologic samples. Delayed analysis of blood or

BOX 1-2. SELECTION OF COLLECTION TUBES**No Anticoagulant: Red-Top Tube**

Serum for chemistry testing
Fluid for cytologic examination

Heparin: Green-Top Tube

Plasma for chemistry testing

EDTA* (Sodium or Potassium): Purple-Top Tube

Binds divalent cations: ↓ Ca and Mg concentrations
Preferred anticoagulant for CBC
Fluid for cytologic examination
Interferes with chemistry testing: methodology dependent
↑ Na or K concentration

Sodium Citrate: Light Blue-Top Tube

Binds divalent cations: ↓ Ca and Mg concentrations
Preferred anticoagulant for hemostasis testing
Interferes with chemistry testing: methodology dependent

Fluoride (Sodium): Gray-Top Tube

Use for glucose or lactate determinations only
Inhibits glycolytic enzymes

*EDTA, Ethylenediaminetetraacetic acid; CBC, complete blood count.

other body fluids causes loss of cells and may make it impossible to identify cell types or evaluate morphology on a smear. Whole blood or body fluids should be kept refrigerated until analyzed. Direct smears of fresh blood and fluid cytology samples should be made promptly following collection and should accompany tubes of blood or fluids. Smears should be air-dried and not be refrigerated. Exposure to excessive heat or cold can cause lysis of cells, leakage of intracellular constituents, or loss of temperature-sensitive analytes. Delayed separation of plasma or serum from blood cells causes leakage of intracellular constituents and consumption of glucose.¹² To avoid breakdown or loss of enzymes or other substances, it is best to keep serum or plasma refrigerated or frozen if it will not be analyzed promptly after collection.

A key to effective sample collection is good communication with referral laboratories. Laboratory personnel will explain how to submit samples properly. When an unsatisfactory sample is received by a laboratory or when instructions or sample labeling are incomplete, someone must either try to contact the busy veterinarian for further instructions or arbitrarily decide what to do with the specimen. This may delay results or may lead to unordered assays being performed. It is important not to waste the effort used in obtaining the sample or lose a diagnostic opportunity that may not be available later. Many laboratories have prepared written information on submission procedures pertaining to their laboratories on their websites (see Appendix I, Listing of Selected Referral and Commercial Laboratories). Questions about sample

handling and submission and routine charges should be directed to the medical technologist, clerk, or secretary who answers them daily. The pathologist's time should be used for questions that are interpretive, diagnostic, or of a policy nature.

PROFILES VERSUS INDIVIDUAL TEST SELECTION

Some criticize profiles for being a “shotgun approach” or a nonspecific “fishing trip.” Profiles instead should be considered cost-effective screens for a large number of common problems. It usually costs less for most laboratories to perform a standard profile of 20 chemistry tests than to perform 3 or 4 individual tests that vary for each individual patient. This is due in part to using the automation of the instruments to perform profiles. Also, labor costs required to enter patient information into the computer system, label tubes, and prepare the instrument are the same if 20 tests are performed per sample or only 1 to 3 tests. A profile of many tests is recommended for those clinicians who have access to a larger laboratory not only for cost-effectiveness but for the reliability and accuracy of labs using QC and experienced laboratory professionals (see also later discussions on stat and point-of-care testing).

Serum chemistry profiles have variable numbers and types of tests included, although the “basic profile” includes the same or similar tests in most laboratories. A well-designed profile should, for a minimal fee and minimal redundancy, have a high probability of detecting the common diseases for the particular situation. A CBC is actually a profile that includes many tests to screen for anemia, inflammatory disease, stress, thrombocytopenia, and various other problems. The urinalysis is a profile of chemical, morphologic, microbiological, and physical tests designed not only to reveal hemorrhagic, inflammatory, or functional deficits in the urogenital tract but also to detect systemic disorders such as diabetes mellitus, hepatic disease, massive acute muscle injury, or intravascular hemolysis. A hemostatic profile for evaluating bleeding disorders is discussed in Chapter 5.

Individual test selection becomes important if the method or instrument locally available requires that each test be individually performed or if certain tests are not offered in an inexpensive profile. Some tests may not be cost-effective enough to include in an initial screening profile. Tests that infrequently have abnormal results or that are expensive are requested only when patient information indicates a problem that justifies their use. After a specific disease has been diagnosed (e.g., diabetes mellitus), only one test may be needed to monitor the treatment process and may be most cost-effective.

STAT TESTS

Very short turnaround time between sample collection and the availability of results is important in some “stat” situations (e.g., designing fluid therapy for acid-base and electrolyte abnormalities). Instruments that can analyze

plasma or heparinized whole blood save the time (e.g., 30 minutes) required for blood to fully clot before harvesting serum. Unlike cost-efficient batches, stat tests are usually analyzed individually. Stat tests require laboratory personnel to interrupt their efficient work routines to perform single tests.

SEND IT OUT OR DO IT YOURSELF?

In-Clinic Laboratory Testing

Veterinarians must decide what testing to do “in clinic” and what to send out. Major advantages to in-clinic testing are rapid turnaround time and potentially improved client satisfaction by eliminating the need to delay treatment or surgery until results are received from a referral laboratory. Other advantages include minimizing preanalytical errors associated with transportation of samples, providing an additional source of revenue for the clinic, and increased incorporation of biochemical profiles into presurgical screens, geriatric screens, and routine health maintenance examinations.¹³ Disadvantages to in-clinic testing include the costs required to purchase equipment and maintain adequate stock of reagents, the need to perform QC testing, and the need for additional staff time to properly perform assays in the laboratory. (See later discussion of calculation of the cost to perform a test based on test volume, etc.) A laboratory can be a revenue-producing unit, but this is seldom true for low-volume, inefficient testing situations.

The quality of in-clinic laboratories varies greatly. Accurate and reliable laboratory results require investment in experienced medical technologists or their equivalent, and a willingness to provide them adequate time to set up and maintain a system of good laboratory testing. Many clinics have nonlaboratory personnel performing their testing who lack the experience and training typical for technical staff in referral laboratories. Technician time must be allotted for training, inventory and maintenance of reagents, equipment maintenance, troubleshooting problems, and performing repeated measurement on samples with suspected artifacts or results that are questionable.

The clinic is responsible for assuring the laboratory's results are reliable. One person should be designated to be in charge of a quality assurance program to ensure that results are accurate, repeatable, and properly reported. QC testing must be performed, recorded, and monitored to prove validity of the answers sold to the client. Practices with low test volume should analyze control samples with each patient's sample and for each type of test performed. Because this is a significant expense in time and money, the frequency and extent of QC testing is often suboptimal and trust in the reliability of test results fades. Standard operating procedures should be in place to assure that testing is properly and consistently performed no matter who performs the test.

Microscopic analysis of cytology and hematology smears rapidly provides useful diagnostic information. Therefore someone in a practice should learn and practice these techniques (see Chapters 2 to 5 and 16). A good-quality microscope is necessary. “Quick” stains (e.g., Diff-Quik or Hemocolor) are easy and provide

consistent staining characteristics of blood smears, bacteria and fungi, and cytologic smears. New methylene blue (NMB) is easy to use for urine sediments, cytology smears, and reticulocytes in blood (see stain and microscope discussions in Chapter 16). Air-dried smears of cytology, blood, or urine sediment should be sent to a clinical pathologist whenever there is any doubt about the diagnosis. Manual erythrocyte and leukocyte counting techniques are discussed in Chapter 2, and platelet counting is discussed in Chapter 5. A refractometer is needed to determine urine specific gravity, plasma total protein concentration, and total protein concentration in various body fluids. With the refractometer, only a drop of urine is needed to determine specific gravity, which is essential for evaluating renal function. Similarly, only the volume of plasma in a microhematocrit tube is needed for an accurate estimate of plasma protein.

Most laboratories need at least two good-quality centrifuges: a single-purpose centrifuge for microhematocrit tubes to ensure consistent PCV determinations and a basic centrifuge for separating serum or plasma. Additional centrifuges to consider include a high-speed microcentrifuge for small (Eppendorf-type) tubes that allows more effective separation of serum or plasma from small samples. A refrigerated centrifuge is needed for some labile substances. If the clinic has a blood bank, a blood banking centrifuge is needed to separate fresh frozen plasma from concentrated red blood cells (RBCs) or platelet-rich plasma. A cytocentrifuge is needed for preparation of cytologic smears with good morphology of cerebrospinal fluid (CSF) or bronchoalveolar lavage (BAL) samples. Serum or plasma may need to be stored in a refrigerator (4°C) or freezer (−20°C) before it is mailed to a referral laboratory. Freezers included in refrigerators and many home freezers may not maintain a consistent temperature below −20°C.

Veterinary Referral Laboratories

Large referral laboratories offer more cost-effective testing (large profiles usually cost less than two to four individual tests), top-end instruments, more experienced personnel specializing in laboratory testing, better quality assurance, better detection of laboratory errors, and a wide range of tests. Many veterinarians purchase in-office analyzers only to find that, without frequent calibration and consistent use of controls, the results are untrustworthy. Over the years, different instrument manufacturers have promoted various in-clinic systems that disappeared (along with reagents) a few years later.

The larger, more sophisticated instruments not only analyze tests more quickly and cheaply but also better detect sample and laboratory errors. Small, simplified instruments often report only numbers, with limited additional information to detect errors. In contrast, larger, automated hematology cell counters have graphic displays to illustrate errors in what is being counted. Analyses may be performed by more than one method (e.g., two types of total white blood cell [WBC] count), and if results for the same parameter do not match, a flag signals the operator to check that value. These more sophisticated instruments usually also provide a much broader range

of data that are more precise, accurate, and rapid than are obtained by small in-clinic instruments (see Chapters 2 to 5).

Referral laboratories employ well-trained, experienced personnel and often board-certified veterinary clinical pathologists. These are specialists focusing only on laboratory testing, analogous to specialists in other fields (e.g., cardiology, ophthalmology, orthopedics). Referral laboratories offer a wider variety of tests, whereas the variety of tests a veterinary clinic may offer is limited by test volume. Offering a wider variety of tests requires more reagent storage and increases the likelihood that reagents will be outdated before they are used in a veterinary practice. *Outdated reagents should never be used*, and loss of unused reagents adds significant cost to testing. Some referral laboratories have courier systems that pick up samples and return results (e.g., by fax) within hours, fulfilling the need for rapid answers.

NOTE: It is recommended to use larger veterinary laboratories with experienced laboratory personnel, better quality assurance, better instrumentation, and lower cost per test as much as possible and practical.

Use of Human Laboratories

Many chemistry tests for animals, such as electrolytes, glucose, urea, and creatinine, are performed accurately in large human laboratories. However, veterinary referral laboratories avoid methods that fail to work with animal samples. Veterinary-specific testing is needed with various protein, hemostasis, immunologic, and hematologic analyses. Occasionally uncommon tests, such as methemoglobin and drug testing, may be only be available at a local human laboratory, because they are too infrequently requested to offer at veterinary laboratories. Human laboratories do not produce their own laboratory-derived reference intervals for animal species and cannot help with diagnosis of animal diseases.

Point-of-Care Chemistry Analyzers

For tests that are truly “stat” (e.g., measuring blood glucose concentration for suspected hypoglycemic seizures), point-of-care testing is necessary. Point-of-care testing is performed in close proximity to the patient; it includes analyzers used at the patient’s side and benchtop instruments found nearby (e.g., in the ward). The test available must match the clinic’s needs and the species being tested. Sample size requirement is important with regard to how little can be collected from dehydrated cats or puppies (Table 1-1). Analyzers that use small volumes of heparinized whole blood may eliminate some artifacts associated with plasma or serum separation. Use of analyzers that require plasma or serum means collection of 2 to 3 times more blood than needed for an instrument using heparinized whole blood, because of “dead space” and the fluid-cell interface involved in separation of serum from blood cells.

Reference values for heparinized whole blood may differ from routine serum values.

The ideal analyzer should be easy to use, provide fast and accurate results, require minimal operator time and training, be easy to maintain, have stable reagents, rarely break down, and be easy to repair or be immediately replaced by the manufacturer as needed. Published objective evaluation is available for relatively few commercially available analyzers used for veterinary testing.^{10,15,16,18} Manufacturers of the analyzers should be able to provide data showing the precision of their assays and how well the results compare with those obtained using a reference method for each species of interest.

The simplest analyzer measures the concentration of only one analyte, such as blood glucose. Advantages of blood glucose analyzers include rapid results, requirement for small quantities of blood (minimum requirement of 3 to 5 μ l whole blood), affordability, and portability. Comparison of results obtained using a variety of portable blood glucose meters (AlphaTrak [Abbott Laboratories], Precision Xtra [Abbott Laboratories], Ascensia Elite XL [Bayer Diagnostics, Inc.], Ascensia Contour [Bayer Diagnostics, Inc.], Accu-Chek Advantage [Roche Diagnostics, Inc.], and OneTouch Ultra2 [LifeScan, Inc.]) with a reference method in dogs revealed differences in the accuracy of the different analyzers. In some samples, this leads to misclassification of the glyemic state of the patient.⁴

Many in-clinic chemistry analyzers have the capacity to measure multiple analytes (see Table 1-1). Those that allow the clinic to measure a single analyte or to customize a biochemical panel are the most flexible but tend to be more labor intensive and require more technician attention when performing multiple tests. Analyzers offering a limited number of biochemical panels that are predetermined by the manufacturer are less flexible but generally cost less per test. They also tend to require less technician time and expertise.¹⁸

Different chemistry analyzers often use different reagents or methods; therefore each analyzer system requires its own species-specific reference intervals. Two or more different instruments analyzing the same substance may be used in the same clinic, which causes confusion in comparing results to a reference interval or results on the same patient from different instruments. Reference intervals from referral laboratories should not be used for in-clinic chemistry testing unless the same analyzer system and reagents are used. If a clinic is unable to generate its own reference intervals, it is important to determine if appropriate reference intervals are available from the manufacturer for the species of interest. Manufacturers and laboratories should provide information about how their reference intervals were calculated (e.g., the number, age, breed, and sex of animals used) upon request. It is also important to know if in-clinic analyzers were validated to perform well with ill patients, because the data provided by manufacturers are often established in healthy animals.

Cost and time required for QC procedures must be considered. Manufacturers should provide recommendations for a QC program. If a QC program from the manufacturer is not recommended, is unavailable, or is inadequate, then that analytical system should be suspect.

TABLE 1-1. COMPARISON OF SOME COMMERCIALY AVAILABLE CHEMISTRY ANALYZERS

ANALYZER DISTRIBUTOR/ MANUFACTURER	I-STAT1 ABAXIS	REFLOVET/ PLUS SCIL	SPOTCHEM EZ SCIL	DRI-CHEM 4000 HESKA	VETSCAN VS2 ABAXIS	VETTEST IDEXX LABORATORIES
Number of available chemistry and electrolyte tests	9 plus acid-base, blood gas, coagulation, troponin assays	15 plus hemoglobin	21	22	23 plus canine heartworm, T ₄	26
Panels or individual tests	6 predetermined panels + individual tests	Individual tests only	1 predetermined panel + individual tests	5 predetermined panels + individual tests	9 predetermined panels	7 predetermined panels plus individual tests
Species with available reference intervals	NA*	Dog, cat, horse, cow, rabbit, guinea pig, hamster	Dog, cat, horse	Dog, cat, horse, mouse, rabbit, ferret, cow	Dog, cat, horse	18 species supported
Sample type	Whole blood	Whole blood, serum, or plasma	Whole blood, serum, or plasma	Whole blood, serum or plasma	Whole blood, serum, or plasma	Serum or plasma
Sample size	<95 µl	32 µl	250 µl (blood); 100 µl (serum/plasma)	1.5 ml (blood); 0.5 ml (serum/plasma)	100 µl	40 µl for 1 test plus 10 µl for each additional test
Recommended quality control procedures	Automatic testing of analyzer and sensors on cartridges; liquid controls available upon request	Liquid controls recommended	Liquid controls available upon request	Liquid controls (daily to weekly depending on test volume)	Check with manufacturer	Monthly liquid controls
Reagent type	Cartridges	Dry test strips	Dry test strips	Slides	Plastic rotors	Dry slides
Assay time	<2 min	2–3 min per test	8–14 min	<13 min for complete panel	<12 min for panel	6 min for panel
Technical support available per day, 7 days per week [†]	Yes	Yes	Yes	Yes	Yes	Yes
Results transmissible to hospital computer system [‡]	Yes	Yes	Yes	Yes	Yes	Yes

T₄, thyroxine.

NA*Reference intervals for dog, cat, and horse are anticipated to be available in 2011.

[†]Current availability as of December 2010; the reader should check with manufacturer for any changes and for specific information about costs associated with technical support.[‡]Check with manufacturer regarding specific information about compatible practice management software.

Electronic QC is provided with some instruments. It adequately tests the electronic circuitry in the instrument and is fine as such, but electronic QC will not detect reagent problems or some types of operator error.⁶

The ease of operation of the analyzer and training requirements of the operator should be taken into account. One must keep in mind the technician time required for sample setup as well as the time it takes to complete testing. Most in-clinic chemistry analyzers can provide panel results within 10 to 20 minutes; however, this time may not include analyzer or reagent warm-up times. Improper warm-up procedures can dramatically alter results. Finally, the linearity range of the assays should be determined. Results above the linear range are inaccurate and require dilution and retesting so that results are in the acceptable testing range. Dilution of patient samples is time consuming and creates a source of operator error. Some analyzers provide automated sample dilution.

Reagent and equipment features are the final considerations. For reagents, the cost per test, shelf life, and storage requirements should be evaluated. If shelf life is short, reagents will often become out-of-date if the number of tests performed is low. Out-of-date reagents must be discarded and can be a significant expense. Equipment considerations include cost (e.g., purchase or lease, maintenance, service contract) and the technician time required for maintenance. If an analyzer malfunction occurs, one should determine the technical support offered by the manufacturer (e.g., times of day or week when available, cost), the expected turnaround time for repairs, and whether a loaner analyzer is available during the repair period. Because many of the analyzers currently available are software driven, one should find out the expected frequency of upgrades and any associated costs.

COST ANALYSIS FOR IN-CLINIC ANALYZERS

In-office instruments have many overlooked costs. Often salespeople discuss only the cost of the instrument and reagents. The final cost per test is affected by many more factors than just the base cost of the instrument and cost for reagents. The cost of validating the instrument, the service contract, establishing reference ranges, QC testing, calibration, repeating questionable tests, diluting and rerunning samples out of range, instrument depreciation, labor, lost interest (if the money for the instrument had been invested), and hospital overhead should be calculated in the lowest and highest possible sample volume situations. Actual cost per test is often much more than what a salesperson suggests. Other hidden costs include repeat testing on problem samples, disposable supplies, waste disposal, and optional and accessory equipment. A realistic cost per test should be used in determining if laboratory testing is actually a profit center for the clinic or if the convenience of having in-clinic testing is worth the true expense.

Volume of patient samples analyzed per day, week, and year is critical in determining the cost per test. The

cost for one test (e.g., glucose or alanine aminotransferase [ALT]) using an in-clinic chemistry analyzer was described in earlier editions of this book to vary from \$3.00 a test (if 10 tests are analyzed per day) to \$17.50 (if 3 tests are analyzed per week). Similarly, the cost for a blood gas analysis (pH, PCO₂, oxygen partial pressure [PO₂], and HCO₃) with a standard blood gas instrument (Nova 4) was only \$9.63 if 4000 tests were analyzed per year versus \$41.71 if 424 tests were analyzed per year.¹⁷ These values are outdated and the tests are likely more expensive now, but the trend holds true.

As an example, the costs for a full Michigan State University (MSU) small animal profile in 2010 can be compared to the cost for individual tests from the same laboratory. A profile for \$40 included: urea, glucose, total protein, albumin, globulin, ALT, AST, SDH (sorbitol dehydrogenase), calcium (Ca), phosphorus (P), magnesium (Mg), sodium (Na), potassium (K), chlorine (Cl), HCO₃, anion gap, total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase (ALP), amylase, cholesterol, CK, creatinine, osmolality, iron, and Na:K ratio. This is 22 analyzed tests and 5 calculated results, at a cost of about \$1.82 per analyzed test. Individually analyzed tests are more expensive because the QC, time, and labor expense to do a batch of 10 to 15 tests is often little more than for 1 or 2 tests. Individual tests from the same MSU laboratory in 2010 were: amylase \$19, urea \$14, bilirubin (total and conjugated) \$26, and AST \$14. Thus 4 individual tests would be \$73 whereas the 22 tests in a profile (actually analyzed) would be only \$40 (55% the cost of 4).

The lower cost per test when profiles are performed is due to efficiency. A laboratory has certain fixed costs per patient whether only 1 test is performed on that sample or 25 tests. The cost per test is obviously lower when these fixed costs are divided by 25 tests compared to only 1 to 4 tests. Information on the sample (e.g., owner, date, animal data, tests requested) must be entered into the laboratory computer system, the sample needs to be labeled and perhaps centrifuged and divided into more tubes and transferred to work stations, the instrument must be programmed to perform the tests, QC testing must be done each day, and a report must be sent to the submitting veterinarian. Laboratories also have regularly scheduled profile runs during the day, but individual tests come in a haphazard fashion.

One estimate for assigning hospital overhead costs (by Dr. Jack Judy, the MSU University Hospital director in 1995) to income-generating areas of a veterinary clinic was \$50.00 per square foot of floor space. If a laboratory floor space is 88 square feet, one should divide \$4400 per year of overhead by the number of tests performed in that area. If one performs five tests per day during 250 working days per year, or 1250 tests per year, the overhead per test would be \$3.52. Income-generating areas should compensate for other areas (e.g., medical records, reception area, office space). The total cost of depreciation of the instruments each year is similarly divided by the number of test analyzed each year to determine this additional cost per test. The labor cost per test can be estimated by dividing the annual salary costs of medical technologists and others working in the lab by the number of tests performed per year.

QUALITY CONTROL

A QC program must be in place to assure accurate results, because all instruments, reagents, and laboratory personnel eventually make errors. Properly performed QC detects these problems so they can be corrected in a timely manner. QC should be a *major* consideration in laboratory testing but is often poorly performed in in-clinic laboratory testing.

Within-laboratory QC is performed by repeated analysis of control reagents containing known amounts of various substances (i.e., analytes) such as glucose or creatinine. Results on control samples must be within the range of expected QC results before patient samples can be analyzed. Control samples with high, low, and normal levels of each substance should be used. In laboratories with a large volume, all three control samples (i.e., high, normal, low) are analyzed with each batch of patient samples. The number of control samples used in small-volume situations is more open to discussion. Using one or two QC samples (low, high, or normal) is better than not doing any QC. Results from QC testing are recorded daily and reviewed to detect sudden shifts or gradual trends suggesting instrument, personnel, or reagent problems. Gradual trends may be the result of slow deterioration of a reagent or slowly decreasing light intensity in an instrument. Rapid shifts may be the result of introducing a new reagent, a sudden change in an instrument component, or human error.

External QC programs document that the results of one's own laboratory's procedures are comparable to those of other laboratories using the same instruments and procedures. Veterinary laboratories are not legally required to participate in external QC programs, but they would certainly benefit from them. The College of American Pathologists (CAP) program is high quality, expensive, and required by laboratories testing human samples. Larger veterinary laboratories may purchase the program. Laboratories in these programs receive test samples, analyze the samples, and report their results. The performance of each laboratory in the testing program is compared with that of others using the same test method. This requires a large database of many laboratories using the same methods. Other external QC programs are available (e.g., from reagent or instrument producers). A practitioner can check a referral laboratory's precision by submitting duplicate samples to that laboratory or by submitting split samples to two or more laboratories to test accuracy.

Although reference is made to certain instruments or products, readers should not favor one particular

company's products discussed in this book or a particular referral laboratory listed in Appendix I over those unintentionally excluded.

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2

The Complete Blood Count, Bone Marrow Examination, and Blood Banking

General Comments and Selected Techniques

Douglas J. Weiss and Harold Tvedten

COMPLETE BLOOD COUNT

The complete blood count (CBC) is a common name for a hematologic profile of tests used to describe the quantity and morphology of the cellular elements in blood and a few substances in plasma. The CBC is a cost-effective profile that detects many abnormalities and disease conditions. Bone marrow examination is used in selected patients to answer questions that the CBC cannot.

NOTE: The CBC is a cost-effective profile of hematologic tests that detects many abnormalities and disease conditions.

This chapter discusses how conclusions are derived from information in the CBC and bone marrow examination, in addition to describing selected hematologic techniques. Explanations about interpretation are brief, because detailed discussions about the use of diagnostic tests for erythrocytes (i.e., red blood cells [RBCs]), leukocytes (i.e., white blood cells [WBCs]), and platelets are presented in Chapters 3, 4, and 5, respectively, and in other references.¹⁶ Technical comments emphasize the basic tests used in small clinics, but they may include more advanced techniques when they clearly illustrate a basic hematologic principle.

CBC results should be evaluated systematically. The first step involves identifying which test results are abnormal. Appropriate scientific terms should be used to describe the abnormalities (Box 2-1). Adjectives such as “mild, moderate, or marked” are applied to reflect the magnitude of the change, which aids in interpretation. Although these adjectives are subjectively based on clinical experience, some published guidelines are available. Greater deviations from reference values allow more confidence that the change is truly a disease change and serious than do mild deviations in results.

NOTE: First determine which test results are abnormal and how greatly they deviate from normal. Then evaluate abnormal results in groups of related types of data.

Test results outside reference intervals for a given species are usually considered abnormal. However, reference values are often not optimal because they are difficult and expensive to establish for each laboratory, and should be revised every time a lab changes instruments or methods. Reference intervals are usually derived from limited numbers of adult animals not segregated by age, sex, or breed (see Chapter 1). These factors can be significant. For example, a general adult reference interval for canine packed cell volume (PCV; hematocrit; Hct) is 37% to 55%. St. Bernards, however, tend to have PCVs that range from 35% to 40%, whereas the PCVs of greyhounds typically range from 52% to 60%; therefore what is normal in these two breeds hardly overlaps. Prominent age-related changes also occur. At birth, canine RBCs are very large, with a mean corpuscular volume (MCV) of about 95 femtoliters (fl). The MCV decreases to adult values by 2 to 3 months of age. At 5 to 6 weeks of age, puppies normally have a PCV around 30%, a plasma protein around 5.3 g/dl, and 3% to 4% reticulocytes. Puppy values could be confused with a regenerative, blood loss anemia if compared with adult reference intervals. Detailed references with information on breed, age, instrument, method, and other variables that affect interpretation should be on hand.¹⁶

The second step in evaluating abnormal results is to group abnormal data within the CBC. For example, a low PCV (i.e., anemia) should be linked to tests of bone marrow erythropoiesis, such as reticulocyte count and polychromasia, and observations on RBC morphology (see Chapter 3) to describe the anemia. Additionally, severe thrombocytopenia, hypoproteinemia, and icterus, often aid in diagnosis of the cause of anemia. The mental process of formulating a good description frequently suggests a diagnosis. For example, deciding an anemia is severe, erythroid regeneration is marked, and identifying moderate to many spherocytes in the smear and autoagglutination in the EDTA blood, clearly leads to a diagnosis of immune-mediated hemolytic anemia (IMHA). Total leukocyte count should be linked with the differential leukocyte count and WBC morphology (see Chapter 4). For example, severe neutropenia with more immature neutrophils than mature neutrophils and marked toxic change, usually indicates gram-negative infection or septicemia. A leukopenia with only lymphopenia and a

BOX 2-1. DEFINITION OF SELECTED HEMATOLOGIC CHANGES

HEMATOLOGIC CHANGE	DEFINITION
Anemia	Decreased red blood cell (RBC) mass, clinically noted by decreased packed cell volume (PCV)
Polycythemia	Increased RBC mass in body (increased PCV)
Polychromasia	Increased number of polychromatophils
Poikilocytosis	Increased variation in RBC shapes
Microcytic	Increased number of small RBCs
Macrocytic	Increased number of large RBCs
Normocytic	RBCs are of normal size
Hypochromic	RBCs have lower hemoglobin (Hgb) concentration (lower mean corpuscular Hgb concentration [MCHC])
Normochromic	RBCs have normal MCHC
Spherocytosis	Increased number of spherical (i.e., small and dense-appearing) RBCs
Echinocytosis	Increased number of RBCs with many spiny projections
Acanthocytosis	Increased number of RBCs with a few elongated, blunt projections
Schistocytes	Small RBC fragments
Rouleaux	Clumping of RBCs into linear formations resembling stacks of coins
Autoagglutination	Immune aggregation of RBCs into grapelike clusters
Heinz bodies	Precipitated Hgb resulting from oxidation
Thrombocytopenia	Decreased number of platelets
Thrombocytosis	Increased number of platelets
Leukocytosis	Increased number of white blood cells (WBCs)
Leukopenia	Decreased number of WBCs
Neutrophilia	Increased number of neutrophils
Neutropenia	Decreased number of neutrophils
Left shift	Increased number of immature neutrophils (nonsegs)
Right shift	Increased number of hypermature neutrophils (hypersegmentation)
Toxic neutrophils	Neutrophils with degenerative changes in their cytoplasm
Reactive lymphocytes	Lymphocytes with morphologic evidence of immunologic activation
Monocytosis	Increased number of monocytes
Monocytopenia	Decreased number of monocytes
Lymphocytosis	Increased number of lymphocytes
Lymphopenia	Decreased number of lymphocytes
Eosinophilia	Increased number of eosinophils
Eosinopenia	Decreased number of eosinophils
Basophilia	Increased number of basophils
Basopenia	Decreased number of basophils
Bicytopenia	Decrease in two cell lines (RBCs, WBCs, or platelets)
Pancytopenia	Decrease in three cell lines (RBCs, WBCs, and platelets)

normal number of leukocytes indicates a chronic stress/steroid process and not inflammation.

The third step in interpretation involves drawing hematologic conclusions from related sets of CBC results (Box 2-2). For example, with anemia one should determine the degree of RBC regeneration by reticulocytes and polychromasia, note RBC morphology, and consider plasma protein concentration and platelet count. If there is a deficiency of one cell type (e.g., thrombocytopenia), then one should check for leukopenia, neutropenia, and nonregenerative anemia to judge bone marrow production of all cell lines.

Magnitude of Hematologic Abnormalities

The magnitude of a change has great diagnostic significance and must be evaluated. Anemia in sick animals is often mild. For example, in Table 2-1, 29% of 737 blood samples indicated anemia; however, 55% of the anemic dogs had mild anemia (i.e., PCV 30% to 37%). Mild anemia is often not a primary hematopoietic disease but secondary to other problems such as inflammatory disease, malignancy, hepatic failure, or renal disease. Diagnostic effort should pursue the primary disease.

BOX 2-2. HEMATOLOGIC CONCLUSIONS THAT MAY BE DRAWN FROM HEMATOLOGIC DATA

HEMATOLOGIC CONCLUSION	HEMATOLOGIC DATA
Regenerative anemia	Anemia with appropriate reticulocytosis
Nonregenerative anemia	Anemia with no or insufficient reticulocytosis
Hemolytic anemia	Strongly regenerative anemia with additional evidence, such as hemoglobinuria, normal to high plasma protein, and one of the causes of hemolytic anemia
Blood loss anemia	Regenerative anemia with normal to decreased plasma protein, evidence of iron deficiency, or proof of blood loss
Immune-mediated hemolytic anemia	Moderate to marked spherocytosis, autoagglutination, and/or positive direct Coombs test
Oxidant-induced hemolytic anemia	Large numbers of Heinz bodies, eccentrocytes, or pyknocytes
Fragmentation anemia	Large number of keratocytes, schizocytes, or acanthocytes (dogs)
Iron deficiency anemia	Microcytic hypochromic anemia with variable RBC regeneration
Inflammation	Leukocytosis, neutrophilia, left shift, eosinophilia
Stress or steroid reaction	Lymphopenia and eosinopenia; often neutrophilia, and occasionally monocytosis
Excitement/epinephrine response	Lymphocytosis, leukocytosis, neutrophilia, and perhaps polycythemia, especially in cats
Toxemia	Significant number of very toxic neutrophils

TABLE 2-1. FREQUENCY (%) OF SELECTED ABNORMALITIES IN TWO SURVEYS OF COMPLETE BLOOD COUNTS PERFORMED AT MICHIGAN STATE UNIVERSITY

ABNORMALITY	DOGS (N = 100)	DOGS (N = 737)	CATS (N = 30)	CATS (N = 159)
Anemia (PCV)	23	29	10	20
Anemia (Hgb)	13	29	10	21
Polychromasia	10	na	0	na
Abnormal RBC morphology	38	na	53	na
Microcytic (low MCV)	na	3	na	7
Hypochromic (low MCHC)	na	6	na	9
Macrocytic (high MCV)	na	5	na	7
Hyperchromic (high MCHC)	na	1	na	8
Polycythemia	5	3	7	9
Hypoproteinemia	9	na	7	na
Hyperproteinemia	24	na	47	na
Hemolysis	4	na	3	na
Lipemia	5	na	3	na
Leukocytosis	16	28	13	19
Leukopenia	na	6	na	22
Left shift	5	na	10	na
Lymphopenia	27	na	43	na
Lymphocytosis	2	na	3	na
Monocytosis	14	na	13	na
Monocytopenia	0	na	4	na
Eosinophilia	17	na	10	na
Neutrophil toxicity	4	na	23	na
Reactive lymphocytes	7	na	7	na
Thrombocytopenia	5	15	7	60
Clumped platelets	6	na	23	na
Thrombocytosis	5	11	7	6

Hgb, Hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; na, not available because of design; PCV, packed cell volume; RBC, red blood cell.

Severe anemia (i.e., PCV <20% in dogs), however, should be evaluated as a primary hematologic problem. An exception is in pancytopenia, where life span of different cells becomes important. RBCs have a long life span compared to neutrophils and platelets, so anemia in pancytopenia can be mild or unapparent early, while neutropenia and thrombocytopenia may already be moderate to severe.

Frequency of Abnormalities

Numerous hematologic abnormalities are detected by routine CBC testing. The frequency data in Table 2-1 were from two surveys of CBC results from consecutive patients at Michigan State University. The larger survey

was a computer search of quantitative values. The smaller survey was a manual search of records that included subjective observations.

Anemia occurred frequently (10% to 29% of hospital patients); thus diagnosis of the cause of anemia is important (see Chapter 3). Hyperproteinemia also is a frequent finding in dogs and cats that may be the result of dehydration, inflammation, and immune stimulation (see Chapter 12).

NOTE: Anemia and hyperproteinemia are frequent findings in hospitalized dogs and cats.

Results from microscopic evaluation of blood smears are subjective and commonly evaluated as 0 to 4+, with 0 indicating the change is absent and 4+ indicating a maximal increase. An alternative is to use adjectives such as “mild, moderate, or “marked.”

Abnormal RBC shape (e.g., poikilocytosis) was frequently reported in cats and dogs; however, the abnormalities were often mild and clinically insignificant (e.g., 1+ crenation). Although reporting small variations in RBC size and shape may be technically correct, it may not be helpful to the veterinarian reading the report because too many details can obscure the diagnostically important findings. Findings such as blood parasites, Heinz bodies, or 2+ to 4+ spherocytes are less common but are of important diagnostic significance.

Left shift and leukocytosis, indicating inflammation, was moderately frequent (5% to 10%) in these hospital populations of dogs and cats. The greater frequency of toxic changes in feline neutrophils (23%) is due to the propensity of cats to form Döhle bodies (a very mild form of toxic change; see Chapter 4).

QUANTITATION TECHNIQUES

Sample Submission

Anticoagulated blood is required for cell counts. Any visible clots in a blood sample will alter WBC or platelet counts, because distribution of cells in blood is then not uniform. Clinical laboratories should not process clotted samples, because results are invalid and clots may plug hematology instruments. Blood smears submitted with a clotted sample may still be evaluated.

NOTE: EDTA is the recommended anticoagulant for routine CBC samples.

Ethylenediaminetetraacetic acid (EDTA) is the best anticoagulant to preserve cell detail. Heparin can cause poor staining with a diffuse blue background. Formalin or formalin fumes cause poor staining of Wright-stained blood smears, resulting in a blue background. One must keep formalin away from blood and cytology smears, both in the laboratory and in packages sent to referral laboratories. Formalin fumes can affect smears without direct contact. A citrate-based anticoagulant that reduces aggregation of feline platelets (Diatube-H; Becton Dickinson, Oxford, UK) was associated with good cell detail on smears.⁸

Commercial blood collection tubes contain a vacuum and should be permitted to fill until flow stops to have a proper blood volume-to-anticoagulant ratio. Moderate to severe underfilling of the tube may result in an excessive concentration of EDTA salt, which draws water out of cells, causing RBC shrinkage and decreased PCV and MCV. However, prominent errors are not expected if at least 1 ml of blood is collected into a 4-ml EDTA tube.

Tubes with less than 1 ml of blood or with visible clots should be rejected. If very small amounts of blood are collected into tubes with liquid anticoagulant, dilution of blood may be significant, resulting in lower cell counts. Overfilling the tube can potentially dilute out the anticoagulant enough to permit clotting. Citrate blood collection tubes, used for hemostasis testing, are marked with a line that shows the proper final volume to give a 1:9 ratio of anticoagulant to blood. A properly filled citrate tube is 10% anticoagulant fluid. It is very important to fill tubes to this line ($\pm 10\%$) to avoid dilution or concentration errors in hemostasis testing.

If not analyzed within 2 to 3 hours, EDTA blood should be refrigerated (4°C). RBC swelling after 6 to 24 hours of storage raises PCV and MCV and lowers mean corpuscular hemoglobin concentration (MCHC). RBC swelling may prevent detection of microcytic hypochromic cells in iron deficiency anemia. The RBC count, hemoglobin (Hgb) concentration, Hct, and RBC indices (i.e., MCV, mean corpuscular Hgb [MCH], MCHC) have minimal changes if blood is refrigerated for up to 24 hours.

NOTE: If not analyzed within 2 to 3 hours, EDTA blood should be refrigerated. A freshly made, air-dried blood smear should be submitted to allow proper interpretation of cell morphology.

Blood smears should be prepared immediately and air-dried to avoid artifacts caused by exposure of cells to anticoagulants and cell deterioration during storage and shipment. A great deal of information can be obtained from a fresh blood smear even if the EDTA tube is too old to analyze. Fresh capillary blood (ear prick) provides a higher concentration of parasitized RBCs if blood parasite examination is needed. Blood from an ear prick is streaked out immediately on a blood smear.

Slides should not be mailed in the thin, cardboard mailing cards that fit into envelopes. These envelopes are often machine processed by the post office, and the machines crush the slides. Instead, they should be mailed in rigid plastic containers (e.g., boxes) too bulky to be machine cancelled.

Microhematocrit

The microhematocrit method for determining PCV (Hct) has several advantages (over hemoglobin concentration or RBC count) to make it the recommended way to estimate RBC mass in evaluation of anemia and polycythemia in small clinics. Additionally, the microhematocrit provides a good quality control check on an automated instrument's hematocrit when analyzed in parallel with automated testing.

NOTE: The microhematocrit method (PCV, Hct) is recommended for evaluation of anemia or polycythemia in small clinics.

PCV, hemoglobin concentration, and RBC count are equivalent methods to estimate RBC mass in the properly hydrated patient. Microhematocrit is more precise and technically easier than a manual RBC count and provides additional useful information over Hgb determination and RBC count. Gross examination of plasma in the microhematocrit tube allows detection of icterus, hemolysis, or lipemia (Figure 2-1). The microhematocrit can even be used to screen for heartworm disease, because actively moving microfilariae are concentrated in the plasma just above the buffy coat. Plasma protein concentration can be quantified using a refractometer on plasma obtained from one or two microhematocrit tubes.

PCV is determined by centrifuging anticoagulated blood in a microcapillary tube to separate cells from plasma. Microhematocrit tubes are filled about two-thirds to three-fourths full. After centrifugation in a special microhematocrit centrifuge, RBCs are well packed at the bottom. WBCs and platelets appear as a thin white line (i.e., buffy coat) between RBCs and plasma (see Figure 2-1). The volume of blood that is packed RBCs (PCV in % or L/L) is determined. Microhematocrit tubes that contain heparin may be used for direct collection of non-anticoagulated blood. To calculate PCV, one divides the length of packed RBCs by the total length of the packed RBCs, buffy coat, and plasma. The clay plugging the bottom of the tube should not be included. Various microhematocrit reading devices are available.

Error in microhematocrit determination is minimal but, when present, is usually related to centrifugation or very fragile RBCs. Microhematocrit tubes should be centrifuged for 5 minutes. When the PCV is greater than 50%, packing of RBCs by the centrifuge is less complete (i.e., less tight). This causes an overestimation of the PCV. If the microhematocrit tubes are filled to more than two-thirds to three-fourths full, cell packing is also less complete. When a PCV is less than 25%, the packing of RBCs

is tighter. This exaggerates the decrease in the PCV and makes the animal seem slightly more anemic. Microhematocrit centrifuges attain high speeds (11,500 to 15,000 rpm), which ensures the proper centrifugal force to pack cells. The speed should be checked periodically, because slower speeds cause poorer cell packing that cannot be compensated for by longer periods of centrifugation. One should check the centrifuge's brushes three to four times per year and replace them if they are worn. Microhematocrit tubes should be evenly balanced in the centrifuge's head to prevent unequal weight distribution. If the head is not properly balanced, the wear on the motor is uneven and eventually causes the head to vibrate. One should not use the brake when the head is still rotating at high speeds, because this also causes excessive motor wear.

Hemoglobin Concentration

Under most circumstances, hemoglobin concentration (i.e., Hgb), RBC count, and PCV are essentially equivalent in estimating the animal's RBC mass.

NOTE: Under most circumstances Hgb, RBC count, and PCV are essentially equivalent measures of RBC mass.

Because the microhematocrit technique is simple, accurate, and inexpensive, most veterinarians interpret PCV results rather than Hgb or RBC count. However, Hgb is provided by most hematology instruments, and redundant information helps ensure a correct diagnosis. Hgb may be more accurate than PCV if poor RBC packing, cell shrinkage, cell swelling, or increased cell fragility is present. Hgb is inaccurate if lipemia interferes with plasma light transmission during photometric analysis. Large numbers of Heinz bodies may cause an erroneous increase in optical density, thus artifactually increasing Hgb concentration.

Total Cell Counts

Total WBC, RBC, and platelet counts can be determined manually using a hemocytometer or with automated instruments. A hemocytometer is an inexpensive, simple glass counting chamber that fits easily in a microscope and does not require calibration and quality control reagents. A hemocytometer count takes more time and is imprecise, but it is a useful method for small clinics that perform WBC or platelet counts only occasionally. Thus this method will be explained. Automated instruments are much more precise, are easier to use, and save time when the number of hematology samples to analyze justifies their purchase.

NOTE: Manual cell counts in a hemocytometer are practical in small clinics with only occasional need to do a cell count.



FIGURE 2-1. The upper two microhematocrit tubes appear normal. The far right side of the tubes has white clay that plugs the tube. The dark red area left of the clay is packed RBCs (PCV; about 45% of blood volume). To the left of the RBCs is a white layer (buffy coat of WBCs and platelets). To the left of the buffy coat is clear plasma (about 55% of blood volume). The next tube down indicates a severe anemia (PCV <10%) with clear plasma. The next tube illustrates anemia with icteric plasma. The next tube illustrates anemia with hemolyzed plasma. The bottom tube illustrates lipemia in the plasma.

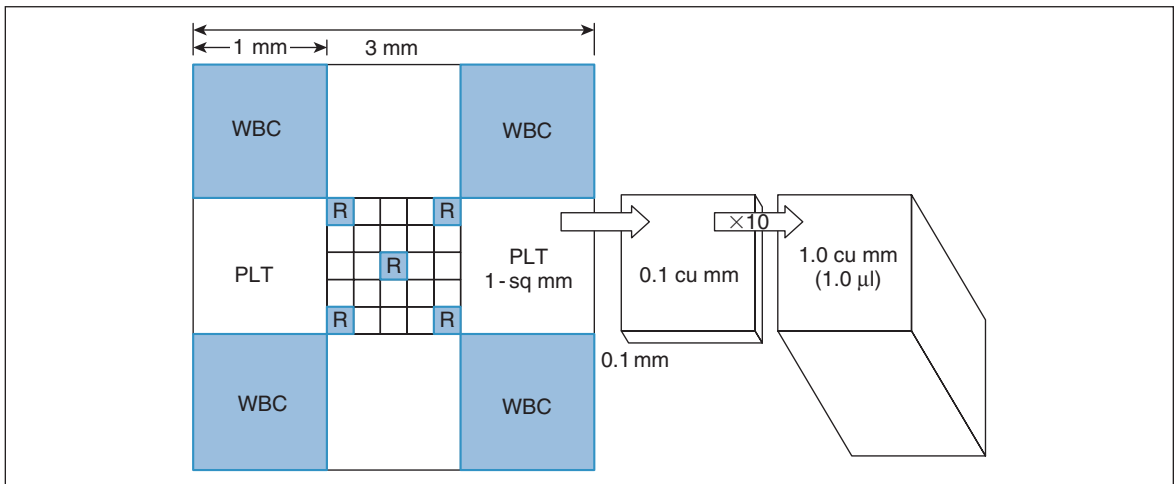


FIGURE 2-2. Dimensions of a hemocytometer. The hemocytometer grid consists of 9 mm² and it is 0.1 mm deep. The central 1 mm² is additionally divided into 25 squares. The areas usually used for erythrocyte, leukocyte, and platelet counts are indicated by R, WBC, and PLT, respectively. To determine the cells in 1 mm³ (which is equal to 1 μ l), conversion factors for RBCs, WBCs, and platelets are listed in Table 2-2.

Hemocytometer

A hemocytometer is a transparent glass chamber that holds a cell suspension for microscopic cell counting. The hemocytometer is 0.1 mm deep and is divided into subunits by a grid with a precise 3-mm by 3-mm surface area (Figure 2-2). The surface area of the grid used in a procedure thus determines volume of the hemocytometer containing the cells counted. One can mathematically determine the number of cells in a cubic millimeter (i.e., mm³; see Figure 2-2) based on this volume. One should understand these calculations so that the hemocytometer can be used to count cells in other fluids such as cerebrospinal and synovial fluid. In the United States, cell numbers are reported in cells/ μ l, which equals cells/mm³. This was likely because originally cells were counted in a hemocytometer designed to represent portions as mm³. Most other countries report hematologic cell concentrations in cells $\times 10^9$ /L. A liter contains 1 million (10⁶) μ l; therefore a liter would have a million times more cells than 1 μ l (see Chapter 1).

The blood (or other fluid) must be diluted so that the number of cells in the chamber is easy to count. A dilution system with plastic containers is easiest. Becton Dickinson (BD) discontinued the Unopette System and now recommends, as an alternative, similar products made by Bioanalytic GmbH (www.bioanalytic.de). The amount of dilution needed to provide a reasonable concentration of cells in the chamber varies with expected concentration of cells in blood (or other fluids such as abdominal fluid). Erythrocytes in blood are very numerous, so blood is greatly diluted (e.g., 1:200). Note that manual RBC counts are so inaccurate and time consuming that a manual RBC count is not recommended. A microhematocrit is more practical if one

does not have an automated hematology instrument. A microhematocrit is even useful for bloody fluids such as abdominal fluid to estimate the amount of hemorrhage. WBC dilution is usually 1:20 and platelets are diluted 1:100. The reciprocal of the dilution (i.e., 1/dilution) is used in calculation of the final conversion factor (Table 2-2).

The portion of grid in which cells are counted varies for each cell type and even type of counting chamber. For WBC counts in the United States, the outer four squares are counted (see Figure 2-2). For platelets, two squares (2 mm²) are counted. The reciprocal of the area is used in calculating the final conversion factor. Because the hemocytometer is only 0.1 mm deep, counting 1 mm² represents one tenth of 1 mm³.

To convert cell counts to number/mm³, cell count must be adjusted for the portion of 1 mm³ counted and the dilution of the blood sample (see Table 2-2). The WBC factor of 50 is based on adjustments for depth of the hemocytometer (0.1 mm), area counted (4 mm²), and dilution factor (1:20). A different factor can be determined if one chooses to vary from the standard approach.

Hemocytometers have two counting chambers (one on each side). Both chambers should be filled and counted. The number of cells counted from each side should vary by less than 10% for WBC counts. If the cell suspension was not evenly distributed, as indicated by greater variation, the test should be repeated.

Manual counts with a hemocytometer have significant error (e.g., 20% error may occur with WBC counts). This magnitude of error should be considered when interpreting day-to-day changes in WBC counts in animals. For example, a count of 2100 WBCs/ μ l varies too little from a count of 1900 WBCs/ μ l the previous day to be considered an improvement. Despite imprecision, manual

TABLE 2-2. HEMOCYTOMETER CONVERSION FACTORS

	DEPTH	AREA	DILUTION	CONVERSION FACTOR
Conversion factor	10/1 ×	1/area (mm ²) ×	1/Dilution ×	
RBC factor	10/1 ×	25/5 ×	200/1 ×	= 10,000
WBC factor	10/1 ×	¼ ×	20/1 ×	= 50
Platelet factor	10/1 ×	½ ×	100/1 ×	= 500

RBC, Red blood cell; WBC, white blood cell.

counts are adequate for most clinical diagnoses for clinicians who do not have need of or access to an automated hematology instrument. Manual counts may be performed when errors in automated counts are suspected.

Corrected White Blood Cell Count

Because nucleated RBCs (NRBCs) are included in manual and automated instrument total WBC counts, it is necessary to reduce the WBC count (actually a nucleated cell count) to account for NRBCs when they are numerous. The correction is made if more than 5 NRBCs are noted while counting 100 WBCs in a WBC differential count (i.e., >5% error). A proportion is used to mathematically adjust the nucleated cell count to a corrected WBC count as follows:

$$\text{Corrected WBC count} = \frac{\text{Nucleated cell count} \times 100}{\text{NRBCs} + 100}$$

Absolute Nucleated Red Blood Cell Count

The absolute NRBC count is the difference between the nucleated cell count and the corrected WBC count. Reporting only the relative ratio of NRBCs/100 WBCs can be misleading if the WBC count is very high or low. For example, NRBCs are released in sepsis and heat stroke, and in these instances there is often neutropenia and leukopenia. For these cases, the absolute NRBC count (per liter or microliter) is easier to interpret.

Erythrocyte Indices

The RBC indices describe the average size and Hgb content of RBCs. Indices were originally calculated from directly determined measurements (i.e., PCV, RBC count, Hgb) by the following equations. Automated cell counters may directly measure cell volume or Hgb concentration of RBCs.

Mean corpuscular volume (MCV) in femtoliters (fl):

$$= \text{PCV} \times 10 \div \text{RBCs} (10^6)$$

Mean corpuscular hemoglobin (MCH) in picograms (pg):

$$= \text{Hgb} \times 10 \div \text{RBCs} (10^6)$$

Mean corpuscular hemoglobin concentration (MCHC) in g/dl:

$$= \text{Hgb} \times 100 \div \text{PCV}$$

MCV indicates the average volume of the RBCs. An increased, normal, or decreased MCV indicates that the average RBC was macrocytic, normocytic, or microcytic, respectively. A normal or decreased MCHC tends to indicate if RBCs were normochromic or hypochromic, respectively. Hyperchromic RBCs (i.e., increased MCHC) indicate an instrument error, such as hemolysis or the presence of Heinz bodies. MCH indicates absolute Hgb content per average RBC. MCHC indicates concentration of Hgb in an average RBC.

Note that these mean values require a large percentage of abnormal RBCs for the MCV or MCHC to be drawn out of the reference interval. Therefore MCV and MCHC often give a misleading picture of the true presence of macrocytic hypochromic or microcytic hypochromic RBCs. For example, only 8.3% of blood samples of 6752 dogs with regenerative anemia had both increased MCV and decreased MCHC despite the fact that regenerative anemia (increased reticulocytes) is truly macrocytic and hypochromic.² Use of MCV and MCHC usually gives the wrong morphologic classification of the animal's anemia.

NOTE: Use of MCV and MCHC often gives an incorrect morphologic classification of the animal's true type of anemia because large numbers of abnormal RBCs are needed to pull mean values out of the reference values.

Automated Hematology Cell Counters Impedance Counters

The impedance principle was the standard method of cell counting in hematologic instruments and remains a common method in many current instruments. Using the impedance counting principle (i.e., Coulter principle), cells are diluted in an electrolyte solution and drawn through an aperture (hole in an electrode). The electrical resistance across the aperture changes as a pulse with each cell because cells are poor conductors of electricity. The frequency of the change in resistance indicates cell number, and the magnitude of the change in resistance indicates cell size. Impedance counters must be

electronically adjusted to count only cells within an appropriate size interval. This size interval is set by adjusting electronic thresholds for each species. Impedance counters directly count cell numbers, measure cell size, and then mathematically calculate the Hct, MCHC, and MCH.

NOTE: Inspection of platelet-erythrocyte histograms can avoid errors in impedance platelet counts.

Platelet-erythrocyte histograms should be inspected for adequate separation between the platelet and erythrocyte peaks to avoid errors. The histogram should show two peaks and indicate which cell types were counted as platelets or RBCs by the impedance instrument (Figure 2-3). There must be a clear separation “valley” between the RBC and platelet peaks. This valley and a dashed line correctly placed between RBC and platelet peaks assures that the counts were accurate. A frequent error is that there is overlap in the size of platelets and RBCs so that the instrument cannot correctly determine the division between them.

Impedance counters count the number of RBCs and determine the size of each RBC in that sample and then calculate a hematocrit (Hct) essentially by multiplying the number of RBCs by their size (e.g., $\text{RBCs} \times \text{MCV} \div 10$) or by a summation of individual RBC sizes. This is unlike the classic method of centrifugation of blood to pack RBCs and determine a PCV. Therefore instrument counts are better called a *hematocrit (Hct)* instead of packed cell volume (PCV). For simplicity, PCV is usually used in this book to indicate Hct or PCV. PCV by the microhematocrit method is a good quality control addition to performing a CBC with a hematology instrument. If the variation between the microhematocrit's PCV and the instrument's Hct is greater than 3% to 5%, this suggests a technical error, which should be clarified.

Newer impedance instruments provide additional information, such as three to four cell differential leukocyte counts, mean platelet volume (MPV), platelet distribution width (PDW), and reticulocyte count, and they may identify the presence of NRBCs.⁴

Laser Light Scatter Cell Counters

Newer automated hematology analyzers often use a laser detection system in a flow cytometer to measure size and internal complexity of cells based on light scatter at different angles.⁷ Stains are added to help differentiate types of leukocytes or reticulocytes. Two veterinary instruments designed for large referral laboratories are the Advia 120/2120 (previously the H-1; Siemens Medical Solutions) and the Sysmex XT-2000iV (Sysmex Corporation, Kobe, Japan). The Sysmex reports both laser (optical; PLT-O) and impedance (PLT-I) platelet counts. These newer instruments provide abundant information about each cell type. The RDW numerically describes the variability in RBC size (anisocytosis), and the hemoglobin distribution width (HDW) describes

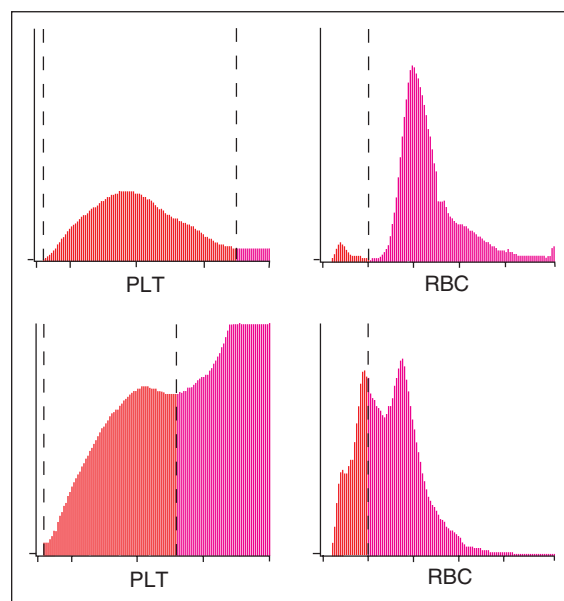


FIGURE 2-3. Two examples of red blood cell (RBC) platelet and platelet (PLT) histograms from an impedance platelet and RBC count from a Cell-Dyn instrument. The upper histograms are from a normal dog. In the upper right histogram, two distinct peaks are seen, a smaller orange platelet peak and a large red RBC peak. The orange platelet peak is repeated on the left as only a PLT histogram, which shows distribution of platelets by size. When the platelets and RBCs are distinctly different in size, an impedance instrument can differentiate and count them accurately. The lower histograms are from a dog with iron deficiency anemia. There are three populations (peaks) of cells in the right histogram, and the abnormal middle peak represents microcytic RBCs. Because there was no clear separation of platelets from RBCs (no valley between two peaks), the instrument could not correctly distinguish between two cell types and drew the border right through the middle of the microcytic RBCs. Part of the microcytic RBCs were counted as platelets (orange) and part as RBCs (red). Thus the platelet count was in error. Usually the relative error with an RBC count is minor because there are a great many more RBCs than platelets, but in a case of severe anemia and strong thrombocytosis, even the RBC count can be prominently in error.

variability in Hgb concentration (see Chapter 3). Tvedten prefers interpreting the morphology of the graphics rather than many numerical results such as RDW, HDW, and several others.

Graphic displays of cells aid in diagnosis and detection of laboratory errors.¹³ The Advia measures and reports the volume and Hgb content of each erythrocyte analyzed (Figures 2-4 and 2-5). The Advia system is the most sensitive and specific way to correctly classify anemia as normocytic normochromic, macrocytic hypochromic, or microcytic hypochromic. As described in RBC indices, the use of MCV and MCHC more often gives an incorrect classification than the true type of anemia. RBC cytograms illustrate normal and abnormal RBC populations based on cell size and Hgb concentration. The Advia

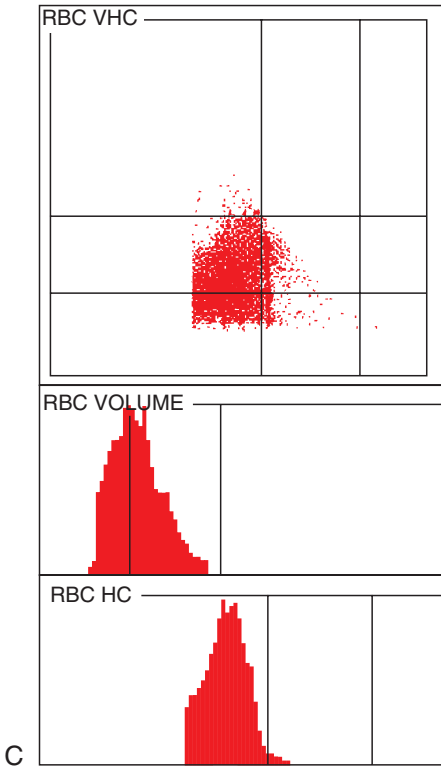
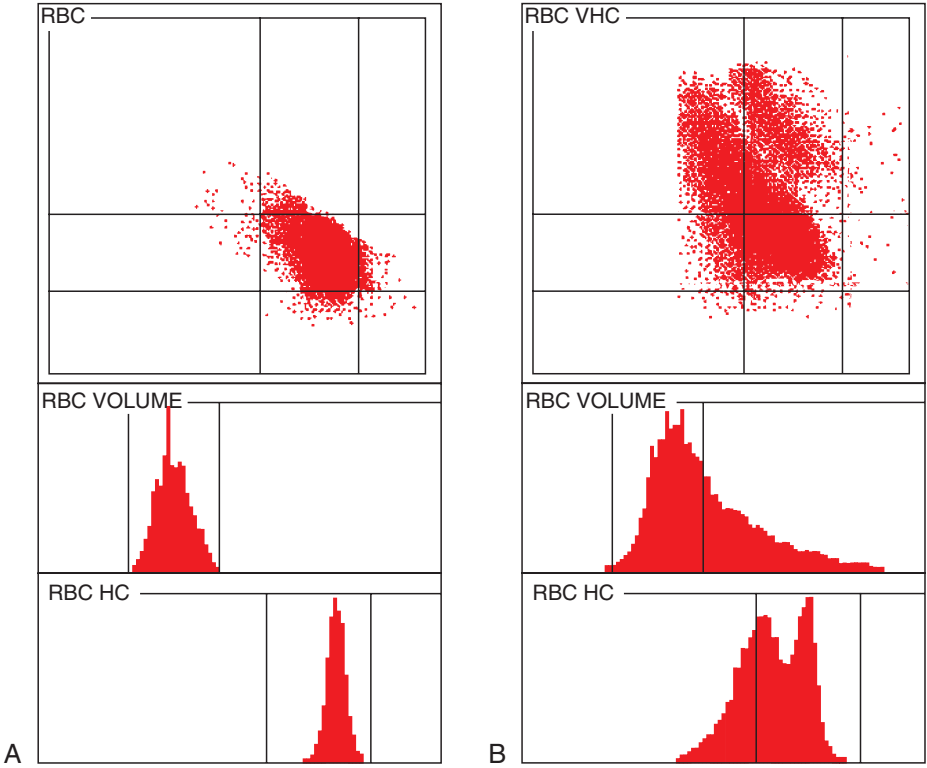


FIGURE 2-4. The Advia 2120 instrument graphics best illustrate the three basic types of anemia, which are normocytic normochromic anemia (**A**), macrocytic hypochromic anemia (**B**), and microcytic hypochromic anemia (**C**). The graphics include a red blood cell (RBC) cytogram, which is a nine-box grid, in the top row. The RBC cytogram displays each individual erythrocyte analyzed based on that cell's volume (V) on the vertical axis and that cell's Hgb concentration (HC) on the horizontal axis. There are two histograms under this nine-box grid. The RBC volume histogram is equivalent to the vertical axis of the cytogram and shows the number of RBCs based on volume. The two vertical lines reflect upper and lower reference limits. The Hgb histogram is equivalent to the horizontal axis of the cytogram and shows the number of RBCs based on cell hemoglobin concentration. **A**, The dog with normocytic normochromic anemia had all RBCs within the central box or vertical lines on the histograms, as would a normal non-anemic dog. **B**, The dog with macrocytic hypochromic anemia was an IMHA case with many immature (macrocytic hypochromic) RBCs that extended up and to the left from the central box on the cytogram. Extension of cells to the right on the RBC volume histogram reflect that they were macrocytic. These RBCs also extended to the left on the cytogram and Hgb histogram, indicating they were hypochromic. Unique to the Advia 2120 is the display of hypochromic RBCs. The Hgb histogram shows two peaks, and the area "under the curve" reflects the relative number (%) of RBCs in the left peak, which were hypochromic. In the IMHA case, there were more hypochromic (left peak) than normochromic (right peak) RBCs. Thus at a glance one can determine the magnitude of a regenerative response of immature RBCs (usually reticulocytes). Additionally, a cluster of RBCs in the upper central box of the cytogram were RBCs in autoagglutination. **C**, This dog had a microcytic hypochromic anemia due to iron deficiency that was so extreme that there were too few normal normocytic normochromic RBCs remaining to show where normal cells should be found. The straight lines at the bottom and left of the cells in the cytogram and a line on the left of the two histograms indicate that the Advia 2120 did not believe canine RBCs could be small or hypochromic and excluded them from analysis (truncated).

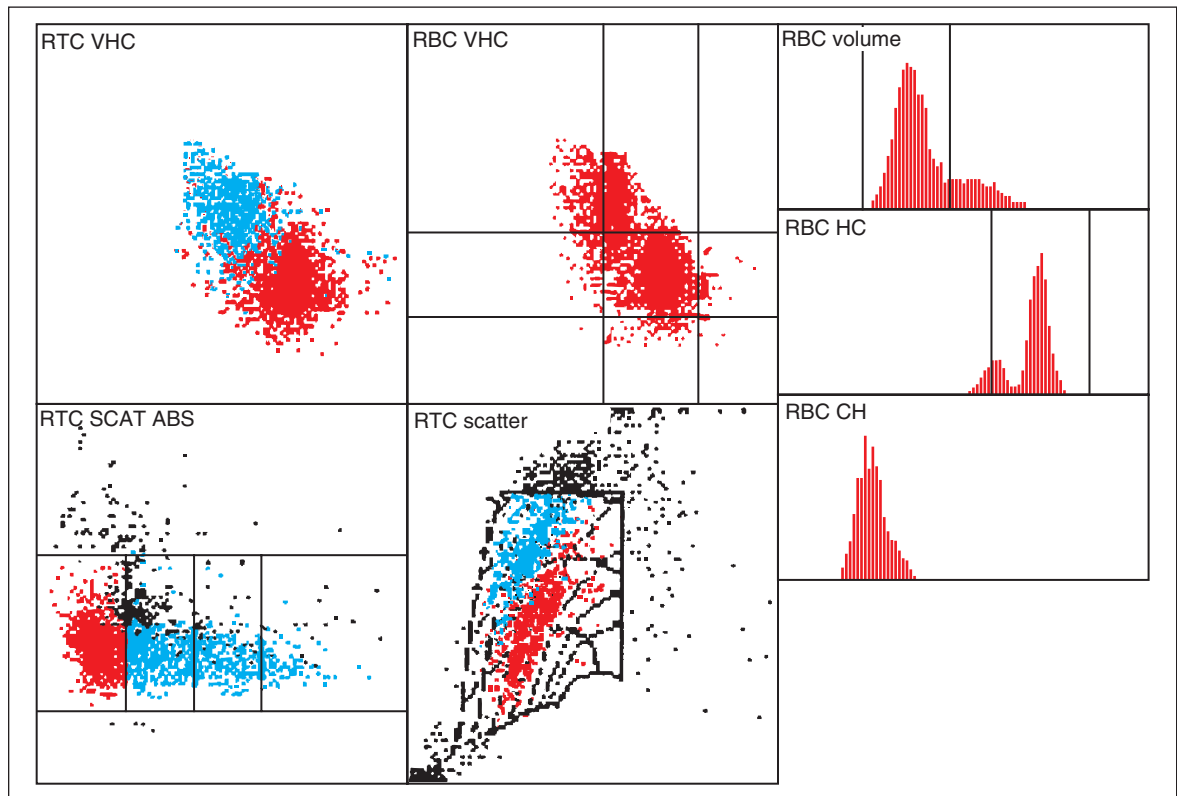


FIGURE 2-5. Erythrocyte and reticulocyte graphics from the Advia 2120 showing both cell types from a dog with a regenerative anemia. Reticulocytes are stained blue. Two separate RBC populations are shown in the four RBC cytograms, including normal RBCs and macrocytic hypochromic RBCs. The upper central cytogram (RBC VHC) was described in Figure 2-4B. The Advia 2120 recalls which RBCs were stained as reticulocytes and displays them as blue dots in various graphics. The upper left cytogram (RTC VHC) displays the macrocytic hypochromic RBCs as blue dots, illustrating that they were reticulocytes and reflect a regenerative response. The RTC SCAT ABS cytogram (lower left) shows the cells that took up the reticulocyte stain and further divides those reticulocytes into three boxes reflecting increasing RNA content to the right. The RBC VOLUME histogram (upper right) shows a smaller second, though prominent, peak of macrocytic RBCs on the right. The RBC HC cytogram shows a similar-sized peak (about one third the size of the normal RBC peak) of hypochromic RBCs to the left of the larger normochromic peak. The area under the curves of the histograms reflects the relative number (%) of normal or macrocytic hypochromic RBCs. Exact numbers of cells in each of the nine boxes are available from the instrument.

120/2120 automated reticulocyte analysis provides not only the absolute and relative reticulocyte counts but even mean reticulocyte volume, which may aid in judging the response of a dog with iron deficiency to treatment. The color-coded reticulocyte graphics in [Figure 2-5](#) show at a glance that the anemia in the dog was clearly regenerative. Advia 120/2120 and Sysmex XT-2000iV reticulocyte analysis works well for canine reticulocytes. The Advia and Sysmex instruments detect mainly the aggregate-type feline reticulocyte but do not detect even great changes in punctate reticulocytes.

The Advia 2120 and Sysmex XT-2000iV provide an automated four-cell differential leukocyte count (total neutrophils, lymphocytes, eosinophils, and monocytes). Neither the Advia, Sysmex, nor LaserCyte correctly identifies basophils in the dog or cat. There are other limitations of currently available automated instrument leukocyte differential counts (Diff), but the automated Diff can be used effectively if its limitations are recognized. Patterns in the automated instrument graphic displays are useful for detecting errors in classification of WBC types and in detecting blast cells in leukemia, NRBCs, eosinophilia, left shifts, and toxic changes in neutrophils.¹³

Laser-type hematology instruments such as the Advia 2120, Sysmex XT-2000iV, and LaserCyte identify platelets with an optical system and not only by size, as with impedance-type instruments (see also [Figure 2-3](#)). With two characteristics such as size and internal complexity (Advia) or size and nucleic acid staining (Sysmex), the instrument can correctly identify platelets larger than small erythrocytes. This is important in cats, where 60% to 74% of the platelets may be too large to be detected by an impedance platelet count.⁶ Platelets up to 60 fl are counted by the Advia instrument, while RBCs are frequently smaller than 60 fl (especially in cats). Platelet cytograms and histograms of the Advia and Sysmex instruments can often identify when nonplatelet particles (such as RBC ghost cells) were miscounted as platelets. [Figure 2-6](#) shows a normal canine platelet histogram and cytogram, and an abnormal histogram and cytogram in which erythrocyte ghost cells lysed in lipemic plasma were erroneously included in the platelet count. The MPV indicates the average size of platelets. The platelet cytograms and histograms illustrate distribution of platelets in the sample based on size and internal complexity. The mean platelet concentration (MPC) decreases in activated platelets. Various flags and cytograms warn of potential errors (e.g., clumped platelets).

Quantitative Buffy Coat VetAutoread

The IDEXX VetAutoread Haematology Analyzer (QBC V) (IDEXX Inc., Westbrook, ME) is an in-office hematology analyzer. Cell counts in the QBC V are determined from the width of various layers of different cell types in an expanded buffy coat. A plastic float spreads out the buffy coat, which is stained so that various layers are visible ([Figure 2-7](#)). Boundaries of the layers must be distinct enough to measure and may be less distinct in abnormal samples. The QBC V determines a two- to three-part WBC differential cell count consisting of mononuclear cells (i.e., lymphocytes and monocytes) and granulocytes, and, in dogs, eosinophils. QBC V analysis is more rapid and precise than manual counts using a hemocytometer but

less precise than an impedance instrument. Correlation to reference methods was only fair for platelet counts, but the QBC V actually measures a “plateletcrit.” As with the hematocrit for erythrocytes, the plateletcrit indicates the percentage of blood volume composed of platelets. The plateletcrit is converted in the QBC V to a platelet count, which veterinarians are accustomed to interpreting. The average size of platelets (MPV) is important in determining the actual number of platelets. For example, Cavalier King Charles spaniels (and Norfolk terriers) often have a hereditary platelet dysplasia in which platelets are very large and few in numbers. Especially with impedance platelet counts, the number of platelets is often so low in these Cavaliers that they seem like they should tend to bleed. Measuring the plateletcrit in these Cavaliers indicates that their plateletcrit is the same as normal dogs and they have no tendency to bleed.¹² The QBC V “platelet count” (actually a number reflecting the plateletcrit) is normal in these Cavaliers, while all other methods that actually enumerate the number of platelets indicate they are abnormal. Thus the QBC V could be called the “Cavalier instrument” because it was the only instrument validated to accurately measure plateletcrit in Cavaliers. The Advia 2120 plateletcrit has just been validated for use with Cavalier’s blood (data in press).

NOTE: The QBC V directly determines a plateletcrit. Plateletcrit is the best way to evaluate the platelet mass in the blood of Cavalier King Charles spaniel and Norfolk terrier dogs with hereditary platelet dysplasia with very large but few platelets.

Feline Platelet Counts

Platelet counts are usually inaccurate in cats (see also discussion of platelet counting in Chapter 5). Low platelet counts ($<200,000/\mu\text{l}$) were frequent in feline samples (71%), but only 3% were true decreases.⁹ Approximately 60% to 74% of feline platelets were too large to be detected by an impedance counter.⁶ With both aggregation and large platelets causing such great and approximately equal error, many believe it is impossible to obtain an accurate platelet count. However, if a true thrombocytopenia is suspected in a cat, use of optical platelet counting (laser-type analyzers) which include even the large platelets and prostaglandin E_1 (PGE_1) to inhibit platelet aggregation, can produce an accurate platelet count even in clinical settings.¹¹ PGE_1 must be frozen until just prior to use and is expensive, but true thrombocytopenia in cats is uncommon, so PGE_1 need not be used often.

NOTE: Feline platelet counts are often inaccurate, because approximately 70% of feline blood samples have clumping of platelets and 60% to 74% of feline platelets are too large to be detected by impedance-type analyzers.

If platelet clumping is observed in blood smears, distribution of platelets in the blood sample is uneven and the accuracy of manual counts, automated counts, and

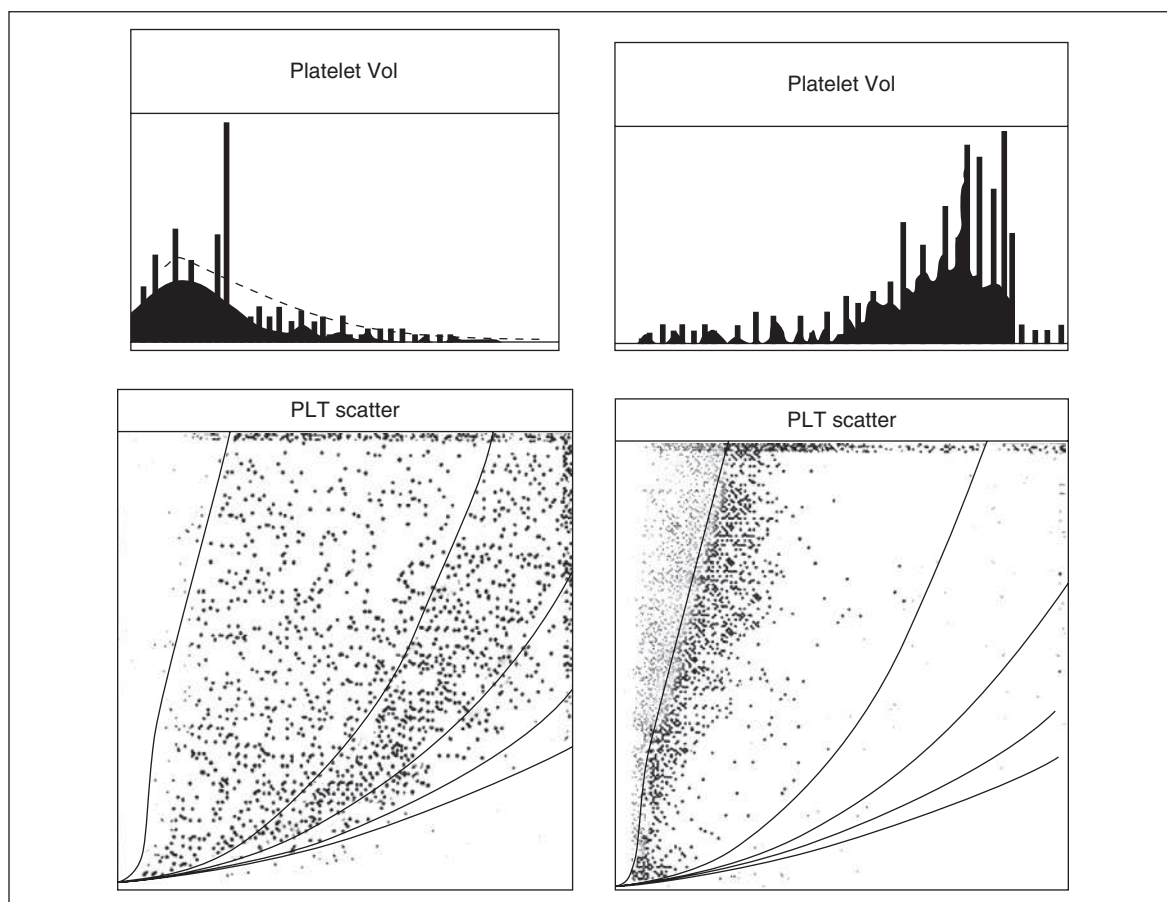


FIGURE 2-6. Advia 2120 platelet histograms and cytograms from two dogs. The histogram and cytogram on the left were from a dog with iron deficiency anemia but look relatively normal. The upper-left platelet volume (*Platelet Vol*) histogram shows the number of platelets of increasing size from left to right along the x-axis. The mean platelet volume (MPV) was about 4 to 6 fl. The platelet histogram and cytogram on the right were from a dog with Cushing's disease, lipemia, and many red blood cell (RBC) ghost cells. This histogram shows a reverse tendency with mainly large platelets and particles shown with a peak far to the right side of the histogram. The platelet cytograms (*PLT Scatter*) below show platelets based on size and optical density. The line that approximately follows the y-axis up to the top indicates increasing size. The upper x-axis is particle size and detects particles from 30 to 60 fl. Most dots (i.e., platelets) are within the counting area. The RBC ghost cells are in a cluster along the left edge of the counting area with some outside of the counting area. This was an instrument error that can be detected by visually inspecting the histograms and cytograms.

estimates from the blood smear are adversely affected. Manual counts are also affected by platelet aggregates; therefore a manual platelet count using the same blood sample with platelet aggregates or clots will also be in error. Manual counts include large feline platelets, which are often not counted by impedance-type hematology instruments, so even if imprecise, manual counts can be more accurate than impedance counts. The presence of platelet clumps on the smear does not disprove that true thrombocytopenia was present. A citrate-based anticoagulant (Diatube-H; Becton Dickinson) reduces pseudothrombocytopenia in feline blood samples. The Diatube-H includes citrate, theophylline, adenosine, and dipyridamole (CTAD), which inhibit platelet clumping better than EDTA.⁸ CTAD tubes do not provide a consistently accurate feline platelet count but do reduce the frequency and

magnitude of the pseudothrombocytopenia. Additionally, collecting blood by placing a needle into a vein and allowing blood to drip directly into a Vacutainer tube (with stopper removed) containing EDTA, or using a two-tube method (collect blood first into a tube not used for cell counting and then later collect blood into an anticoagulated tube for cell counts), helps to prevent platelet clumping.

BLOOD SMEAR ANALYSIS

Automated analysis of blood by hematology instruments validated for a given veterinary species provides abundant and useful information. However, blood smear evaluation is an important complement to hematology

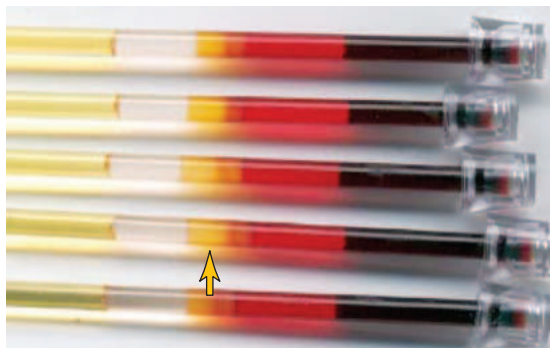


FIGURE 2-7. Five blood samples from Cavalier King Charles spaniel dogs with an autosomal recessive dysplasia of platelets (hereditary asymptomatic macrothrombocytosis) in which the number of platelets is low but the dog's plateletcrit is normal because the platelets are very large. The plateletcrit indicates the mass of platelets in blood and can be reported as a percentage of blood volume that is platelets (similar to the hematocrit). The IDEXX VetAutoread Haematology analyzer (QBC V; IDEXX Inc., Westbrook, ME) converts this plateletcrit to a platelet count. The width of the yellow layer (platelets, yellow arrow) in the five tubes reflects the plateletcrit, and all five Cavaliers had a normal plateletcrit despite platelet counts sometimes even less than 10,000/ μ l by impedance instruments. The clear area to the left of the platelets is the top of a clear plastic float that spreads out the buffy coat into layers of different cell types. The darker yellow-to-orange layer to the right of the platelets is the layer of leukocytes.

instruments and often provides diagnostic information that an instrument does not. Evaluation of a blood smear by a skilled observer is a rapid source of abundant information even in the absence of a hematology instrument. No currently available hematology instrument can identify canine or feline basophils nor even consistently identify all cell types in all species. A full five- to six-cell differential leukocyte count, including immature neutrophils, basophils, and atypical cells, is only possible by microscopy (manual Diff). Immature neutrophils, toxic change in neutrophils, reactive lymphocytes and monocytes, basophils, eosinophils, and leukemic cells typically are inconsistently identified by instruments. Detection of many morphologic alterations in RBCs, WBCs, and platelets requires morphologic evaluation in a microscope. Blood smear evaluation can be used to quickly detect clinically significant changes in WBC and platelet numbers. RBC estimates are inconsistent, so the PCV or another method should be used for enumeration of RBCs. Instrument cell counts are more precise and usually more accurate, but gross errors in instrument counts can be detected by blood smear estimates.

A full CBC includes automated instrument data and subjective evaluation of the blood smear and should be done at least for the first hematology examination of a sick patient. Use of the results of automated instruments alone allows great time savings and can be adequate to follow a previously well-defined hematologic problem. Several qualitative morphologic observations of WBCs, RBCs, and platelets permit clinically useful conclusions (see Chapters 3, 4 and 5).

NOTE: Blood smear evaluation is a rapid source of abundant information that, together with data from an automated hematology instrument, is an essential part of the initial CBC in a sick animal.

Making the Smear

One or more freshly made and quickly air-dried blood smears must accompany any complete hematologic examination and blood sample submission. Cells in tubes of anticoagulant have artifactual changes such as vacuolization and increased basophilia of neutrophils, degeneration of any cell type, and platelet aggregation, which occur over hours and days. These can be confused with disease changes or prevent some subjective evaluations. Cells in a freshly made smear retain proper morphology, and platelet aggregation is less likely. Blood smears should be mailed with the EDTA blood to a referral laboratory. EDTA blood is required for cell counts.

Smears must have a thin area where cells are distributed in a monolayer. This area provides optimal morphologic detail and reasonable cell distribution (Figure 2-8). RBCs in the monolayer should be close together but infrequently touching. RBCs in dogs should show central pallor to show they lay flat. Cells should not be distorted. Cells at the far edge (feathered edge) of a smear are often physically distorted, which can confuse or prevent interpretation. WBCs must lie flat and expose a large surface area for viewing (see Figure 16-2). Cells in the thin monolayer are stained best and lay flat and wide on the glass, allowing good evaluation of cytoplasmic and nuclear detail.

NOTE: Freshly made blood smears with a thin "monolayer" area are essential to allow proper identification of cells and make several diagnostic decisions.

Two common errors in reading blood and cytologic smears are (1) trying to identify cells in thick areas of the smear and (2) trying to identify damaged or distorted cells. Only cells with sufficient morphologic detail for evaluation should be included in any report. All smears have some damaged cells with distorted shapes and staining characteristics, which should not be included in the differential cell count. A large percentage of these cells is a cause for concern, and their origin (e.g., artifact, toxic change, dysplastic or neoplastic change) should be pursued. These cells may be placed in an "other" classification if more than 5% of WBCs were not interpretable.

The technique of making a smear should be practiced to produce good, readable smears. A wedge-type blood smear method starts by placing a proper-sized drop of blood on a glass slide near the frosted edge and using another spreader slide to make the smear. The spreader slide should be drawn back across the slide, going through the drop of blood. Without pausing, the spreader slide is

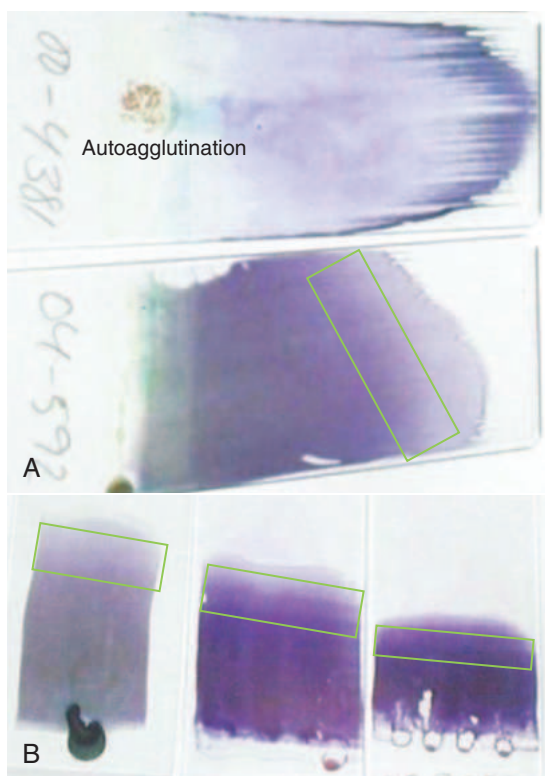


FIGURE 2-8. Examples of blood smears. **A**, The upper of the two blood smears has autoagglutination and an uneven distribution of cells, unlike the normal blood smear under it. Note that the upper smear had aggregated RBCs in the unstained original drop of blood on the left and ridges of cells at the feathered edge at the right. Smears should be properly labeled with lead pencil on the frosted end with the animal's identification, date, and other information needed to attribute any abnormality to the correct patient and sample. **B**, The three blood smears vary in length but have an evenly distributed thin (i.e., monolayer) area shown by green boxes near the ends of smears where cell evaluation should be performed. The monolayer area is also shown as a green box in the lower of the two smears in **A**. The upper smear with autoagglutination in **A** has no monolayer area, which makes it difficult to evaluate RBC morphology, such as the presence of spherocytes.

pushed forward with a single smooth stroke (stopping to permit the blood to spread along the spreader slide will result in altered WBC distribution in the smear). The angle of the spreader slide can be changed to vary the length of the smear (i.e., a lower angle produces a longer smear). A common mistake is to make a smear that extends to the end of the glass slide, causing the monolayer area to be lost. The size of the drop(s) can also be regulated to alter the length of the smear. If the drop of EDTA blood is too large, it is likely that the smear will go too long and off the end of the glass slide. The smear should only extend to the middle or distal two thirds of the slide so that the monolayer is in an area of the slide that is easily stained and examined. To prevent an irregular smear, one should use good-quality glass slides that have smooth edges and a clean surface, and learn to make a calm, even stroke during the smear.

Stains

Stains for hematology and cytology are discussed in Chapter 16.

Evaluating Blood Smears

A routine should be established to consistently evaluate and describe blood smears. All three cell types (i.e., WBCs, RBCs, platelets) should be evaluated for distribution, quantity, and morphologic characteristics.

Platelet Estimation

Platelet numbers can be estimated more accurately than WBC or RBC numbers (see Figure 5-5). The average number of platelets in a 100× oil immersion field in the monolayer area is counted. Between approximately 8 and 29 platelets/100× oil immersion field is expected on normal canine blood smears. The number varies with the field of view of the microscope used and what part of the blood smear (how thick) was examined. One estimate is to multiply the average number of platelets/100× oil immersion field times 15,000 to 20,000 platelets/μl. However, platelet estimates should be evaluated in semi-quantitative terms (e.g., very low, low, normal, high, very high). Precise instrument platelet counts should be used for judging response to treatment. One should check for platelet clumping, especially at the distal end of the smear. If platelets are prominently clumped, they are not evenly distributed; therefore, neither an estimate nor an actual platelet count (instrument or manual) is accurate. It has not been well determined what severity of platelet clumping (how many aggregates or how large the platelet clumps) is too severe to accept a clinically useful platelet count. The presence of few and small clumps does not seem to cause clinically inaccurate counts, but large aggregates or many aggregates should prevent reporting a platelet count. (See previous discussion of platelet counts in this chapter and Chapter 5.)

NOTE: Platelet numbers can be estimated reasonably well on blood smears. An average of 8 to 29 platelets per oil immersion (100×) field indicates a normal platelet count. Multiplying the average number of platelets/100× oil immersion field by approximately 15,000 to 20,000 platelets/μl also gives a rough estimate of the platelet count. Finding very large or many platelet aggregates indicates that an estimated or actual platelet count will be in error.

Platelet Morphology

Larger-than-normal platelets are consistently seen with thrombocytopenia and usually indicate active platelet production. However, the presence of large platelets on a blood smear—and, more precisely, an increased MPV or PDW—does not discriminate among different causes of thrombocytopenia in dogs, including immune-mediated thrombocytopenia, bone marrow disease, and disseminated intravascular coagulation in several retrospective studies. Large platelets and increased MPV are seen even

in dogs without thrombocytopenia, perhaps because of generalized bone marrow stimulation caused by another problem.

Leukocyte Estimation

Estimating the WBC count from a smear is less precise than estimating with platelets, because the larger WBCs are not as evenly distributed and fewer in number. Estimation is made by scanning the smear using the 10× microscope objective and subjectively estimating whether the number of WBCs is more or less than normal. One should look throughout the smear and especially at the end of the smear (i.e., feathered edge), where many WBCs may be unevenly distributed. Appropriate adjectives (e.g., “slight,” “moderate,” “marked”) are added to an estimated leukocytosis or leukopenia. Another method is to count the number of WBCs in several 10× objective fields in the monolayer area, where the platelet estimate and WBC differential are performed. In the authors’ laboratory, counting between 18 and 51 WBCs/10× objective field indicated a normal WBC count for canine blood smears. Correlation of the WBCs/10× field and the actual WBC count was good, as indicated by an *r* value of 0.87. Because accuracy deteriorates when the number of WBCs/10× field is greater than 60 (too numerous to count accurately), one should stop counting at 60 and not attempt to differentiate the magnitude of a leukocytosis based on the number of WBCs/10× field. This method depends on even distribution of WBCs in the smear and the field of view of the microscope lens. Uneven distribution, such as WBCs being pulled to the feathered edge of the smear, can causing falsely low WBC estimates.

Leukocyte Aggregation

Rare dogs and cats have strong aggregation of WBCs in EDTA blood tubes. Collection of blood in other anticoagulants (e.g., heparin, citrate) sometimes prevents or slows the aggregation and allows a more accurate total WBC count. Usually the error from WBC aggregates is minor, but in one cat with large WBC aggregates, the WBC count was 36,920/μl in EDTA but 64,650/μl when blood was collected in heparin and immediately analyzed. The cat’s WBCs also aggregated in heparin after 15 to 30 minutes. The agglutination is thought to result from antibodies that are affected by anticoagulants, and the tendency for WBCs to aggregate can be absent at the next examination.

Leukocyte Differential Count

WBCs are reported by absolute (cells/μl) and relative (%) differential leukocyte counts (Diff). A Diff can be from manual or automated methods. (See section on hematology analyzers in this chapter or Chapter 4 for more on the automated Diff.) The manual Diff is determined by classifying 100 to 200 or more WBCs on a blood smear to determine the percentage of each type of WBC present. The percentage of each type (e.g., 18% eosinophils) is multiplied by the total WBC count/μl to obtain the absolute count of each WBC type (e.g., if the total WBC count is 15,000/μl, then the absolute eosinophil count with 18% eosinophils is 2700 eosinophils/μl). Absolute counts are more consistently interpreted than percentages of cells (see Chapter 4).

Leukocyte Morphology

Left Shift • One of the major conclusions from a CBC is that the patient has an inflammatory disease. A left shift indicates an inflammatory disease with rare exceptions (i.e., granulocytic leukemia, Pelger-Huët anomaly).

NOTE: Documenting a left shift indicates the patient has an inflammatory disease. A manual Diff is required to document a left shift (increased immature neutrophils).

Identifying immature nonsegmented neutrophils (nonsegs or bands) to detect a left shift requires blood smear examination and a manual Diff. The criteria used to identify segmented neutrophils (segs) and nonsegs are subjective and vary with the observer. Just as no one criterion consistently identifies a person as mature, no one criterion consistently delineates the transition of a band into a seg. Segs acquire various morphologic markers of maturity that distinguish them from bands, including:

- Nuclear shape: lobe formation with focal narrowing (i.e., indentation) of the nuclear margin and loss of parallel sides
- Thin, dark nucleus
- Elongated nucleus
- Coarse clumping of nuclear chromatin (heterochromatin)
- Rough nuclear margin (where chromatin clumps protrude)

When uncertain if a cell is a nonseg or a seg, one should call it a seg, because segs are usually much more common than bands and younger nonsegs. Toxic neutrophils develop abnormally (i.e., asynchronous maturation; [Figure 2-9](#)), making consistent identification of various stages of maturation difficult or impossible. A description of the blood picture as, for example, a severe left shift back to metamyelocytes with severe toxic change may be more honest than trying to report a specific number (%) of nonsegs.

The magnitude of the left shift reflects the severity of inflammation. Neutrophils younger than segs include bands, metamyelocytes, myelocytes, promyelocytes, and myeloblasts. All immature neutrophils are nonsegs. Nonsegs in blood are usually bands, because the bone marrow preferentially releases the most mature forms of neutrophils. If the left shift includes nonsegs younger than bands, each type should be noted to reflect increased severity of the left shift.

Toxic Neutrophils • Significant toxic change in neutrophils (see [Figure 2-9](#)) indicates more severe disease and a worse prognosis (see Chapter 4). Toxic neutrophils usually are associated with left shifts, leukopenia, and leukocytosis, but they may be present while the WBC count is normal, in which case a blood smear is required to document the abnormality. Toxic change should be reported both by the number of neutrophils affected and by the severity. When toxic change is mild, or few neutrophils are affected (or both), abnormal clinical signs may not be noted. When toxic changes are moderate to severe

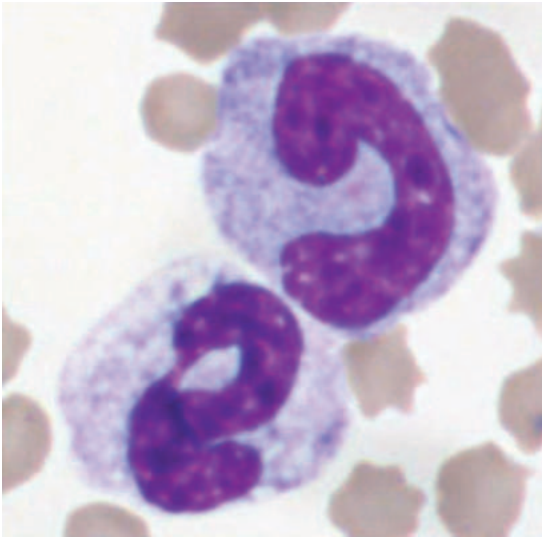


FIGURE 2-9. Toxic neutrophils from a cat with pyothorax. The dark blue (not neutral-colored) neutrophil cytoplasm indicates moderate to severe (3+) toxic change. The lower cell has indistinct clearing (lighter colored areas), indicating toxic vacuolation. Both are bands (nonsegs) even though the lower neutrophil has a distinct narrowing of the width of the nucleus (asynchronous maturation). However, the rest of the nucleus has smooth parallel sides and the chromatin density (clumping) is similar to the classic U-shaped band above.

and many cells are affected, signs such as fever, vomiting, diarrhea, depression, shock, and sepsis are more common; hospitalization days are longer; and prognosis is worse.^{1,10}

NOTE: Moderate to severe toxic changes in moderate to many neutrophils indicates a worse prognosis, longer stays in the hospital, and more frequent signs such as fever, vomiting, diarrhea, depression, shock, and sepsis.

Enteric disease (e.g., parvoviral diarrhea) and bacterial infections cause the most severe toxic changes and often degenerative left shifts. Toxic change is not restricted to bacterial toxemia and may be seen in diseases such as IMHA and metabolic diseases such as diabetes and hepatic lipidosis.

The following guidelines may be used for uniformity in reporting. The number of neutrophils that appear toxic is reported as few, moderate, or many. No comment is made with 0% to 4% toxic neutrophils; few indicate 5% to 10%; moderate indicates 11% to 30%; and many indicate that greater than 30% of neutrophils appeared toxic. Döhle bodies in feline neutrophils appear frequently and may be seen in cats without severe clinical signs. However, Döhle bodies are a morphologic change most consistently recognized by microscopists and thus important in documenting toxic change in contrast to artifact (e.g.,

BOX 2-3. SIMPLE GRADING SCHEME FOR REPORTING APPEARANCE OF TOXIC NEUTROPHILS

SEVERITY	MORPHOLOGIC CHARACTERISTICS OF TOXICITY
1+	Only few to moderate number of Döhle bodies are present in clear cytoplasm
2+ or 3+	Mainly variable intensity of cytoplasmic basophilia, cytoplasmic foaminess, but also giant neutrophils or toxic granulation
4+	Cytoplasm is too blue, vacuolated, and nuclei are too rounded to differentiate toxic neutrophils from reactive monocytes or reactive lymphocytes

EDTA basophilia and distinct vacuoles). Finding only a few Döhle bodies in clear cytoplasm indicates a mild change. More severely toxic neutrophils have several changes, including cytoplasmic basophilia, vacuolization, and, more often in cats, giant nuclei. Grading cytoplasmic basophilia or vacuolization is subjective and affected by the type of stain used and how long the neutrophils remained in EDTA. EDTA-induced vacuoles (artifact) have distinct membranes around them, whereas toxic “vacuolization” is a more diffuse and irregular clearing in bluish cytoplasm. A simple reporting system for severity of toxicity is provided in Box 2-3. A more complex grading system for a research project on feline toxic neutrophils is shown in Table 2-3.¹⁰ Total scores from that system were mild

TABLE 2-3. SEGEV'S GRADE FOR TOXIC CHANGE IN FELINE NEUTROPHILS

MOPHOLOGIC CHANGE INTENSITY	Cells Affected with Change		
	<10%	10%-30%	>30%
Döhle bodies			
Mild	1	1	1
Moderate	1	1	2
Marked	2	2	3
Cytoplasmic basophilia			
Mild	1	1	2
Moderate	2	2	3
Marked	2	3	3
Cytoplasmic vacuolation			
Mild	1	1	2
Moderate	2	2	3
Marked	2	3	3
Giant toxic neutrophils	3	3	3

(1 to 6 points), moderate (7 to 12 points), and marked (>13 points) toxic change.

Reactive Lymphocytes • Lymphocytes vary greatly in type and appearance. Immune stimulation of lymphocytes occurs in both healthy and diseased animals. Stimulated lymphocytes are called *reactive lymphocytes*. Occasional reactive lymphocytes are common in blood smears of both sick and healthy animals. Numerous reactive lymphocytes in sick animals suggest strong antigenic stimulation, but the number of reactive lymphocytes does not reliably indicate the strength of an immune reaction. They have prominent dark-blue cytoplasm that reflects increased protein synthesis and increased messenger RNA (mRNA) in the cytoplasm. The nucleus may undergo blast transformation and have a convoluted shape. Reactive lymphocytes may be only slightly enlarged with slightly bluer cytoplasm or may transform into large blast cells with dark-blue cytoplasm. Reactive lymphocytes tend to be more numerous in young animals. Blast-transformed lymphoid cells resulting from immune stimulation (e.g., *Anaplasma phagocytophilum* infection) can erroneously mimic acute lymphoblastic leukemia (ALL).

NOTE: Reactive lymphocytes may be large, immature, and dark. Increased numbers of reactive lymphocytes that are large and immature may be confused with leukemia. Reactive lymphocytes are commonly seen in sick animals.

Leukemia • Most dogs and cats with leukemia have acute leukemia. Acute leukemia is usually characterized by marked leukocytosis with many blast cells (see Chapter 4). The cell type of neoplastic cells may be hard to identify, and they may simply be called “blasts” or “atypical cells” unless specific staining procedures are done. The number of leukemic cells in peripheral blood can vary from many to none. In some leukemia patients, the blast cells are retained in the bone marrow.

Erythrocyte Estimation

Estimating RBC numbers from a blood smear is not consistent due to variability in the thickness of manually prepared blood smears. The severity of an anemia may be reflected by the gross staining intensity of the slide and greater length of the monolayer area. Blood smears from severely anemic animals grossly appear pale against a white background, whereas blood smears from patients with a normal PCV appear red or orange. In blood smears from animals with a normal PCV, the monolayer area is a small to moderate elliptical area just behind the feathered edge of the smear (see Figure 2-8A and 2-8B). In smears of very anemic blood, the monolayer area extends farther back, even to where the drop of blood was initially applied in severe anemia. In hemoconcentrated blood (e.g., dehydration), the monolayer area is small or absent.

Erythrocyte Morphology

Many RBC morphologic changes are described with Latin and English names. Some morphologic changes are diagnostically useful, such as polychromasia (i.e., reticulocytosis), true hypochromasia, spherocytes, autoagglutination, rouleaux formation, Heinz bodies,

eccentricocytes, blood parasites, and RBCs with distemper inclusion bodies (Figure 2-10 and 2-11).

NOTE: The morphologic changes in RBCs that are diagnostically useful include polychromasia (i.e., reticulocytosis), hypochromasia, spherocytes, autoagglutination, rouleaux formation, Heinz bodies, eccentricocytes, and blood parasites, or viral inclusions.

Many common RBC changes are of little clinical significance (e.g., anisocytosis, echinocytes, elliptocytes, codocytes, leptocytes), especially in low numbers (see Chapter 3). Although it is technically correct to report these, excessive details may distract the clinician who is reading a report from concentrating on the more diagnostic findings.

RBC morphology should be evaluated on the thick edge of the monolayer area. On the thin edge of the monolayer area (i.e., near the feathered edge), RBCs are distorted and lose their original shape. Canine RBCs, in the thick edge of the monolayer area of the smear, should display central pallor. It is particularly important in identifying spherocytes to have normal RBCs with distinct central pallor near the spherocytes for comparison. RBCs lack central pallor near the feathered edge and may be misidentified as spherocytes, causing a misdiagnosis of IMHA.

The frequency of the morphologic change in the RBC population is usually related to its significance and important in interpretation of the change. Abnormal RBCs may be found in low numbers on blood smears from normal animals. One should disregard rare abnormal RBCs.

Polychromasia • Polychromasia, as seen in Wright- or Giemsa-stained (i.e., Romanowsky-type stains) blood smears, indicates increased numbers of larger, bluer RBCs called polychromatophils. Polychromatophils correspond fairly well to canine reticulocytes or feline aggregate reticulocytes. However, different Romanowsky stains vary in how well the polychromatophils appear clearly darker blue than erythrocytes and thus how well they can be identified. Reticulocytes have dark granules representing ribosomal material and RNA when stained with new methylene blue (NMB) or other reticulocyte stains (see Chapter 3). Polychromasia indicates increased release of reticulocytes from the bone marrow in regenerative anemias (see Chapter 3). Reticulocytes are macrocytic hypochromic RBCs.

NOTE: Polychromasia indicates that increased numbers of reticulocytes were released from bone marrow in regenerative anemia. Polychromatophils correspond to canine reticulocytes and feline aggregate reticulocytes.

Relative guidelines to quantify reticulocytes and polychromatophils in dogs are as follows: normal, less than 1%; slight increase, 1% to 4%; moderate increase, 5% to 20%; and marked increase, 21% to 50% (see Chapter 3). For feline aggregate reticulocytes and polychromasia,

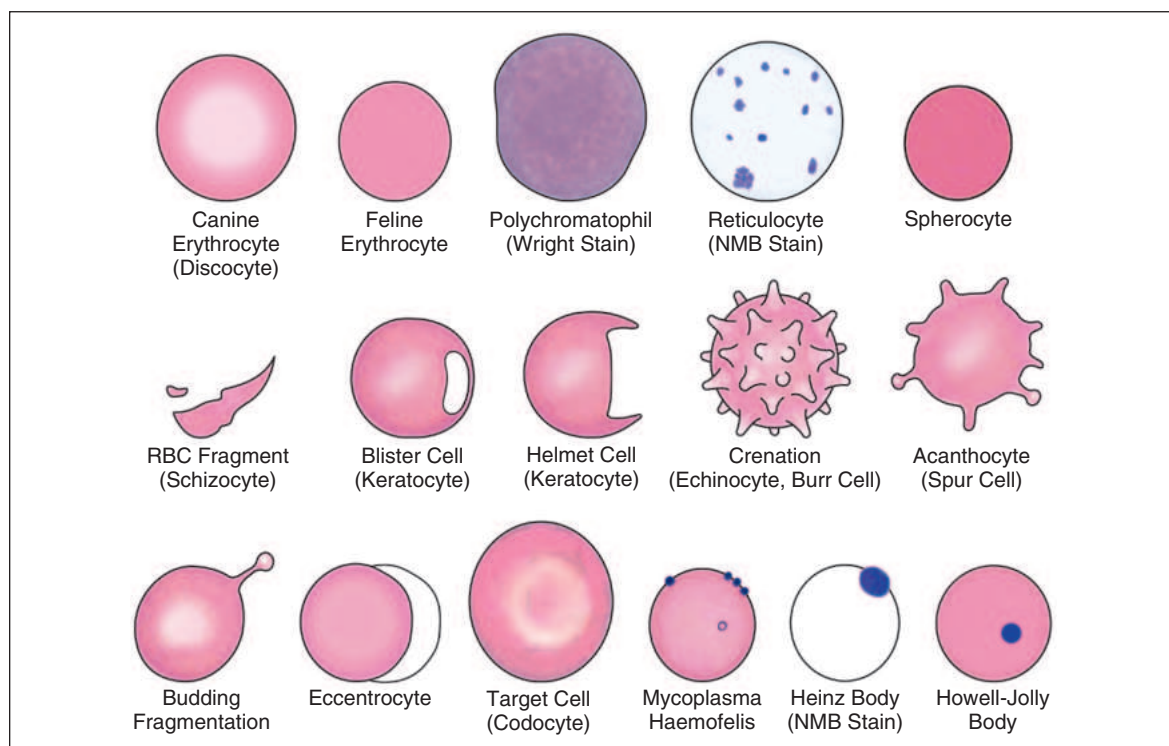


FIGURE 2-10. Selected erythrocyte inclusions and shape changes. Some common terms and synonyms are given beneath drawings of selected morphologic alterations of red blood cells (RBCs). These are illustrated as they appear on Wright-stained blood smears, except for reticulocytes and Heinz bodies, which are preferentially stained with new methylene blue (NMB). Two normal erythrocytes are shown first for comparison. (See text for descriptions and diagnostic significance.)

guidelines are as follows: normal, less than or equal to 0.4%; slight increase, 0.5% to 2%; moderate increase, 3% to 4%; and marked increase, greater than or equal to 5%.

Spherocytes • Blood smear analysis is frequently diagnostic in hemolytic anemias. Several morphologic changes in RBCs are specific for certain disease conditions (see Chapter 3). Moderate to abundant spherocytes or autoagglutination, or both, are good indicators of IMHA. Since IMHA is the most common severe anemia of dogs, these changes must be correctly identified and interpreted (see Chapter 3).

NOTE: Good indicators of IMHA are finding moderate to abundant spherocytes in blood smears and/or autoagglutination remaining after mixing EDTA blood with 3 to 5 times more saline.

To identify spherocytes in canine blood smears, it is important to examine only the monolayer area where normal biconcave canine erythrocytes typically lie flat and exhibit central pallor (see Figures 2-8 to 2-11). By comparison to normal disc-shaped canine erythrocytes, spherocytes are more spherical and do not lay flat on the smear so they appear smaller in diameter, are darker in color, and lack central pallor (see Figure 2-11C). Spherocytes have normal cell volume and hemoglobin concentration but appear smaller because they are

spherical and do not lay as flat as discs on the smear. Because they are spherical, they are thicker and thus appear darker (though not really hyperchromic). In thick areas and near the feathered edge of blood smears, normal canine RBCs do not lay flat so lack central pallor and mimic spherocytes, so this area should be avoided. Feline RBCs normally lack central pallor; therefore spherocytosis is difficult to detect.

Autoagglutination • True autoagglutination is immune-induced aggregation of RBCs into grapelike clusters. True autoagglutination is equivalent to a positive Coombs' reaction and strong evidence for IMHA. Incompatible transfused blood is removed by immune-mediated agglutination, opsonization and phagocytosis, or hemolysis and may have the features of IMHA. Both autoagglutination and strong rouleaux formation may be visible grossly as RBCs clumping in EDTA collection tubes and may be difficult to differentiate on blood smears.

Rouleaux formation is the linking of RBCs into chains resembling stacks of coins. Some rouleaux is normal in dogs, and more occurs in normal cats. Increased rouleaux formation in canine blood smears is associated with an increase in fibrinogen or acute phase proteins and is usually seen in inflammatory diseases. Rouleaux formation is also associated with excessive antibody production seen in some cases of lymphoid neoplasia (e.g., plasma cell myeloma), but this is an uncommon cause. Autoagglutination may be differentiated from rouleaux

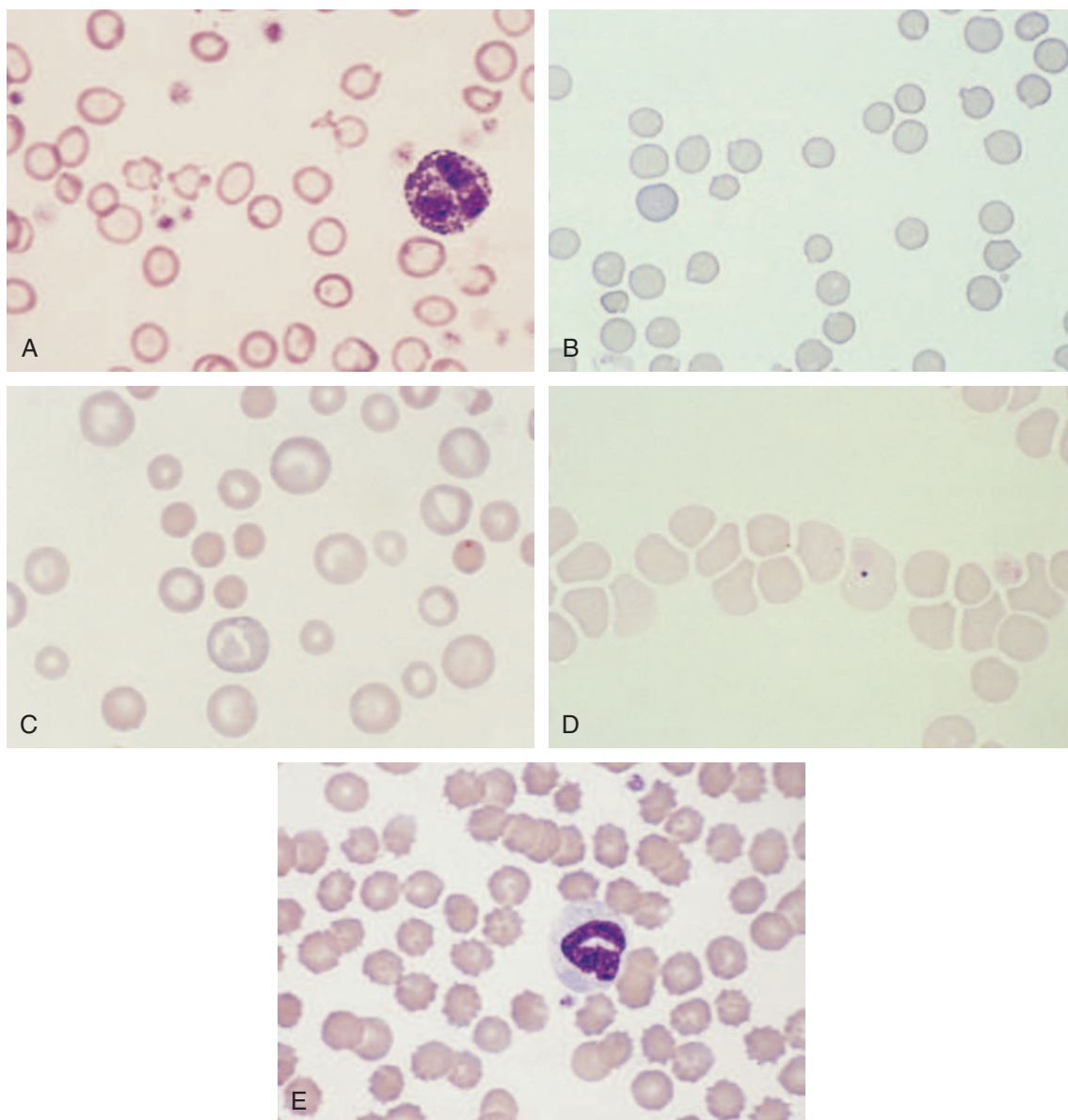


FIGURE 2-11. **A**, Canine iron deficiency anemia. The erythrocytes have marked central pallor (hypochromasia) with only thin rims of hemoglobin. Erythrocyte fragmentation is also visible. **B**, Feline Heinz body anemia. Many of the erythrocytes have a round, usually lighter staining Heinz body at the margin, with about half of the Heinz body extending above the surface. A free, round Heinz body has the same color as the hemoglobin in erythrocytes. **C**, Canine immune-mediated hemolytic anemia. There is a cluster of four spherocytes, slightly up and left of center, and two spherocytes to the far right of center. Note that this is in an area where other erythrocytes show central pallor, so it is easy to see the smaller diameter, darker color, and lack of central pallor in the spherocytes. **D**, Canine distemper. The rounded viral inclusion bodies vary in color from gray to reddish. The most obvious inclusion body is above the dark-blue, smaller Howell-Jolly body. **E**, Canine distemper. There is a large, gray, viral inclusion body in the cytoplasm of the neutrophil at about 7 o'clock at the cell margin.

formation by vigorous mixing of EDTA blood with a greater amount of saline (3 to 5 times more saline) and observing it as a wet mount under the microscope. Saline causes RBCs to disperse from gross and microscopic clumps resulting from rouleaux formation but does not cause dispersal if the clumping is from autoagglutination.

NOTE: Both autoagglutination and strong rouleaux formation may be visible grossly in test tubes. Autoagglutination may be differentiated from rouleaux formation by mixing blood with 3 to 5 times more saline and observing it as a wet mount under the microscope.

Poikilocytosis • Poikilocytosis is a nonspecific term for variation in RBC shape and should be further classified as to the type of shape change present (see Figure 2-10). *Echinocytes* are frequently artifacts (e.g., crenation) but may be physiologic/pathologic changes. Echinocytes may be more numerous in some metabolic diseases. Echinocytes have numerous, uniform, usually pointed or occasionally rounded projections from the RBC surface. These projections, when viewed from above the cell, resemble dots and tiny letter o's that may mimic the ring forms of *Mycoplasma haemofelis* or *Mycoplasma haemocanis* (previously *Haemobartonella*). Canine distemper inclusion bodies are rare in RBCs and WBCs (see Figures 2-11D and 2-11E) but are diagnostic when present.

Acanthocytes • Acanthocytes have a few irregular projections with rounded ends often forming a bud. Some RBCs resemble both acanthocytes and echinocytes in having sharp pointed projections that are irregular in length and few in number. They have been called echinoacanthocytes. In dogs, acanthocytes are associated with RBC fragmentation and with altered lipid metabolism, such as what occurs in hepatic disease. In cats, acanthocytes are often associated with liver disease, including hepatic lipidosis and cholangiohepatitis. Some cats have prominent poikilocytosis with acanthocytes but appear clinically healthy (anecdotally associated with Persian cats).

Red Blood Cell Fragmentation • RBC fragmentation results when RBCs are forced through altered vascular channels, or occurs as a result of turbulent blood flow. RBC fragmentation has been associated with disseminated intravascular coagulation, iron deficiency anemia, myelofibrosis, congestive heart failure, glomerulonephritis, hemangiosarcoma, and hemophagocytic histiocytosis in dogs.

NOTE: In the dog, RBC fragmentation has been associated with disseminated intravascular coagulation, iron deficiency anemia, myelofibrosis, congestive heart failure, glomerulonephritis, hemangiosarcoma, and hemophagocytic histiocytosis.

Vascular disorders such as disseminated intravascular coagulation cause fibrin strands in circulating blood that can split RBCs by hitting them. The small, irregular RBC fragments are called schistocytes (also termed schizocytes), keratocytes (helmet cells), or RBC fragments (see Figure 2-10). The acanthocyte is the most frequent shape change associated with RBC fragmentation in dogs.

Leptocytes • Leptocytes are flexible RBCs with seemingly excessive membrane. When they lie flatter than normal on the slide, one sees an enlarged area of central pallor often misclassified as hypochromasia. This wider area of central pallor of leptocytes is differentiated from true hypochromasia of iron deficiency by the thickness and color of the rim of Hgb and by RBC indices. Leptocytes have a dark rim of Hgb, whereas iron-deficient

cells have a thin, faint rim of Hgb (see Figure 2-11A). A common form of leptocyte is the codocyte (i.e., target cell), which has a small circle of Hgb in the middle of the area of central pallor (see Figure 2-10). Leptocytes and codocytes are nonspecific findings and may be associated with regenerative anemias and splenic or hepatic disease.

Other RBC Morphologic Changes • Many other RBC shapes have descriptive names, such as elliptocyte (i.e., oval RBCs or ovalocyte) and dacryocyte (i.e., teardrop-shaped RBCs). If RBCs have abnormal shapes that do not easily fit the common classifications, one should simply report the presence of poikilocytosis and quantify it from 1+ to 4+. Prominent poikilocytosis indicates an abnormality, but a cause may not be apparent.

OTHER DETERMINATIONS

Plasma Protein Determination

Plasma total protein can be determined from the plasma layer in a centrifuged microhematocrit tube. The tube is scored with a file just above the buffy coat and broken, and the plasma is placed into a refractometer. Most refractometers have an internal scale for plasma total protein and a scale for urine specific gravity. If the scale is graduated for only refractive index, a conversion chart is necessary to convert the index to a protein concentration. Plasma protein concentration is useful in interpretation of anemia or polycythemia (see Chapter 3), dehydration, and other problems (see Chapter 12).

NOTE: Because both plasma protein and PCV are affected by hydration status, it is helpful to determine both plasma protein and PCV to evaluate anemia, polycythemia, or protein disorders.

Acute Phase Proteins

The total and differential leukocyte counts (with left shift) are usually used for documenting inflammation in dogs and cats, but acute phase protein responses are an additional and very sensitive way to document inflammation. Some feline infectious peritonitis (FIP) profiles for cats include an acute phase protein such as α 1-acid glycoprotein or serum amyloid A. C-reactive protein is more commonly used in dogs to document and monitor inflammatory diseases. Fibrinogen is a plasma acute phase protein that begins to increase with the onset of inflammatory diseases, continues to increase for several days, and remains elevated until the inflammatory process resolves. C-reactive protein, α 1-acid glycoprotein, and serum amyloid A have much greater and earlier increases during inflammation than fibrinogen. C-reactive protein, haptoglobin, and serum amyloid A are globulins that migrate in the α 2 region on a serum protein electrophoretogram (SEP) are positive acute phase proteins that increase as the result of inflammatory mediators. Albumin is a negative acute phase protein that decreases in synthesis during inflammation.

NOTE: C-reactive protein, α 1-acid glycoprotein, and serum amyloid A are acute phase proteins that are very sensitive indicators of inflammation in the dog and cat. Fibrinogen and haptoglobin also increase in response to inflammation but to a lesser degree.

Increased fibrinogen or other globulins will cause increased rouleaux formation on canine blood smears as an additional indicator of inflammation. Rouleaux formation causes RBCs to sediment more rapidly. RBC sedimentation can be measured by determining the erythrocyte sedimentation rate (ESR) to monitor inflammation, but this is more often done now in human patients than dogs and cats. Rouleaux formation is prominent in blood from healthy cats; thus it is harder to note increased rouleaux formation.

Lipemia, Hemolysis, and Icterus

Lipemia (turbid, white plasma), hemolysis (red color), and icterus (yellow to orange color) can be visually detected in plasma of the microhematocrit tube and aid in diagnosis (see [Figure 2-1](#)).

NOTE: Lipemia (turbid, white plasma), hemolysis (red color), and icterus (yellow to orange color) can be visually detected in plasma of the microhematocrit tube.

Lipemia occurs with recent ingestion of a fatty meal, pancreatitis, diabetic ketoacidosis, hypothyroidism, hepatic disease, and primary lipid disorders (e.g., in schnauzers; see Chapter 8). Lipemic plasma causes an altered refractive index, so plasma protein determination in a refractometer or hemoglobin concentration in hematology analyzers may be erroneous. RBCs are more fragile in lipemic plasma and tend to lyse *in vitro*, so hemolysis frequently accompanies lipemia (turbid red plasma). Lipemia will affect the erythrocyte fragility test. RBCs appear fuzzy on blood smears, and the background appears blue and foamy.

Hemolysis is often an artifact of collection and handling but may also be an indicator of intravascular hemolysis. Hemolytic anemia must be acute and massive to cause true intravascular hemolysis and should be associated with hemoglobinuria. Artifactual hemolysis will falsely decrease PCV and MCV values and increase the MCHC. Hyperchromasia (i.e., increased MCHC) is always an indication of laboratory error that may be associated with lipemia, free Hgb in plasma, Heinz bodies in the RBCs (laser counters), or Heinz bodies in suspensions of lysed RBCs (Hgb determination).

Icterus suggests either hemolytic anemia or a hepatic problem (see Chapters 3 and 9). Some laboratories perform an icterus index on plasma to roughly quantify icterus. The icterus index compares the color of plasma with a set of color standards.

Color of Blood

Abnormal color of blood may be evaluated by putting a drop on white filter paper. Brown blood (or perhaps only darker-than-normal blood) suggests methemoglobinemia. To obtain normal red color of oxyhemoglobin in normal blood, one should expose EDTA blood to air. A drop of blood on filter paper is well exposed to air, but to oxygenate blood in a tube, one should remove the cork and pour blood back and forth between 2 tubes. Blood with methemoglobin remains brown after airing. In cyanide poisoning blood may be cherry red, and in carbon monoxide poisoning blood should be bright red.

BONE MARROW EXAMINATION

Bone marrow is usually examined to answer specific questions that were not answered by evaluation of routine CBC examinations.

NOTE: Indications for bone marrow examination include nonregenerative anemia, persistent neutropenia, persistent thrombocytopenia, bicytopenias, pancytopenias, unexplained polycythemia or thrombocytosis, atypical cells in blood, unexplained hypercalcemia, hyperproteinemia, monoclonal gammopathy, and staging of lymphomas.

Indications for bone marrow examination include moderate to severe nonregenerative anemia, persistent neutropenia, persistent thrombocytopenia, bicytopenia, pancytopenia, unexplained polycythemia or thrombocytosis, atypical cells in blood, unexplained hypercalcemia, hyperproteinemia, monoclonal gammopathy, and staging of lymphomas. Mild anemia with a likely primary cause such as inflammation or a regenerative anemia in which the bone marrow appears to be responding appropriately are not indications to undertake the effort of obtaining bone marrow samples. A brief summary of general bone marrow conclusions is given in [Box 2-4](#). For more specific details on diagnostic approaches in certain disorders of RBCs, WBCs, and platelets, see Chapters 3, 4, and 5, respectively.

The conclusions obtained from evaluation of a bone marrow aspirate and a biopsy are quantitative and qualitative. The quantity of various cell types is determined. In animals with peripheral cytopenia, bone marrow examination should indicate whether decreased bone marrow production is the cause. With blood loss (e.g., hemorrhage) or blood destruction (e.g., hemolysis), the numbers of marrow precursor cells should be increased (i.e., erythroid hyperplasia) after 1 to 3 days. With decreased cell production, the numbers of the particular cell type in bone marrow may be decreased (i.e., hypoplasia) but quite often the bone marrow conclusion is ineffective hematopoiesis, in which the number of precursor cells in the marrow is normal to increased despite no effective increased release of cells. Cells are destroyed before being released into the blood (e.g., apoptosis). In order to make a proper conclusion from bone marrow examination, the

BOX 2-4. SELECTED BASIC BONE MARROW CONCLUSIONS

BONE MARROW CONCLUSION	DEFINITION
Erythroid hyperplasia	Increased marrow cellularity with decreased M/E ratio
Erythroid hypoplasia	Decreased marrow cellularity with increased M/E ratio
Myeloid hyperplasia	Increased marrow cellularity with increased M/E ratio
Myeloid hypoplasia	Decreased marrow cellularity with decreased M/E ratio
Megakaryocytic hyperplasia	Increased number of megakaryocytes in marrow
Megakaryocytic hypoplasia	Decreased number of megakaryocytes in marrow
Aplastic anemia (also termed aplastic pancytopenia)	Marked decrease in erythroid, myeloid, and megakaryocytic cells in marrow
Lymphoid hyperplasia	Increased proliferation of lymphocytes and plasma cells in marrow
Myelodysplastic syndrome	Anemia, bicytopenia, or pancytopenia in blood; variably cellular marrow; variable increase in blast cells (5%-20%); and consistent prominent dysplastic features in blood or marrow
Acute leukemia	Hypercellular marrow with greater than 20% blast cells

M:E ratios is the percentage of myeloid cells divided by percentage of erythroid cells.

number of cells in the blood and bone marrow should be known; therefore a CBC should be performed the same day as a bone marrow examination.

Hypoplasia and Aplasia

The marrow may have decreased numbers (i.e., hypoplasia) or severe deficiency (i.e., aplasia) of one or more cell types. Hypoplasia or aplasia indicates that cytopenia is due to insufficient marrow production of that cell type. When all cell lines (erythroid, granulocytes, and megakaryocytes) are decreased, the condition is termed aplastic anemia or aplastic pancytopenia. Specific causes of marrow hypoplasia and aplasia are described in the appropriate chapters.

Hyperplasia and Neoplasia

A proliferation of one or more cell types in the bone marrow may be a normal response to an increased need for RBCs, WBCs, and platelets, or it may be an abnormal neoplastic or dysplastic process.

NOTE: Proliferation of cell types can result from a normal need for increased production or from an abnormal neoplastic or dysplastic process.

In hemolytic or blood loss anemia, erythroid hyperplasia is the expected and appropriate physiologic response in bone marrow. Inflammatory disease stimulates myeloid hyperplasia in bone marrow. Consumption of platelets in immune-mediated thrombocytopenia or disseminated intravascular coagulation induces megakaryocytic hyperplasia. Additionally, megakaryocytic hyperplasia often accompanies erythroid hyperplasia in response to a hemolytic anemia. A systemic immune response may result in lymphocytic and plasmacytic hyperplasia. A marked increase in immature (i.e., >20% blast cells) cells in any cell lineage indicates hematologic neoplasia with rare exceptions. Myelodysplastic syndromes have large numbers of dysplastic (i.e., abnormal development) cells and frequently have a mild to moderate (i.e., 5% to 20%) increase in blast cells (see Chapter 4).

Myelodysplastic Syndromes

Myelodysplastic syndromes identify qualitative bone marrow defects that result from acquired genetic mutations in hematopoietic stem cells.

NOTE: Myelodysplastic syndromes identify qualitative bone marrow defects that result from acquired genetic mutations in hematopoietic stem cells.

Bone marrow cellularity is usually normal or increased, but cells are destroyed before entering the blood (see Chapters 3 and 4). Myelodysplastic syndromes are characterized by cytopenias in the blood, variable mild increases in numbers of immature cells (i.e., 5% to 20%) in bone marrow, and prominent morphologic alterations (dysplastic changes) in precursor cells. Myelodysplastic syndromes have been subclassified according to a World Health Organization classification system into categories, including refractory anemia (RA), refractory anemia with ringed sideroblasts (RARS), refractory anemia with multilineage dysplasia (RAMD), and refractory anemia with excess blasts (RAEB).¹⁵ Dysplastic changes associated with myelodysplastic syndromes must be differentiated from secondary dysmyelopoiesis that is associated with certain diseases and with exposure to certain drugs and toxins.¹⁵

Acute Leukemia

Acute leukemias are caused by genetic defects similar to those seen in myelodysplastic syndromes. The number of blast cells in bone marrow or blood differentiates the disorders.

NOTE: The number of blast cells in blood or bone marrow differentiates leukemia from myelodysplastic syndrome. Myelodysplastic syndromes have less than 20% blast cells and leukemia has greater than 20% blast cells.

Normally, blast cells account for less than 5% of the total nucleated cell population in bone marrow. In myelodysplastic syndromes, blast cells are less than 20%

of nucleated bone marrow cells. In leukemia, blast cells exceed 20% (see Chapter 4). Myelodysplastic syndromes may progress to acute leukemias.

Bone Marrow Histopathology

When a bone marrow core biopsy is examined, several disease conditions can be identified that cannot be identified on bone marrow aspiration smear. Major pathologic changes include myelofibrosis, myelonecrosis, and acute inflammation.

NOTE: When a bone marrow core biopsy is examined, several disease conditions can be identified that cannot be identified on bone marrow aspiration smear. Major pathologic changes include myelofibrosis, myelonecrosis, and acute inflammation.

Myelofibrosis is bone marrow replacement by fibroblasts, collagen, or reticulin fibers. Myelofibrosis can be primary or secondary. Bone marrow aspirates from a fibrotic marrow are often poorly cellular and not diagnostic, so a core biopsy for histopathologic evaluation is required.

Various chemicals (e.g., chemotherapeutic drugs, estrogen, phenobarbital) and infectious agents (e.g., gram-negative bacteria, panleukopenia virus) may directly or indirectly damage the marrow, resulting in ischemic injury and cell death. Marrow necrosis is often diagnosed in acute toxicity studies of new drugs or chemicals at pharmaceutical or chemical companies. Myelofibrosis may follow necrosis of marrow tissue. Acute inflammation in bone marrow has been associated with infectious diseases, immune-mediated diseases, and some drug treatments.

BONE MARROW BIOPSY AND ASPIRATE

Complete evaluation of the bone marrow’s status requires all of the following: a current CBC, blood smear, bone marrow aspirate, and bone marrow core biopsy.

NOTE: Complete evaluation of bone marrow evaluation requires: a current CBC, blood smear, bone marrow aspirate, and bone marrow core biopsy.

Different types of information are obtained from these procedures (Box 2-5). The humerus and iliac crest are good sites for biopsy and aspiration. Cortex-to-cortex biopsies through the iliac crest contain a sufficient volume of bone marrow in which to judge total bone marrow cellularity and architectural changes. Bone marrow biopsy needles (e.g., pediatric Jamshidi needle for core biopsy and Illinois sternal needle for aspiration) have stylets locked in the needle to prevent plugging with bone. Core biopsies of bone taken for histopathologic examination

BOX 2-5. INFORMATION PROVIDED BY THREE TESTS USED IN EXAMINATION OF HEMATOPOIESIS	
TEST	INFORMATION PROVIDED
Complete blood count (CBC)	Quantitative information on all three cell types in peripheral blood and excellent morphology of blood cells
Bone marrow aspirate	Excellent morphology of individual marrow cells, best determination of percentages and ratios of cell types (e.g., M/E ratio and % lymphocytes)
Bone marrow biopsy for histopathology	Best for determination of cellularity of marrow and iron stores, architectural patterns, and identification of pathologic changes, including myelofibrosis, myelonecrosis, and inflammation

are often undiagnostic because they lack actual bone marrow (e.g., are only cortical bone). Therefore the surgeon should take two or more cores or be certain the biopsy includes the marrow area. The histopathology report is not available for 2 to 3 days, and if the biopsies are not diagnostic, the surgery must be rescheduled.

The bone marrow smears can be prepared from aspirated marrow or cores can be rolled on glass slides before exposure to formalin. Core samples are later placed in neutral-buffered 10% formalin or other fixative and sent to a referral laboratory for sectioning. A drop of aspirated marrow is placed on a glass slide. To prepare smears for cytologic evaluation, a spreader slide is touched to the drop, then picked up, and a wedge-type smear is made on another clean slide (i.e., similar to the way a blood smear is made). In this way several thin slides can be made from one drop of marrow. An aspirate should only obtain a drop of bone marrow; excessive aspiration collects more blood that dilutes out the bone marrow.

Smears should be inspected for small white fatty specks (i.e., unit particles) on the feathered edge of the smears. If not present, the slide (containing the drop of blood) should be tilted to allow excessive blood to flow away from bone marrow particles before making a squash preparation. A second slide or coverslip is placed on the marrow particle, and marrow is allowed to spread between the slides. The slides are gently pulled apart in the same horizontal plane. Smears are stained with a Wright’s type of stain, but longer staining times than blood smears are required to allow adequate stain penetration of thicker, more cellular, smears.

Evaluation of Bone Marrow Aspiration Smears

A consistent pattern should be followed for describing the quantitative and qualitative aspects of the cells present in bone marrow aspiration smears. Major steps in evaluation include assessment of cellularity, megakaryocyte

numbers, myeloid/erythroid (M/E) ratio, and whether maturation in the myeloid and erythroid series is orderly. These observations can be made via the 10× scanning objective.

NOTE: Major steps in evaluation of a bone marrow aspirate include assessment of cellularity, megakaryocyte numbers, M/E ratio, and whether maturation in the myeloid and erythroid series is orderly.

Cellularity

Cellularity should be evaluated as normal, increased, or decreased by observing overall cellularity and cellularity of tissue fragments (i.e., unit particles) present on most smears. Aspirates from a normally cellular marrow should have many more individual nucleated cells than are usually visible in blood and should include immature forms. Low cellularity may result from dilution of samples with peripheral blood. Therefore when cellularity appears low, cellularity of unit particles should be evaluated. Unit particles are present in most good smears and are frequently found near the feathered edge. Within the unit particle, the percentage of space occupied by hemic cells (i.e., hematopoietic cells that stain dark blue) is compared with the percentage of space occupied by fat (i.e., clear, nonstaining). In normally cellular marrow, hemic tissue occupies roughly 25% to 75% of the unit particle.

Megakaryocyte numbers can be readily evaluated using the 10× objective. Although normal numbers vary among samples and among slides from the same aspirate, cellular smears containing several unit particles usually contain 10 to 60 megakaryocytes.

Myeloid/Erythroid Ratio

The M/E ratio is akin to the CBC's differential leukocyte count. To accurately evaluate cell populations, one should count 500 to 1000 cells. For clinical use, the M:E ratio may be determined by performing two or three 100-cell counts on different smears. Some experienced hematologists estimate an M/E ratio by examining smears using the 10× objective. Erythroid cells tend to be smaller than myeloid cells, have coarse dark nuclear chromatin, and have cytoplasm that is dark blue converting to orange in more mature stages. Myeloid cells have nuclei with fine chromatin and gray cytoplasm with various granules and band- to polymorphonuclear-shaped nuclei in more mature cells. Normal M/E ratios for dogs are 0.75:1 to 2.5:1 and for cats are 1.2:1 to 2.2:1.

Maturation of Myeloid and Erythroid Lines

In normal marrow, 80% of nucleated erythroid cells should be relatively mature (i.e., rubricytes or metarubricytes). These cells can be identified by a densely clumped nuclear chromatin and variable hemoglobinization of the cytoplasm as identified by a gray to pink cytoplasm. Eighty percent of myeloid cells should be metamyelocytes, bands, and segmented granulocytes (Table 2-4). These cells can be identified by their indented to fully lobed nuclei.

TABLE 2-4. APPROXIMATE PERCENTAGE OF DIFFERENT CELL TYPES IN CANINE AND FELINE BONE MARROW

CELL TYPE	DOGS	CATS
Rubriblasts	0.2	0.2
Prorubricytes	3.9	1.0
Rubricytes	27	21.6*
Metarubricytes	15.3	5.6
Total erythroid	46.4	28.7
Myeloblasts	0.0	0.8
Progranulocytes	1.3	1.7
Myelocytes [†]	9.0	5.0
Metamyelocytes [†]	9.9	10.6
Bands [†]	14.5	14.9
Granulocytes (mainly segs)	18.7	13.5
Total myeloid	53.4	45.9
M:E ratio	1.15:1.0	1.6:1.0

Information modified from Jain NC: *Schalm's veterinary hematology*, ed 4, Philadelphia, 1986, Lea & Febiger.

*Rubricytes include basophilic and polychromic forms.

[†]Neutrophilic and eosinophilic forms are combined for myelocytes, metamyelocytes, and band forms.

Hemosiderin

Hemosiderin in macrophages appears as blue-green granules. One should report the amount of hemosiderin. Its absence in canine marrow is suggestive of iron deficiency anemia. Excessive hemosiderin is found in bone marrow of dogs with anemia of inflammatory disease. Hemosiderin can also be excessive in dogs with persistent nonregenerative anemias, dogs given multiple transfusions, or dogs with hemolytic anemia. Hemosiderin is not usually observed in cat bone marrow; therefore its absence cannot be used as an indication of iron deficiency.

Cellular Morphology

Cell morphology is of diagnostic significance in some cases. Dysplastic features occur in myelodysplastic syndromes, iron deficiency, some acute leukemias, and proliferative disorders of plasma cells and macrophages.

Summary of Bone Marrow Smear Evaluation Procedure

In evaluating a bone marrow smear, the following steps are performed:

1. Quantify cell density of the smear or smears.
 - a. Total nucleated cells
 - b. Density of nucleated cells in unit particles
 - c. Number of megakaryocytes
2. Determine M/E ratio.
3. Verify that maturation of each cell line is orderly and complete.
4. Estimate iron (i.e., hemosiderin) stores.
5. Identify morphologic abnormalities such as neoplasia, dysplasia, infections, and inflammation.
6. Summarize information into conclusions.

- Correlate results with the clinical signs, CBC, blood smear morphology, and bone marrow core biopsy and make a final conclusion.

BLOOD BANKING

Comments here are restricted to a few tests that may be performed in small clinics. Hospitals that set up full-service blood banks should refer to more detailed information.¹⁶ Two tests that should be available are crossmatching and blood typing. Crossmatching should be performed prior to a blood transfusion to avoid transfusion reactions.

Crossmatching

The principle is mainly to see if the recipient has antibodies against the donor's cells (major crossmatch). The minor crossmatch checks to see if the donor has antibodies in the plasma against recipient's RBC. An autocontrol (recipient's plasma and own RBCs) is necessary to see if preexisting autoagglutination is present, which prevents interpretation of the major crossmatch. Crossmatching is performed as follows:

- Take EDTA blood tubes from recipient and donor. Centrifuge them for 5 minutes. Separate plasma and RBCs and transfer them to correctly marked small test tubes. Note if there is any hemolysis or autoagglutination.
- Wash RBCs three times with phosphate-buffered saline (PBS) by adding 4 to 5 ml PBS to tubes of RBCs, mixing, centrifuging 1 to 2 minutes, and washing again.
- After washing RBCs, mix them with a few drops of PBS to make a 3% to 5% solution (estimated) of donor and recipient RBCs. Label tubes as major, minor, and recipient autocontrol.
 - Place 1 drop of donor's RBC suspension and 2 drops of recipient's plasma in major crossmatch tube.
 - Place 1 drop of recipient's RBC suspension and 2 drops of donor's plasma in minor crossmatch tube.
 - Place 1 drop of recipient's RBC suspension and 2 drops of recipient's plasma in autocontrol tube.
- Mix tubes gently and incubate in water bath at body temperature for 15 minutes. Centrifuge 15 seconds and note any hemolysis.
- Agitate the pellet of RBCs at the bottom of the tubes by tapping the tube over a mirror. Look for agglutination of RBCs as the pellet breaks up. Report agglutination as a few small clumps, moderate to large aggregates, or no agglutination. Agglutination indicates an incompatible reaction.

Blood Typing

Blood typing in cats is very important. Over 95% of American domestic shorthair cats are type A. Some breeds have high incidence of type B blood (British shorthair, Cornish rex, Devon rex, and exotic cats). These less common

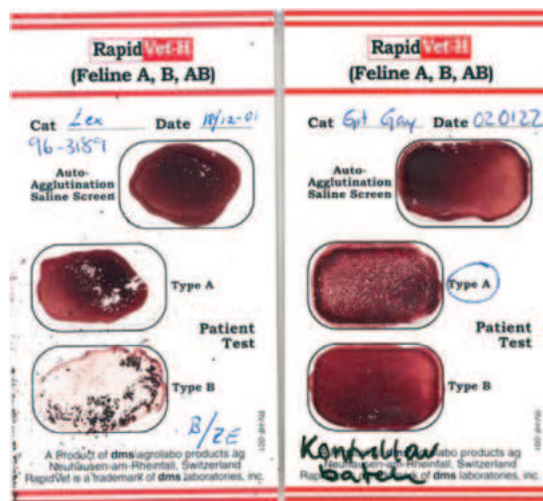


FIGURE 2-12. Blood typing is very important before blood transfusion, especially in cats. These two cards show a positive type-B cat on the left (agglutination in the Type B window) and a type-A cat on the right (moderate agglutination in the Type A window). There is an autoagglutination control window at the top of the cards. If the patient has autoagglutination, one cannot determine if the agglutination seen in the Type A or Type B windows was due to the blood type reaction or preexisting autoagglutination.

breeds are at most risk to receive blood from an A-type cat and die of a transfusion reaction. Type AB is rare and has no alloantibodies to RBCs from type A or B cats. Both recipient and donor cats should be blood typed prior to a blood transfusion. Blood typing cards are available for in-clinic testing (RapidVet-H Feline blood typing cards; DMS Laboratories, Flemington, NJ) (Figure 2-12). No test is 100% sensitive and 100% specific, but blood typing in cats will minimize risks for severe transfusion reactions in type B cats.

Blood typing in dogs is more complex, because they have several different blood types. Canine blood types are currently termed dog erythrocyte antigen (DEA) and previously were called canine erythrocyte antigen (CEA). Types include DEA 1.1, 1.2, 3, 4, 5, and 7. DEA 1.1 and 1.2 are the blood types that can give the strongest transfusion reaction, with hemolysis and/or agglutination of RBCs. Blood typing cards for canine DEA 1.1 are available so donors and recipients may be typed to at least avoid this type reaction. Because several other blood types may be incompatible, it is recommended to crossmatch donors and recipient dogs prior to a blood transfusion, especially if the patient may have been exposed to canine blood products earlier.

References and Suggested Readings

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3

Erythrocyte Disorders

Douglas J. Weiss and Harold Tvedten

ANEMIA DIAGNOSIS

Anemia is the most common erythrocyte (red blood cell [RBC]) disorder. Anemia can cause various clinical signs (e.g., weakness, lethargy, exercise intolerance, heart murmur, hemoglobinuria, pica, shock, death) or may be subclinical and detected only during a general diagnostic workup. Causes of anemia are listed in [Box 3-1](#). An approach to anemia diagnosis is outlined in [Box 3-2](#). A detailed description follows in specific sections.

NOTE: Anemia is very common and detected in 10% to 30% of canine and feline patients.

Anemia classification considers basically production of RBCs from the bone marrow in balance with destruction of RBCs (hemolysis) or loss by hemorrhage. Erythrocyte production may be reduced, normal, or increased, and bone marrow production may be “effective or ineffective.” Removal of RBCs by the monocyte phagocytic system may be normal or accelerated in anemia. Treatment of anemia often aims to reduce macrophage removal of RBCs. The three basic causes of anemia are (1) decreased effective RBC production by bone marrow, (2) loss from the body (i.e., external hemorrhage), and (3) increased destruction of RBCs in the body (i.e., hemolysis). Physiologic anemia can occur, for example, with methemoglobinemia, where the total mass of hemoglobin in the body is normal but oxygen-carrying capacity is reduced. Anemia is often caused by a combination of effects, such as decreased RBC production, shortened RBC life span, and hemorrhage.

NOTE: Hematocrit (PCV), hemoglobin concentration (Hgb), and RBC count are usually equivalent indicators of the severity of anemia. PCV is recommended for interpretation.

The presence and severity of anemia is usually documented by determining a packed cell volume (PCV; hematocrit) (see Chapter 2). Hemoglobin concentration (Hgb) and RBC count are equivalent indicators of the

presence and severity of anemia, but for simplicity, only the PCV is used in the following discussions. Exceptions to the rule that PCV, Hgb, and RBC count decrease in parallel in anemia occur if the blood has many, very small (microcytic hypochromic) RBCs or many, very large (macrocytic hypochromic) RBCs.

Hydration status and blood volume affect assessment of the severity of anemia. Clinical dehydration (i.e., hemoconcentration) reduces plasma volume, which causes a relative increase in PCV (relative polycythemia). Dehydration may mask detection of a mild to moderate anemia, most often early in diagnosis. An animal's hydration status must be normal before the PCV properly reflects the degree of anemia. After hemorrhage, for example, it may take 1 to 2 days to refill the blood volume, based on intake of water and other fluids, and then the PCV will reflect the severity of the anemia. Hydration status is usually assessed by considering plasma protein (PP) concentration and PCV in combination. Disease-induced changes in PP or PCV (e.g., anemia or hypoproteinemia) interfere with the use of these values in evaluation of hydration status. Severe hypoproteinemia may be due to protein loss via hemorrhage, intestinal disease, glomerular disease, or the like (see Chapter 12). PP determination is not a sensitive test of dehydration but is easily performed and commonly available. Additionally, the spleen can affect PCV (and Hgb and RBC count) because it contains 20% to 30% of the RBC mass, and splenic contraction causes rapid changes in distribution by releasing a concentrated bolus of stored RBCs, more so in the dog than cat. The splenic effect is immediate and seen mainly in normal animals. To a lesser extent, the spleen can relax to store RBCs, removing them from circulation (e.g., under anesthesia).

Severity of anemia must be considered during interpretation of complete blood count (CBC) results. Mild anemia is frequently secondary to other disease conditions (e.g., anemia of inflammatory disease; neoplasia; and renal, hepatic, nutritional, or endocrine diseases), and these anemias typically resolve with correction of the primary disease condition. Age and breed variations and statistical chance should also be considered when slight variations from reference intervals are encountered. Greyhounds, for example, normally have greater PCV (45% to 65%) than most dogs, so an anemic greyhound can have a PCV within the usual adult dog reference values. Puppies

BOX 3-1. COMMON TYPES OF ANEMIA**Regenerative Anemia****Blood Loss Anemia**

External blood loss

Internal blood loss

Hemolytic Anemia

Immune-mediated anemia

Cold hemagglutinin disease

Blood parasites

*Mycoplasma haemofelis**Mycoplasma haemocanis**Babesia canis**Cytauxzoon felis*

Heinz body anemia and methemoglobinemia

Zinc or copper toxicity

Hypophosphatemia

Hereditary hemolytic anemia

Pyruvate kinase (PK) deficiency

Phosphofructokinase deficiency

Nonregenerative Anemia**Secondary Anemia**

Anemia of inflammatory diseases

Anemia of chronic renal disease

Anemia of chronic hepatic disease

Hypothyroidism and hypoadrenocorticism

Iron Deficiency Anemia**Bone Marrow Disorders**

Nonregenerative immune-mediated hemolytic anemia

Pure red cell aplasia

Myelodysplastic syndromes

Congenital dyserythropoiesis

Aplastic anemia

Bone marrow necrosis/inflammation

Myelofibrosis

Leukemia

Macrophage proliferative disorders

Drug-Induced Hematologic Dyscrasia

Estrogen toxicity

Sulfadiazine toxicity

Phenylbutazone toxicity

Infections*Ehrlichia*

Feline leukemia virus (FeLV)

Feline immunodeficiency virus (FIV)

Feline panleukopenia virus

Canine and feline parvovirus

between 2 and 8 weeks of age may normally have a mean PCV of around 28%, which appears anemic by adult reference values.

Moderate to severe anemias require more active and directed diagnostic and therapeutic attention. The following PCV ranges arbitrarily classify severity of canine anemia: mild, 30% to 37%; moderate, 20% to 29%; severe, 13% to 19%; and very severe, less than 13%. In cats, arbitrary classifications are mild, 20% to 26%; moderate, 14% to 19%; severe, 10% to 13%; and very severe, less than 10%. Rapid, severe worsening of anemia should stimulate prompt action. Animals with chronic, severe but stable anemia (e.g., PCV of 5% to 8%) can survive for weeks to months with minimal clinical signs because of physiologic adaptations to hypoxemia.

NOTE: Mild anemia is frequently secondary to disease processes in nonhematologic organs or systems. Moderate to severe anemia more often indicates a primary hematologic disease and should be more aggressively diagnosed.

After documenting the presence of anemia and grading its severity, one should evaluate the bone marrow's erythroid production to determine if the bone marrow appears to be contributing to the anemia or responding appropriately to decreased RBC mass. A key

question in anemia is, "Is the bone marrow responding as expected to the anemia, or is it the cause of the anemia?" Bone marrow effective erythropoiesis is primarily judged by its release of reticulocytes (Tables 3-1 and 3-2). Bone marrow function should respond normally in blood loss or hemolytic anemia, and reticulocyte production should be increased in proportion to the severity of the anemia. Anemia with appropriately increased erythropoiesis is classified as regenerative anemia (see Box 3-2). A nonregenerative anemia is indicated by finding clearly less than an expected reticulocyte response for the time after onset of an anemia and the severity of the anemia. A moderate

TABLE 3-1. DEGREE OF ERYTHROID REGENERATION IN ANEMIA

DEGREE OF STIMULATION	Percentage of Reticulocytes	
	DOGS	CATS*
Normal	1	0-0.4
Slight	1-4	0.5-2
Moderate	5-20	3-4
Marked	21-50	5+

From Perman V, Schall WB: Diseases of the red blood cells. In Ettinger SJ, editor: *Textbook of veterinary internal medicine: diseases of the dog and cat*, ed 2, vol 2, Philadelphia, 1983, WB Saunders.

*Indicates the percentage of aggregate reticulocytes in cats.

BOX 3-2. APPROACH TO ANEMIA DIAGNOSIS**I. Determine Severity of the Anemia** (see text)

- A. Mild anemia (PCV > 30% dog, > 20% cat)
 1. Consider age, breed, and statistical chance of normality
 2. Check for laboratory or sample error; repeat venipuncture
 3. Often secondary anemia, go to IV
- B. Moderate to severe anemia, go to II

II. Determine Bone Marrow Responsiveness

- A. No reticulocytosis or polychromasia expected during first 2-3 days or in mild anemia (PCV > 30% dog, > 20% cat)
- B. Reticulocytosis and polychromasia peaks 4-5 days if bone marrow function is normal
 1. Marked canine reticulocytosis (>500,000/ μ l)
 2. Marked feline aggregate reticulocytosis (>200,000/ μ l)
- C. Later-stage responsiveness at 7-14 days, use:
 1. Feline punctate reticulocytosis, marked (>1,500,000/ μ l)
 2. Dogs: use increase in macrocytic hypochromic RBCs
 - a. RBC cytograms and histograms illustrate amount
- D. Classification by RBC indices and hematology instrument graphics
 1. Macrocytic hypochromic: regenerative anemia
 2. Normocytic normochromic: nonregenerative or pre-regenerative anemia
 3. Microcytic hypochromic: usually iron deficiency anemia
 4. Macrocytic normochromic (see text)
- E. If adequately regenerative, go to III; if inadequately regenerative, go to IV

III. Regenerative Anemia Diagnosis

- A. Blood smear analysis critical in hemolytic anemia diagnosis
 1. Spherocytes, autoagglutination, Heinz bodies, polychromasia, blood parasites, eccentrocytes, RBC fragmentation (for interpretation, see text)
- B. Hemoglobinuria is best proof of intravascular hemolytic anemia. Icterus and splenomegaly suggest extravascular hemolysis.
- C. Internal blood loss resembles hemolytic anemia
 1. Document hemorrhage with cytology, etc.
- D. External blood loss
 1. Often in history
 2. Tendency toward hypoproteinemia, hypoalbuminemia, or both
 3. Check for thrombocytopenia or bleeding tendency (see Chapter 5)

IV. Nonregenerative Anemia Diagnosis

- A. Way to a diagnosis varies with case presentation
- B. Use history and severity of anemia to re-evaluate reticulocyte numbers to see if anemia is truly nonregenerative; duration exceeding 3-4 days excludes pre-regenerative anemia; reticulocyte response is weak or absent 2 weeks after the cause of an anemia ceases; mild anemia will not stimulate significant reticulocytosis
- C. CBC Evaluation
 1. Microcytic hypochromic RBCs; usually indicates iron deficiency anemia
 - a. RBC cytograms and histograms more sensitive than MCV and MCHC
 - b. Half of iron deficiency anemia cases regenerative
 2. Normocytic normochromic anemia most common but nonspecific
 3. Macrocytic normochromic feline RBCs without reticulocytosis; suggests FeLV-induced myelodysplasia (see text)
 4. Evidence of inflammation (see Chapter 4); anemia of inflammatory diseases is very common (i.e., mild, normocytic normochromic anemia)
 5. Evidence of leukemia or dysplastic hematopoiesis (see Chapter 4) usually indicates bone marrow evaluation; go to H
 6. Thrombocytopenia (see Chapter 5); consider *Ehrlichia* or other infections (see Chapter 15)
 7. Pancytopenia or bicytopenia; indicates bone marrow disease and bone marrow evaluation; go to H
- D. Clinical chemistry profile
 1. Evidence of renal or hepatic failure; causes secondary anemia (see Chapters 7 and 9)
 2. Evidence of systemic diseases; variable causes of anemia
- E. Virology, serology if infection is likely (e.g., fever, lymphadenopathy)
- F. Endocrinologic examination; hypothyroidism or other dysfunction (see Chapter 8) (e.g., mild, normocytic normochromic anemia)
- G. Toxicity
 1. Check for testicular neoplasm or access to estrogen
 2. Withhold any current drug therapy and monitor for recovery
 3. Check for toxicants in environment
- H. Bone marrow examination reveals many diagnoses (see text and Chapter 2)
 1. Myelofibrosis, aplastic anemia, bone marrow necrosis/inflammation, dyserythropoiesis, leukemia, metastatic neoplasia, myelodysplastic syndromes, etc.

CBC, Complete blood count; FeLV, feline leukemia virus; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; RBC, red blood cell.

TABLE 3-2. RETICULOCYTE GUIDELINES*

DEGREE OF REGENERATION	CANINE RETICULOCYTES/ μ l	FELINE AGGREGATE RETICULOCYTES/ μ l	FELINE PUNCTATE RETICULOCYTES/ μ l
None	60,000	<15,000	<200,000
Slight	150,000	50,000	500,000
Moderate	300,000	100,000	1,000,000
Marked	>500,000	>200,000	1,500,000

*Used at Michigan State University and University of Minnesota as modified by Dr. D.J. Weiss.

to severe anemia should have a maximal response 3 to 7 days after onset of the anemia.

DETERMINING ERYTHROID REGENERATION

Reticulocyte Evaluation

Reticulocyte enumeration in blood is the most consistent way to evaluate the strength of erythropoiesis, but one must consider time after onset of the anemia (Figure 3-1) and magnitude of the reticulocytosis (see Table 3-2) during interpretation. A reticulocyte count should be performed when the PCV is less than 30% in dogs and less than 20% in cats.

NOTE: Enumerating the absolute number of reticulocytes is the most consistent way to evaluate the strength of erythropoiesis.

Reticulocytes are immature RBCs released in increased numbers from normal bone marrow in response to anemia. Reticulocytes have ribosomes (ribonucleic acid [RNA]) for continued hemoglobin synthesis. Ribosomal material appears as dark-blue granules when stained with new methylene blue (NMB) (Figure 3-2). Feline reticulocytes should be subdivided into aggregate and punctate forms, because the cat is unique in having large numbers of punctate reticulocytes. Reticulocytes are reported as absolute reticulocyte numbers per microliter of blood, reticulocyte index (RI), reticulocyte percentage, and corrected reticulocyte percentage, or they can be estimated by inspection of the number of polychromatophilic RBCs on blood smears (see later). Reticulocytes are larger (i.e., macrocytes) and have a lower hemoglobin concentration than mature RBCs. Therefore documenting increased numbers of macrocytic hypochromic erythrocytes usually reflects reticulocytosis and bone marrow responsiveness.

Manual Reticulocyte Counting

For microscopic reticulocyte determination, ethylenediaminetetraacetic acid (EDTA) blood is mixed with a vital stain. Stains used most often are new methylene blue (1% NMB plus 1.6% potassium oxalate anticoagulant) and brilliant cresol blue (BCB; 1% in saline). Blood and stain are mixed in equal amounts and incubated for 10 minutes

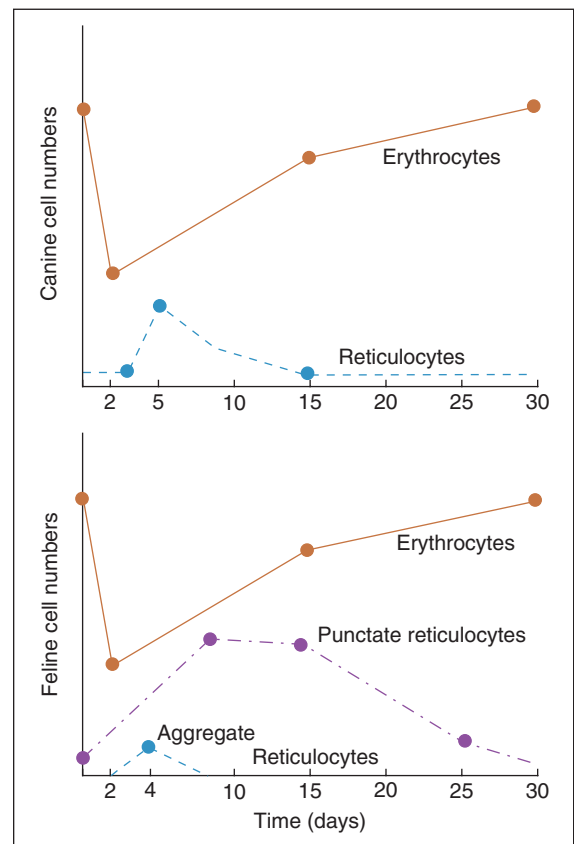


FIGURE 3-1. Reticulocyte and red blood cell (RBC) responses in blood loss anemia in dogs and cats. The reestablishment of the circulating RBC mass as indicated by *Erythrocytes* (RBC count) is similar in both species after one episode of blood loss. Increase in hematocrit (or hemoglobin concentration or RBC count) is rapid in the first 2 weeks and is slower during the next 2 weeks. The reticulocyte response of the cat is more complex than the dog's. The feline aggregate reticulocyte response is weaker than in dogs, similar in onset, but of shorter duration. The feline punctate reticulocyte response has greater numbers and persists much longer than the aggregate response.

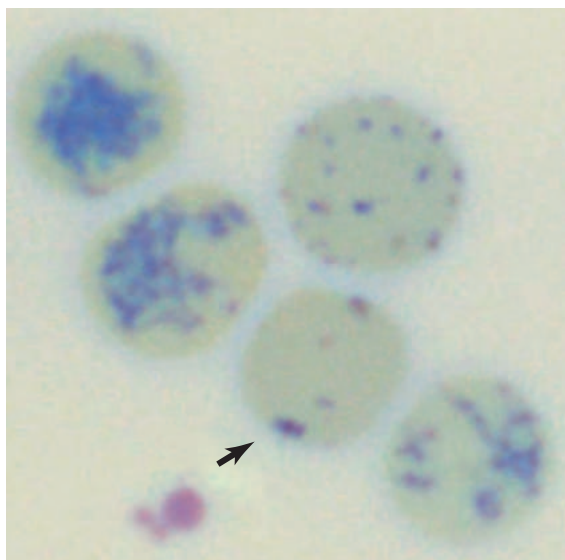


FIGURE 3-2. Five feline reticulocytes are shown, including four aggregate reticulocytes with more than 12 to 15 dots of ribosomal material and one punctate reticulocyte with fewer dots (arrow). Canine reticulocytes look mainly like aggregate feline reticulocytes but also have a small number of reticulocytes with as little ribosomal material as punctate reticulocytes.

for NMB and 15 to 30 minutes for BCB. For birds, one can mix three drops NMB to one drop EDTA blood and incubate 10 minutes. Residual endoplasmic reticulum in the reticulocytes appears as dark-blue granules. A smear of the mixture is air dried, and 1000 non-nucleated RBCs are counted to determine the percentage of reticulocytes. Mature erythrocytes lack RNA and are unstained. Nucleated RBCs (NRBCs) are not included in the reticulocyte count but are reported separately. The manual reticulocyte method is much more imprecise than an automated method, and variation among microscopists is significant. However, no automated instrument currently detects feline punctate reticulocytes; therefore only the manual method provides this information. At least an estimate of the percentage of punctate reticulocytes (10%, 25%, 50%, 75%, or 90%) should be done from a stained smear to permit proper interpretation of the feline regenerative response. Definition of a feline punctate reticulocyte is that it has 3 to 12 dots. An aggregate reticulocyte has more than 12 to 15 dots or too many dots in an aggregate to count those dots. A canine reticulocyte is defined as having more than two dots. Canine reticulocytes can have few dots, but dogs do not have so many punctate reticulocytes that they affect interpretation, thus only a total reticulocyte count is determined.

Automated Reticulocyte Counting

Various veterinary hematology instruments use different reticulocyte stains for an automated reticulocyte count. Sensitivity of reticulocyte detection varies among instruments and methods.¹⁷ The Advia 2120 and Sysmex XT-2000iV reticulocyte counts are approximately correct for canine reticulocytes and correlate best with feline aggregate reticulocytes (see Figure 2-5). Sysmex's reticulocyte staining is slightly more sensitive and detect

about 6% punctate reticulocytes in its automated reticulocyte count in addition to aggregate reticulocytes. The Sysmex automated reticulocyte count still should be considered mainly an aggregate reticulocyte count. Feline punctate reticulocytes must be determined by manual evaluation of reticulocyte stained smears to identify prominent changes in punctate reticulocyte numbers. The LaserCyte (IDEXX Laboratories) uses NMB to stain reticulocytes and is less sensitive than the Advia or Sysmex instruments. The LaserCyte has a negative bias and proportional error compared to manual or automated (Advia) methods. Instrument-specific values for considering LaserCyte reticulocyte results nonregenerative ($<35,000/\mu\text{l}$) or regenerative ($>75,000/\mu\text{l}$) were suggested. A cutoff for canine regenerative anemia is often stated to be greater than $60,000/\mu\text{l}$, but $60,000/\mu\text{l}$ should instead be considered a mean reference value and not maximum value. Reference values for Advia automated canine reticulocyte counts are 11,000 to $111,000/\mu\text{l}$ and 0.1% to 1.5%.²⁹ Reference values for Sysmex XT automated canine reticulocyte counts are 19,400 to $150,100/\mu\text{l}$ and 0.3% to 2.4%.³⁰ With availability of routine automated reticulocyte counts, many nonanemic dogs are noted to have many more than $60,000$ reticulocytes/ μl . Excitement and splenic contraction may cause reticulocytosis. The most common error in interpretation of reticulocytes is to conclude an anemia is regenerative based on slight increases in one of the ways of reporting reticulocytes.

NOTE: The most common error in interpretation of reticulocytes is to conclude an anemia is regenerative based on slight increases in one of the ways of reporting reticulocytes.

Reticulocyte volume can be measured and used in cases of iron deficiency anemia to document response to treatment. Reticulocytes are smaller in iron deficiency but increase in volume with iron therapy. For example, after experimental iron replacement in iron-deficient dogs, the mean cell volume of reticulocytes (MCVr; Advia 2120) increased from 65 fl before iron treatment to 82 fl after 11 to 12 days of iron supplementation, and hemoglobin per reticulocyte (cell hemoglobin reticulocyte, CHR) increased from about 16 up to 22 pg.⁹

Absolute Reticulocyte Count

The percentage of reticulocytes may be reported alone (see Table 3-1), but this can be misleading because percentage is a ratio of reticulocytes to mature RBCs. In anemia, the mature RBCs are variably reduced, thus the reticulocyte percentage overestimates the hematopoietic response (Figure 3-3). The absolute reticulocyte count is calculated by multiplying the reticulocyte percentage by the RBC count, so it adjusts the reticulocyte percentage for the severity of the anemia. Absolute reticulocyte count is the more consistent indicator of bone marrow production and is recommended as the best single indicator of regeneration in the dog and cat (see Table 3-2). If an RBC count is not available, a corrected reticulocyte percentage (CRP) and RI (also termed *reticulocyte production index*) can be determined.

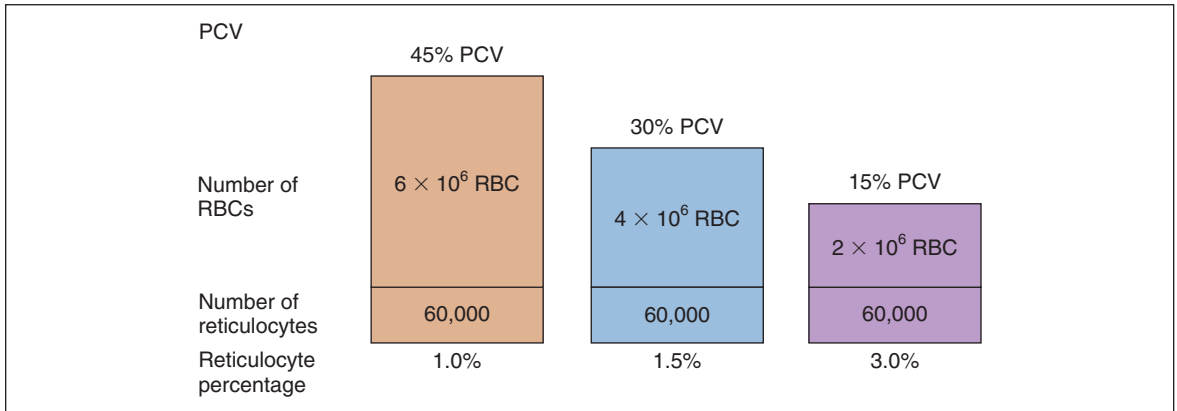


FIGURE 3-3. Increasing the severity of the anemia and thus decreasing the number of mature red blood cells (RBCs) increases the relative percentage of reticulocytes even when the absolute number of reticulocytes per volume of blood remains constant. PCV, Packed cell volume.

NOTE: In anemia, the percentage of reticulocytes overestimates the strength of erythropoiesis. The absolute reticulocyte count is a more consistent indicator of the true reticulocyte response.

Reticulocyte Index and Corrected Reticulocyte Percentage

Discussion of CRP and RI is included here, but only to illustrate some principles. RI and CRP are not recommended for clinical diagnosis interpretation. The CRP is adjusted for the degree of anemia (Box 3-3). The RI is further adjusted for the life span of the canine reticulocyte in peripheral blood. With increasingly severe anemia, reticulocytes are released earlier from the bone marrow and live longer in blood before maturing into RBCs. The longer life span increases the reticulocyte percentage, but that portion is not the result of release of increased numbers of reticulocytes from bone marrow. Feline reticulocyte life spans in blood with increasingly severe anemia are not well determined, so the RI is not recommended for cats.

An RI greater than 1 in dogs indicates regenerative anemia. An RI of 3 or greater indicates a marked regenerative response. Hemolytic anemia tends to have a greater reticulocyte response than does external blood loss anemia, because the various nutrients for erythropoiesis remain in the body. Recent loss of large volumes of blood (e.g., 4 to 5 days ago) or internal blood loss, however, may also be highly regenerative, so not all anemias with an RI greater than 3 are hemolytic. Specific guidelines for interpreting the CRP are not available.

An example is as follows (see Box 3-3): A dog with a PCV of 22.5% and 4% reticulocytes had an RI of 1 that denoted an inadequate regenerative response. The reticulocyte percentage of 4% did not indicate a fourfold increase in reticulocyte production.

Canine Reticulocyte Response

Canine reticulocytes normally mature to RBCs in about 1 day after release into the blood. As a result of this rapid maturation, reticulocytes are primarily aggregate

reticulocytes (i.e., they contain a large mass of precipitated RNA with few punctate forms). Canine reticulocytes are not subdivided because the small number of punctate reticulocytes in the dog is clinically insignificant given the great variability and error potential in microscopic counts. Most laboratories do not count RBCs with only one or

BOX 3-3. STEPS IN CALCULATION OF THE RETICULOCYTE INDEX

Step 1: Corrected reticulocyte percentage (CRP)

CRP =
reticulocyte % (patient's hematocrit / normal hematocrit)

Example: Dog with packed cell volume (PCV) of 22.5% and 4% reticulocytes

$$\text{CRP} = 4\% (22.5\% \div 45\%) = 2\%$$

Step 2: Dogs are similar to people in releasing reticulocytes from their bone marrow earlier than normal when they are anemic. These "shift" reticulocytes live longer than the usual 1 day, as is shown in the table below. This exaggerates the reticulocyte percentage, so the CRP is further adjusted by dividing it by the expected maturation time in days, which varies with the severity of the anemia:

RI = CRP / life span of reticulocytes

HEMATOCRIT	EXPECTED RETICULOCYTE LIFE SPAN (DAYS)
45	1.0
35	1.5
25	2.0
15	2.5

The reticulocytes in the previous example should live about 2 days when the PCV = 22.5%. Remaining in circulation twice as long doubles the percentage of reticulocytes in blood. The reticulocyte index (RI) is adjusted for this to reflect more truly marrow release by dividing by 2:

$$\text{RI} = 2\% \div 2 \text{ days} = 1$$

two individualized granules as reticulocytes, to be certain not to include RBCs with granular artifact or precipitate. Canine reticulocytes approximately equal polychromatophils seen on Wright-stained blood smears, though different stains vary in how well they display polychromatophils. Some guidelines assess the degree of activity of bone marrow according to the percentage of reticulocytes (see Table 3-1). This has been modified to provide absolute reticulocyte numbers to judge the magnitude of bone marrow regeneration (see Table 3-2).

Feline Reticulocyte Response

Cats vary from dogs in having large numbers of punctate reticulocytes both normally and in regenerative anemias. A total feline reticulocyte count does not provide the same interpretation as it does in dogs. A feline reticulocyte count should be subdivided into punctate and aggregate reticulocytes to reflect significant differences in stages of maturation (see Figure 3-2). Aggregate reticulocytes mature rapidly, in about half a day, into punctate reticulocytes. Punctate reticulocytes mature slowly over 10 to 12 days and thus accumulate in blood in much greater numbers than do aggregate reticulocytes. Reports that do not identify the type of reticulocyte counted in a cat are worthless! The duration of a regenerative anemia may be suggested by the pattern of reticulocyte response. For example, at about 4 days into a regenerative response, the aggregate reticulocyte response peaks and punctate reticulocytes are still relatively low (see Figure 3-1). Thereafter, aggregate reticulocytes decrease and punctate reticulocytes continue to increase. Polychromatophils on feline Wright-stained blood smears reflect the number of aggregate reticulocytes (Figure 3-4).

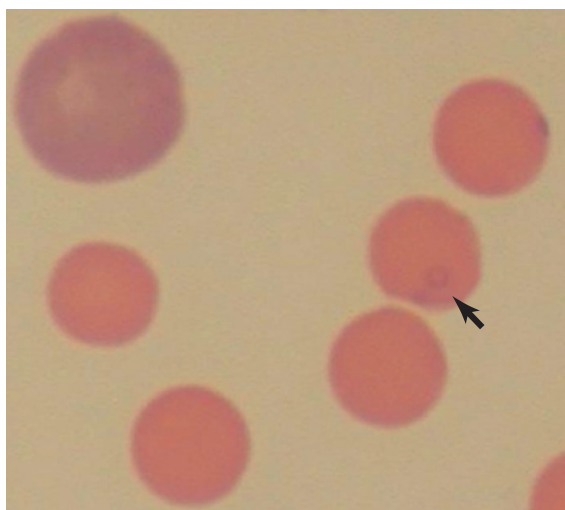


FIGURE 3-4. The appearance of *Mycoplasma haemofelis* on blood smears can be difficult to distinguish from stain precipitate. Seeing a ring form (like a letter o at arrow) on an RBC and a more dull blue color to the bacteria than the refractive metallic color of stain precipitate helps ensure correct diagnosis. Note a polychromatophil at the upper left, which is an aggregate reticulocyte that has enough RNA in it to stain bluer than other non-nucleated erythroid cells.

In persistent hemorrhage or a hemolytic process, the combined punctate and aggregate reticulocyte count (mainly punctate) may approach 100%. This makes the performance of reticulocyte counts tedious. Even with more normal numbers of reticulocytes, no Heinz bodies, and little or no stain precipitate, microscopic reticulocyte determinations are imprecise. Flow cytometric analysis of feline blood in which the RNA of reticulocytes was stained with thiazole orange was a more sensitive and reliable assay of feline reticulocytes than manual microscopic evaluation.¹⁸ Reference intervals for 38 clinically normal cats by this thiazole orange method were 0.1% to 0.5% (8500 to 42,000/ μ l) for aggregate reticulocytes and 2% to 17% (22,500 to 1,270,000/ μ l) for punctate reticulocytes.¹⁶ Manual reference values should be similar.

NOTE: Dogs release aggregate reticulocytes that normally mature to erythrocytes in about 1 day, whereas cats release aggregate reticulocytes that rapidly become punctate reticulocytes, which persist for 10 to 12 days.

Polychromasia

Polychromasia denotes an increased number of polychromatophils observed on Wright-stained blood smears. Canine polychromatophils are equivalent to reticulocytes, and polychromasia indicates reticulocytosis. These are larger than mature RBCs and have slightly bluer staining because of ribosomes in the cytoplasm (see Figure 3-4). Polychromatophilic refers to multiple (“poly”) colors with the orange staining of hemoglobin plus the blue staining of RNA. Feline aggregate reticulocytes appear as polychromatophils, but punctate reticulocytes do not. Therefore polychromasia on feline CBC reports reflects only aggregate reticulocyte numbers, whereas polychromasia on canine blood smears reflects total reticulocyte numbers. Increased polychromasia in both species denotes active bone marrow erythropoiesis 3 to 7 days earlier. The magnitude of the polychromasia reflects the strength of the erythropoiesis.

Macrocytosis

Larger than normal RBCs (i.e., macrocytes) are documented by the mean corpuscular volume (MCV) or by RBC cytograms and RBC volume histograms (see Figures 2-3 to 2-5). Reticulocytosis is the most frequent cause of macrocytosis, especially at 4 to 5 days after the onset of anemia, but mature macrocytes can also be released during accelerated erythropoiesis. Tvedten believes macrocytosis—and especially the percentage of macrocytic hypochromic RBCs—is a more sensitive indicator of increased erythropoiesis late (e.g., 7 to 14 days) in the regenerative response, when polychromasia and reticulocytosis are declining.²²

Other causes of macrocytosis are artifactual swelling of RBCs in EDTA tubes during prolonged storage, congenital dyserythropoiesis in poodles, stomatocytosis, and feline leukemia virus (FeLV) infections in cats. The macrocytosis caused by swelling of RBCs during storage in

EDTA is common in samples mailed to laboratories or samples analyzed the day after collection.

Anisocytosis, Red Blood Cell Distribution Width, Hemoglobin Distribution Width

Anisocytosis is variation in RBC size. Red blood cell distribution width (RDW) is reported by automated hematology counters to describe the amount of anisocytosis. A frequent cause of increased anisocytosis is regenerative anemia with release of reticulocytes, which are macrocytic. Hemoglobin distribution width (HDW) numerically describes variation in RBCs based on hemoglobin concentration. Increases in RDW or HDW are indicators that the RBC population has increased variation. Increased RDW or HDW may be used as an indication to review RBC morphology on a blood smear and not rely only on automated results.

Nucleated Red Blood Cells, Basophilic Stippling, Howell-Jolly Bodies

Other hematologic findings expected in regenerative anemia include Howell-Jolly bodies, nucleated RBCs (i.e., NRBCs; metarubricytosis), and basophilic stippling. These are visible on blood smears but are neither as quantitative nor as specific an indicator of RBC regeneration as reticulocytes or macrocytic hypochromic RBCs.

Circulating NRBCs are reported as the number of NRBCs per 100 WBCs or absolute number of NRBCs per volume of blood (see Chapter 2). The absolute number is more consistent for interpretation because severe neutropenia, such as in sepsis and heat stroke, can exaggerate the number of NRBCs/100 WBCs. NRBCs may be released in regenerative anemia, but NRBC numbers inconsistently reflect bone marrow erythropoiesis in dogs and cats. NRBC release into blood may occur independently of increased erythropoiesis in situations such as splenic disease, extramedullary hematopoiesis, heat stroke, sepsis, lead poisoning, hyperadrenocorticism, leukemia, and various bone marrow diseases.

NOTE: NRBCs are often increased in regenerative anemia but may be increased due to other factors, and therefore are not specific evidence of increased erythropoiesis.

Basophilic stippling is most often associated with regenerative anemias and is no cause for alarm. Basophilic stippling may be caused by lead poisoning but is neither a specific nor a sensitive method for diagnosing lead poisoning in dogs in our experience. Toxicologic testing should be used to evaluate suspected lead poisoning (see Chapter 17 and Appendix I).

Howell-Jolly (H-J) bodies are small round nuclear remnants in RBCs. They may be seen with increased erythropoiesis or with decreased splenic removal of them. Feline spleens are less effective in grooming RBCs, and therefore H-J bodies are more common in normal cats than dogs. Steroid treatment (or Cushing disease) reduces phagocyte function and can increase the number of H-J

bodies. H-J bodies seen in poodle macrocytosis may be numerous and dysplastic.

Siderocytes, Sideroblasts

Siderocytes are abnormal RBCs with basophilic granules (i.e., Pappenheimer bodies) resembling basophilic stippling in Wright-stained blood smears. Prussian blue stains the iron within Pappenheimer bodies but not granules associated with basophilic stippling. Sideroblasts are nucleated erythroid cells in blood or bone marrow with iron-positive granules. Although some sideroblasts are considered normal, increased numbers and abnormal sideroblasts are considered abnormal. Abnormal sideroblasts have more numerous and larger granules that may form a ring around the nucleus (i.e., ringed sideroblasts). Siderocytes and abnormal sideroblasts indicate abnormal erythropoiesis (i.e., dyserythropoiesis). Chloramphenicol therapy, sideroblastic anemia, and some types of myelodysplastic syndromes are causes of increased siderocytes in blood and abnormal sideroblasts in blood or bone marrow.²⁴

MORPHOLOGIC CLASSIFICATION OF ANEMIA

One of the two anemia classification systems most frequently used in veterinary medicine is called “morphologic classification of anemia,” which is the traditional name for classification by use of RBC volume and hemoglobin concentration. The three important classifications are normocytic normochromic, macrocytic hypochromic, and microcytic hypochromic anemia. The other system is classification based on the presence or absence of bone marrow response to anemia (i.e., reticulocytosis, regenerative anemia). Evaluation of blood smear morphology is an essential component of all approaches to evaluation of anemia, but use of those morphologic changes is different than the morphologic classifications described here.

Classification of Anemia by Use of Erythrocyte Volume and Hemoglobin Concentration

Morphologic classification of anemia traditionally uses RBC indices (i.e., MVC, MCHC). MCV and MCHC are mean values of all RBCs. Mean values are not a sensitive indicator of small to medium increases of macrocytic hypochromic or microcytic hypochromic RBCs. DiNicola et al. reported that only 8% of blood samples of 6752 dogs with regenerative anemia had both increased MCV and decreased MCHC despite the principle that regenerative anemia has increased numbers of macrocytic and hypochromic RBCs, which is true and important in diagnosis.⁶

The identification of increased numbers of macrocytic and hypochromic RBCs is best performed by the Advia automated hematology system. The Advia instrument measures the volume and hemoglobin concentration of each individual RBC in its sample and displays each erythrocyte by size and hemoglobin concentration

(see Figure 2-4). In this way, even a few abnormal RBCs can be identified on the RBC cytogram. A better estimate of the number (%) of abnormal RBCs may be made from the RBC volume and Hgb concentration histograms or from precise numbers of cells in the nine boxes of the RBC cytogram that are available from a research screen. The morphologic classification of anemia works best with the Advia instrument and unfortunately poorly with only the MCV and MCHC results that are provided by other instruments. Use of MCV and MCHC alone in diagnosis often gives incorrect classifications of the true changes in the anemia, and this can cause incorrect diagnosis.

Normocytic Normochromic Anemia

Normocytic normochromic anemia is seen in nonregenerative anemias with bone marrow release of no or too few macrocytic reticulocytes. Most RBCs in the blood are those normocytic normochromic RBCs remaining from prior production. Anemia that is due to hemorrhage or hemolysis that is of such recent onset (e.g., 1 to 2 days) as to preclude a regenerative bone marrow response is also normocytic normochromic during that time and is best classified as pre-regenerative.

Macrocytic Hypochromic Anemia

Macrocytic hypochromic anemia usually indicates a regenerative anemia with increased numbers of reticulocytes. Reticulocytes are relatively larger (i.e., macrocytic) than mature RBCs. Reticulocytes are hypochromic because they have not completed hemoglobin synthesis. Tvedten believes that macrocytic hypochromic RBCs that lack ribosomes (are not reticulocytes) still remain late in a regenerative response, reflecting earlier increased erythropoiesis. There are other causes of macrocytic hypochromic RBCs, such as swelling of RBCs during storage (e.g., in mailed samples).

Microcytic Hypochromic Anemia

Microcytic hypochromic anemia occurs most often in iron deficiency, which prevents adequate production of hemoglobin. The RBCs are small (i.e., low MCV) with insufficient hemoglobin production (i.e., low MCHC). Microcytosis and altered iron metabolism are common in dogs with portosystemic shunts (PSSs) and hepatic atrophy. Microcytic hypochromic anemias are also associated with sideroblastic anemias and some types of myelodysplastic syndromes in dogs. Note that Japanese Akita, Shiba and Chow breeds normally have small RBCs (i.e., MCV of about 60 fl).

NOTE: Normocytic normochromic anemia indicates a non-regenerative anemia. Macrocytic hypochromic anemia indicates a regenerative anemia. Microcytic hypochromic anemia indicates iron deficiency.

Macrocytic Normochromic Anemia

Too much emphasis on vitamin B₁₂ or folate deficiency causing macrocytic hypochromic anemia has come into veterinary medicine as a result of reading human literature that has great emphasis on diagnosis of pernicious

anemia in people. Macrocytic normochromic RBCs in cobalamin deficiency are not seen in dogs and cats but are seen in people and primates. Most often the classification of macrocytic normochromic anemia in dogs and cats has come from erroneous classification due to the insensitivity of MCV and MCHC in reflecting true changes in the patient's RBCs. A regenerative anemia may have enough macrocytosis to increase the MCV but not enough hypochromic RBCs to drop the MCHC out of the reference interval. So some regenerative anemias may be misclassified macrocytic and normochromic if only MCV and MCHC results are interpreted. Cobalamin (vitamin B₁₂) malabsorption has been described as a hereditary disorder in giant schnauzers and as acquired gastrointestinal disorders in dogs and cats. Measurement of serum cobalamin is recommended for diagnosis of a deficiency.

There are other causes of macrocytosis besides regenerative anemia. Some poodles have a congenital dyserythropoiesis (i.e., "poodle macrocytosis"; MCV > 80 fl) but are not anemic and show no other signs of hematologic disease. (See also stomatocytosis discussed under Other Hereditary Hemolytic Anemias.) Macrocytic normochromic anemia in cats without reticulocytosis suggests FeLV infection or myelodysplastic syndromes and is not related to vitamin B₁₂ or folate deficiency.

Classification of Anemia by Regenerative Response

Evaluation of bone marrow response to anemia divides anemias into those with variably reduced or ineffective erythropoiesis (nonregenerative) and those with effective and adequate erythropoiesis (regenerative). The anemia is classified as regenerative only if signs of increased erythropoiesis (i.e., reticulocytosis) are sufficient to match the severity of anemia seen in the blood (see Table 3-2). Too often a moderate to severe anemia is misclassified as regenerative with only a slight increase in reticulocytes.

NOTE: The anemia is classified as regenerative only if signs of increased erythropoiesis (i.e., reticulocytosis) are adequate for the degree of anemia seen in the blood.

REGENERATIVE ANEMIA

Anemia caused by RBC destruction (i.e., hemolysis) or blood loss should have normal bone marrow able to respond to the anemia. Regenerative anemia thus suggests the cause was hemolytic anemia or blood loss. (See Box 3-1 for a listing of types of regenerative anemia.) Greater than 500,000 reticulocytes/ μ l frequently occur in dogs with hemolytic anemia, internal hemorrhage, or recent external blood loss (see Tables 3-1 and 3-2). Many anemias, however, are neither markedly regenerative nor completely nonregenerative. Mild anemia (e.g., canine PCV 30% to 35%; feline PCV 20% to 26%) may not stimulate reticulocytosis, because the mildly stimulated bone marrow responds by releasing mature RBCs. Note that puppies and kittens have active growth, including erythropoiesis, and have increased signs of regeneration

such as mild reticulocytosis when very young. Demands for expansion of blood volume also make puppies and kittens more susceptible to iron deficiency if they have blood loss.

Mild to moderate regenerative states (see [Box 3-2](#)) must be interpreted in terms of duration of anemia, severity of anemia, and potential for multiple causes. An intestinal neoplasm is an example of a situation with multiple causative factors contributing to anemia. An intestinal tumor may have external blood loss from hemorrhage into the intestine. Such external blood loss initially should stimulate good bone marrow regeneration, but persistent bleeding may lead to iron and protein deficiency. An intestinal neoplasm is often inflamed, and anemia of inflammatory disease interferes with erythropoiesis, resulting in reduced RBC production. Thus the degree of regeneration could vary and be less than expected from just external blood loss.

Blood Loss Anemia

External blood loss is often obvious from the history or physical examination. However, external blood loss into the gastrointestinal tract or internal blood loss into a body cavity may be occult. Gastrointestinal bleeding may be indicated by a black, tarry stool or fresh red blood in the stool. Tests for occult blood in the stool are of questionable value (see Chapter 9), because myoglobin present in meat diets frequently gives false-positive results. Fluid cytology can document bleeding into body cavities (see Chapter 10). PCV and volume of the fluid in body cavities indicates the amount of hemorrhage.

Hematologic patterns in blood loss vary greatly according to when hemorrhage occurred, severity of blood loss, whether bleeding was one acute episode or was persistent, whether hemorrhage was internal or external, and species variation. Time-related changes and species variations are illustrated in [Figure 3-1](#) and in the following discussions.

External Blood Loss

PCV in a dog does not fully reflect the severity of acute blood loss anemia for 1 to 3 days, until fluid volume of the vascular space is replaced and the remaining RBCs and PPs are diluted by fluid replacement (thirst, redistribution of body fluids, or fluid treatment). Splenic contraction in the first few hours releases stored, concentrated RBCs into the circulation and may initially mask the severity of anemia. Release of reticulocytes should be noticeable by 3 days after hemorrhage, and peak aggregate reticulocytosis occurs 4 to 5 days after hemorrhage (see [Figure 3-1](#)). Improvement in the PCV occurs rapidly over the first 2 weeks until PCV reaches the low-normal reference interval. Thereafter, hypoxia is too mild to stimulate strong erythropoietin production, so the PCV increases slowly and may take a month to return to the original value.

NOTE: After a single episode of blood loss, improvement in the PCV occurs rapidly over the first 2 weeks. The PCV increases slowly thereafter and may take a month to return to the original PCV.

In adult animals, chronic hemorrhage over several weeks causes iron deficiency (see [Figure 3-12](#)) and a negative protein balance impairing erythropoiesis. Thus blood loss anemia initially (e.g., in the first 1 to 2 days) has no reticulocytosis in the blood (i.e., pre-regenerative), becomes most regenerative, and then over time becomes poorly regenerative or nonregenerative because of iron deficiency. Puppies and kittens are born with small iron reserves and with maximum erythropoiesis to match growth rate; iron depletion occurs more rapidly with blood loss (e.g., hookworms or coccidia). Even normal kittens just before weaning often have sub-clinical iron deficiency while drinking milk (an iron-poor diet).

A low or low-normal PP level in regenerative anemia is frequently associated with external blood loss and is a useful diagnostic feature. PP is lost with external blood loss. In hemolytic anemia and internal blood loss, PP concentration tends to be normal to slightly increased, because no protein is lost from the body. The PP reference interval may be too wide for the PP concentration to seem abnormal, but values may be in the lower end of the reference interval. PPs are replaced by the liver and lymphoid tissues more quickly than the bone marrow can replace RBCs, so hypoproteinemia less consistently reflects the presence or severity of external hemorrhage than does PCV.

Feline PCV response to blood loss somewhat parallels the canine response for the first 2 weeks. Feline aggregate reticulocyte response reaches a peak about 4 days after hemorrhage (see [Figure 3-1](#)). The maximum number of feline aggregate reticulocytes is much lower than the maximum number of canine reticulocytes in strong regenerative responses. Similarly, less polychromasia is needed on a feline blood smear to indicate strong regeneration than on a canine smear. Punctate reticulocytes peak about 1 week after hemorrhage and may remain elevated for 3 weeks or more. Punctate reticulocytes may remain increased after the PCV has returned to the reference interval. Therefore, a punctate reticulocytosis probably reflects accelerated erythropoiesis sometime within the previous 2 weeks.

Feline aggregate and punctate reticulocyte numbers can help determine when an anemia began. Moderately to markedly increased numbers of aggregate reticulocytes with few punctate reticulocytes indicates recent anemia (e.g., 2 to 4 days). Increased punctate reticulocytes without increased aggregate reticulocytes indicate anemia of 1 to 3 weeks' duration or anemia too mild to stimulate an aggregate reticulocyte response.

Age-related changes in puppies and kittens must be considered. The PCV in healthy 2- to 6-week-old puppies is approximately 28%, and the PP normally may be less than 6 g/dl. Puppies have approximately 3% to 7% reticulocytes at 2 months of age or younger, with the highest percentage (7%) occurring at 0 to 2 weeks of age. Conversely, adult dogs normally have less than 1.5% reticulocytes. Using adult reference intervals, one would incorrectly conclude that a puppy has anemia, reticulocytosis, and hypoproteinemia, which indicate external blood loss. Therefore age-related reference intervals should be used.²⁸

NOTE: Healthy puppies at 2 to 6 weeks old have a PCV of 28, PP less than 6 g/dl, and 3% to 7% reticulocytes. Age-based reference values should be used.

Young animals are less able to respond to blood loss than are adults. Puppies and kittens already have high rates of bone marrow erythropoietic activity because of the need to expand blood volume as they grow. When bone marrow erythropoiesis is already at a high rate, one cannot expect as great an increase with blood loss as occurs in adults.

Internal Blood Loss

The hematologic pattern of internal blood loss is similar to that associated with hemolytic anemia, because RBCs lost into tissues are destroyed and their constituents conserved to allow maximal bone marrow response. Iron deficiency and hypoproteinemia are not expected because iron and plasma proteins are not lost from the body. Some RBCs lost into the body cavities may be recirculated intact via the lymphatics.

Hemolytic Anemia

Hemolytic anemia is usually a very regenerative anemia without hypoproteinemia or other evidence of blood loss. Careful evaluation of the blood smear is essential to identify evidence for specific types of hemolytic anemia, such as spherocytes, autoagglutination, blood parasites, Heinz bodies, and eccentrocytes.

Hemolytic anemia may be subclassified as intravascular or extravascular, which mainly indicates rate and severity of hemolysis. Intravascular hemolysis is due to rapid and such severe damage to RBCs that they break down in the vascular system. The best proof of intravascular hemolysis is hemoglobinuria (Figure 3-5; see also Chapter 7). Hemolysis in plasma or serum also supports intravascular hemolysis (see Figure 2-1). Extravascular hemolysis alone, without intravascular hemolysis, is more common. Extravascular hemolysis is removal of damaged RBCs by macrophages in the spleen, liver, and bone marrow. Splenomegaly, hepatomegaly, and icterus are evidence of extravascular hemolysis. RBCs damaged during intravascular hemolysis are also removed by macrophages, so extravascular hemolysis always accompanies intravascular hemolysis or can exist by itself during slower rates of hemolysis. Lysed RBCs (ghost cells) seen on blood smear or on instrument graphics (Figure 3-6; see also Figure 2-6) also reflect very fragile RBCs and hemolytic anemia. The more severe intravascular hemolytic anemias with hemoglobinuria include some immune-mediated hemolytic anemias, Heinz body anemias, *Babesia* infection, zinc toxicity, phosphofructokinase deficiency, and snake (viper) bite.

NOTE: Intravascular hemolysis with hemoglobinuria indicates very severe, often peracute hemolytic anemia. Specific causes include Heinz body anemias, complement-fixing immune-mediated anemias, zinc toxicity, *Babesia* infection, phosphofructokinase deficiency, and viper bite.

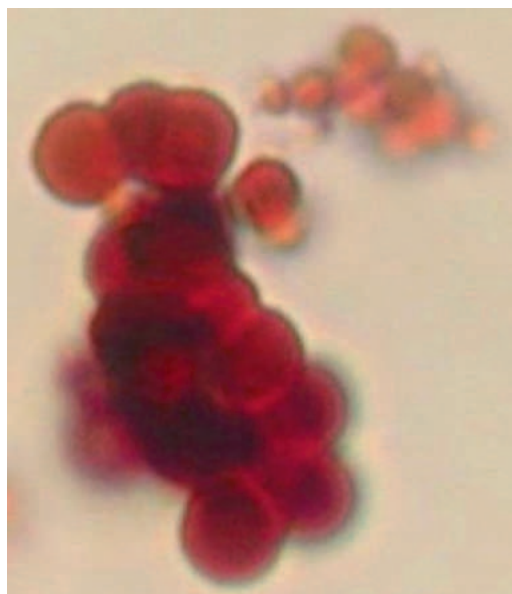


FIGURE 3-5. Hemoglobinuria may be seen as hemoglobin droplets in the urine sediment. Hemoglobin droplets are often not correctly identified. This sediment was from a dog with phosphofructokinase deficiency after an intravascular hemolytic crisis.

Icterus (see Figure 2-1) occurs with intravascular and extravascular hemolysis. Increased unconjugated bilirubin production from RBC destruction in macrophages temporarily exceeds the capacity of the liver to remove it. In severe anemia, the liver may also have hypoxic or toxic damage that causes decreased bilirubin metabolism and cholestasis. Bilirubin concentrations are often greater in intravascular hemolysis than in extravascular hemolysis because of the more rapid rate of hemolysis. Serum bilirubin determinations (i.e., total and conjugated) are of limited use in differentiating anemias from hepatic disease because many exceptions to the expected patterns occur. One should use specific hepatic tests to document hepatic disease (see Chapter 9) or hematologic tests for hemolytic anemia, as noted in this chapter.

Immune-Mediated Hemolytic Anemia

Immune-mediated hemolytic anemia (IMHA) is the most common severe anemia of dogs and is less frequent in cats.^{13,19} IMHA without an apparent cause is often assumed to be due to antibodies directed against autoantigens on RBCs (i.e., primary IMHA); however, infectious agents and certain drugs may induce IMHA directly or indirectly as a result of exposure of foreign antigens on RBCs (i.e., secondary IMHA). Propylthiouracil has been documented in cats and levamisole, carprofen, potentiated sulfonamides, and cephalosporins have been incriminated in dogs as inducers of IMHA. Additionally, a variety of triggers for IMHA have been identified, including lymphoma, myeloproliferative diseases, and infectious diseases (babesiosis, ehrlichiosis, leishmaniasis, rickettsioses) in dogs and lymphoma, FeLV, *Mycoplasma haemofelis*, myelodysplastic syndromes, and acute myelogenous leukemia

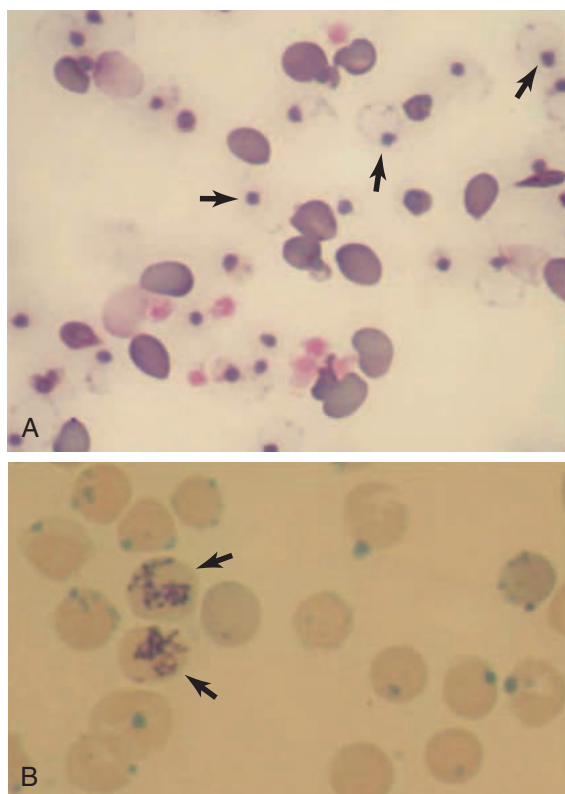


FIGURE 3-6. **A**, Heinz bodies and ghost cells in a cat with onion toxicity from eating baby food with onion powder. The ghost cells appear as faint circles (cell membranes) with one round Heinz body (arrows) that has the same color as hemoglobin in the intact RBCs. This cat had intravascular hemolysis, hemoglobinuria, and hemoglobin nephropathy. **B**, Heinz bodies in a new methylene blue–stained smear from a dog with onion toxicity. The Heinz bodies are lighter blue than dark-purple granules in the two reticulocytes (arrows). Most Heinz bodies are single round inclusions, but there are also smaller, more granular, blue-staining Heinz bodies in several RBCs. These small numerous Heinz bodies may be mistaken on reticulocyte stained smears for reticulocytes.

in cats. The removal of incompatible transfused RBCs is an immune-mediated hemolytic process and thus a form of IMHA, though most authors exclude dogs with blood transfusions from classification of IMHA. Inability to document a specific cause of IMHA may be due mainly to a current lack of specific tools or knowledge to do so.

Direct Coombs Test • The direct Coombs test identifies the presence of antibodies or complement on RBCs. The antibodies and complement on an RBC may or may not be directed toward the RBC itself and may or may not damage the RBC. The screening test is a “polyvalent direct antiglobulin test” in which a polyvalent Coombs reagent is mixed with the patient’s RBCs. This reagent contains species-specific antibodies against various classes of antibodies and complement. If the patient’s RBCs have enough antibody or complement to be detected and the ratio of these antibodies to the antiglobulin is in the

proper proportion, gross or microscopic hemagglutination occurs (i.e., a positive reaction). Some laboratories use monovalent antiglobulin to classify IMHA as either immunoglobulin M (IgM) or immunoglobulin G (IgG) types and detect the presence of complement on RBCs. The pattern found in 48 dogs at the University of Minnesota was: 71% IgG positive, 10% IgG and IgM positive, and 19% IgG, IgM, and complement positive. The effect of steroid treatment *in vivo* is unpredictable. Dogs remain Coombs-positive for variable lengths of time during corticosteroid treatment of IMHA.

The direct Coombs test is neither highly specific nor sensitive for IMHA. The test is positive in only 60% to 70% of canine IMHA cases. Possible reasons for false-negative results include insufficient quantity of antibody on RBCs, temperature at which the test was performed, improper antigen:antibody ratio, and elution of low-avidity antibodies from RBCs during washing. To ensure that the proper antigen:antibody ratio is achieved, serial dilutions of the antiglobulin reagent should be routinely used. Over time, antibody and complement elute off the RBCs in blood samples. The time that an EDTA blood sample may be stored before a Coombs test is unknown. Therefore results obtained from samples sent to laboratories by mail are questionable. Alsever’s solution is good for storing antibody-coated RBCs but is not readily available to most veterinarians. Positive Coombs reactions are expected in RBC parasite infections, incompatible blood transfusions, and drug reactions, which cause RBCs to appear foreign to the immune system.

The specificity of the Coombs test for IMHA is often considered good, but positive reactions frequently occur in the absence of evidence of IMHA. These false positives are not detected as often as false-negative results, because a Coombs test is usually not requested unless the patient has signs of IMHA. Of Coombs-positive anemias in 134 dogs, half were positive for the third component of complement (C3), but there was infrequent evidence of intravascular or extravascular hemolytic anemia.²¹ Therefore a positive Coombs test should be supported by other strong evidence of IMHA.

Canine Immune-Mediated Hemolytic Anemia • Diagnosis of IMHA is typically based on identifying moderate to large numbers of spherocytes, autoagglutination, or both in a dog.¹⁹ A typical CBC pattern in canine IMHA (mean figures from an author’s retrospective study) is: moderate to severe anemia (e.g., PCV 16%), marked reticulocytosis (e.g., 625,000/ μ l), polychromasia (e.g., 3+ or 4+), normal to slightly increased PP (e.g., 7.2 g/dl), marked leukocytosis (e.g., 54,000/ml), significant spherocytosis (e.g., 2+ or more) in most (i.e., 82%) dogs, and autoagglutination and thrombocytopenia (e.g., 29%) in some dogs. The RI is usually greater than 3, suggesting hemolytic anemia. The mean RI in one study of IMHA was 4.8. Moderate to marked spherocytosis (e.g., spherocytes > 50% of RBCs) in dogs is diagnostic for IMHA with rare exceptions, such as viper bite. RBC agglutination, persisting after saline dilution (i.e., autoagglutination), is also diagnostic of IMHA. Thrombocytopenia may be immune mediated or caused by a thromboembolic (disseminated intravascular coagulation [DIC]) problem. Some IMHA cases are nonregenerative if the immune

reaction damages erythroid precursor cells in the bone marrow. A positive Coombs test is also good evidence for IMHA.

NOTE: The diagnosis of canine IMHA is typically based on identifying moderate to large numbers of spherocytes, autoagglutination after saline dilution, or a positive direct Coombs test in a dog with a moderate to severe, regenerative anemia.

Spherocytosis (i.e., increased spherocytes) is a subjective observation made from blood smears that is the most common, and important, diagnostic feature of canine IMHA. Errors in identifying spherocytes are common, especially when evaluating RBC morphology in poor smears or improper areas of blood smears, such as near the feathered edge. However, spherocytes can be consistently identified in high-quality canine blood smears by experienced observers (Figure 3-7). Autoagglutination can interfere with making a good blood smear (see Figure 2-8A). A “squash” preparation can be used as an alternative to a routine wedge smear when blood has autoagglutination. One can lay a second objective glass or coverslip over a drop of blood and the pull the two glass slides apart to attempt to get a better monolayer.

The number of spherocytes is important in diagnosis. A normal animal’s blood may have occasional spherocytes. A moderate to large numbers of spherocytes are required to diagnose IMHA. Spherocytes can be quantified in the monolayer area of a blood smear using the

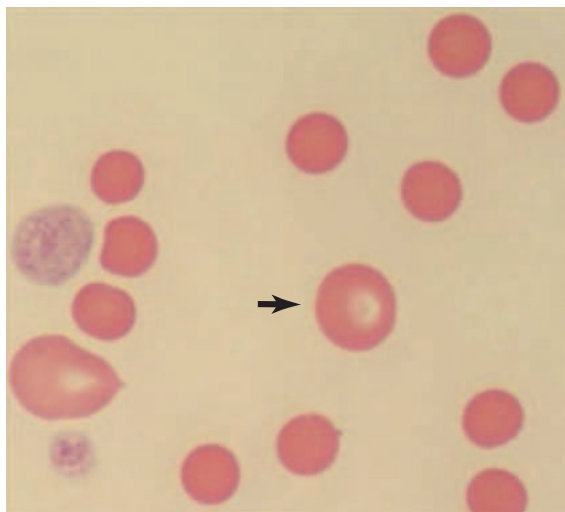


FIGURE 3-7. Identifying moderate to many spherocytes on blood smears is excellent evidence of IMHA. This canine blood smear photo illustrates 11 spherocytes and 2 more normal RBCs. Compare the more normal, biconcave disc-shaped erythrocyte (arrow) with central pallor to the 11 spherocytes, which being spherical and not flat discs have a smaller diameter despite having the same volume. Spherocytes are thicker and so look darker but are not really hyperchromic. There are also two large platelets. One platelet has a wider diameter than most RBCs.

100× oil objective (1000× magnification). One can find an area of the blood smear where normal RBCs have central pallor and compare spherocytes to the normal RBCs. A scale of 1+ to 4+ may be used; 1+ equals 5 to 10 spherocytes per 100× oil field (2% to 4%); 2+ equals 11 to 50 (4% to 20%); 3+ equals 51 to 150 (20% to 60%); and 4+ equals more than 150 spherocytes per field (>60%). Note that less than 2% spherocytes is not reported.

Spherocytosis has been reported in zinc toxicity. Zinc toxicity can cause Heinz body anemia with pyknocytes but may induce immunoglobulin binding to RBCs as well. The spherocytic portion of eccentrocytes has led to their misidentification as spherocytes in vitamin K₃ and zinc toxicity of dogs, but these cells should be called pyknocytes instead of spherocytes to avoid misdiagnosis. Certain cases of snake venom intoxication may have spherocytosis or echinospherocytosis (Figure 3-8).

Autoagglutination is usually diagnostic for IMHA (Figure 3-9). Spontaneous autoagglutination of a blood sample is equivalent to a positive Coombs test result because the end point of the Coombs test is agglutination of the RBCs. Antibodies causing RBC agglutination without need for addition of Coombs reagent are called complete antibodies. Autoagglutination must be differentiated from prominent rouleaux formation resulting from acute phase protein reaction in inflammatory diseases and even paraproteinemia of lymphoid neoplasia. Autoagglutination on a wet mount preparation should resemble grapelike clusters of RBCs. Rouleaux formation microscopically resembles linear stacks of coins and should disperse when blood is mixed with equal or preferably larger amounts of saline in a wet mount. A drop of blood is mixed with one or three drops of saline on a glass slide, coverslipped, and evaluated grossly and microscopically (see Figure 3-9). Some perform a more precise 1:4 or 1:9 dilution and mixing of EDTA blood to saline in tubes to check for persistent agglutination. Note that

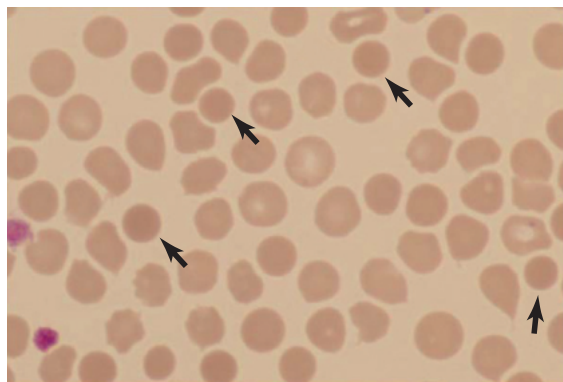


FIGURE 3-8. Spherocytes are in the blood of a dog bitten by a Swedish viper. Spherocytes (arrows) and echinospherocytes may be caused by snake venom. Even though there are spherocytes in this photo, it does not look like IMHA, because it lacks polychromasia, anisocytosis, and leukocytosis. There are only two platelets (lower left), suggesting thrombocytopenia. Disseminated intravascular coagulation (DIC) is common in snake bite patients.

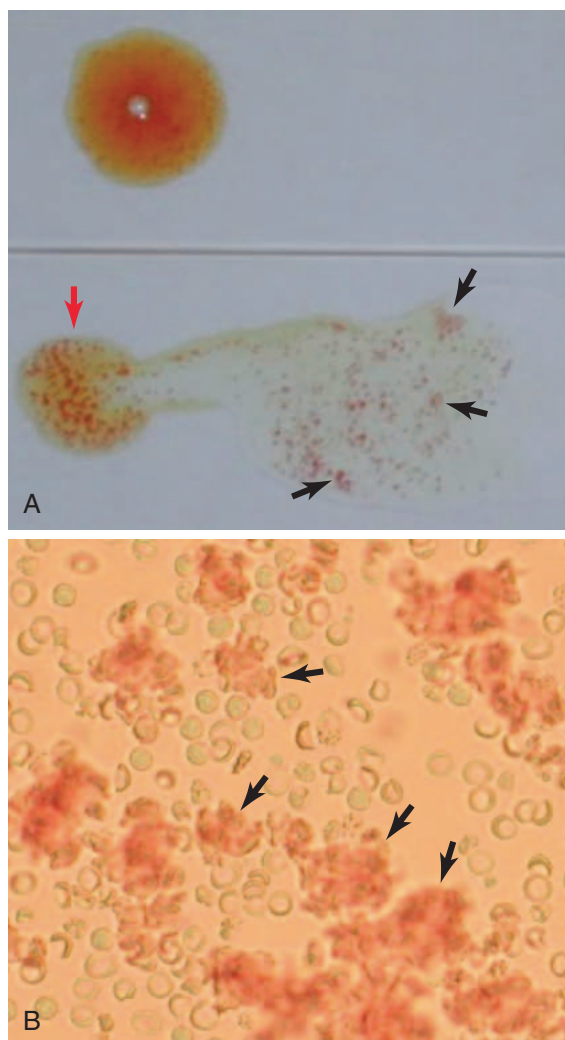


FIGURE 3-9. Autoagglutination is excellent evidence of IMHA. **A**, The upper slide has a drop of EDTA blood with icterus and clumps of RBCs. The lower slide has a drop of 100% EDTA blood (red arrow) on the left side of a glass slide with part of that blood drawn into three drops of saline on the right side of the slide and mixed. There is prominent RBC agglutination even in the well-diluted blood (black arrows), which indicates autoagglutination and not rouleaux. The plasma was very icteric, which supports a diagnosis of hemolytic anemia. **B**, If the agglutination before or after saline dilution is not grossly apparent, then the blood should be examined as a wet mount with a microscope. This photomicrograph of EDTA blood mixed with saline shows individual RBCs in focus and several aggregates of RBCs just out of focus (arrows). Retention of aggregates after saline dilution indicates true autoagglutination.

the equivalent of this saline dilution occurs in hematology analyzers. Electrolyte diluent for the analyzer disperses RBC clumping caused by rouleaux formation, but autoagglutination persists. Autoagglutination can be identified on RBC cytograms and causes a variety of instrument errors in the CBC.

Feline Immune-Mediated Hemolytic Anemia • Feline IMHA is more difficult to diagnose than canine IMHA.¹³ Unlike dogs, cats with IMHA are typically young (<3 years). Spherocytosis is difficult to identify in cats because feline RBCs often lack central pallor, and the marked leukocytosis that is often seen in dogs with IMHA does not occur in cats. Lymphocytosis in blood or bone marrow is frequently associated with feline IMHA. A positive Coombs test and autoagglutination are common findings in affected cats. However, autoagglutination must be differentiated from rouleaux formation. Cats normally have more rouleaux than dogs and rouleaux is increased during inflammation. Whole blood should be diluted 1:4 with isotonic or phosphate-buffered saline to disperse rouleaux formation, and blood should be examined both grossly and microscopically. In the absence of autoagglutination or a positive Coombs test result, a diagnosis of IMHA depends on resolution of anemia in response to immunosuppressive therapy.

NOTE: Unlike dogs, cats with IMHA are typically young, do not have a marked leukocytosis, and spherocytes are difficult to identify.

Causes of IMHA in cats include FeLV, *Mycoplasma haemofelis*, *Babesia felis*, lymphoma, and propylthiouracil or methimazole treatment. Additionally, myelodysplastic syndromes and acute myelogenous leukemia appear to act as triggers for initiation of IMHA in cats. Specific testing should be directed toward detecting these conditions. *M. haemofelis* may be identified in blood smears (see Figure 3-4), but polymerase chain reaction (PCR) tests are more sensitive and can distinguish among types of feline hemoplasmas (*M. haemofelis* and *Candidatus Mycoplasma haemominutum* or newer strains).

Cold Hemagglutinin Disease • Rare animals have clinical signs associated with antibodies that preferentially bind to RBCs below body temperatures (i.e., in peripheral capillary beds: ears or paws). If these antibodies agglutinate RBCs and occlude capillaries, poor blood flow and ischemic necrosis ensue. Cold agglutinin disease may or may not be associated with chronic hemolytic anemia. The antibody expected in cold hemagglutinin disease is IgM.

Test results for cold hemagglutinin disease should be interpreted cautiously. The disease occurs rarely enough that most laboratories are inexperienced with proper evaluation of cold agglutinins. More is involved than just refrigerating the blood sample and performing agglutination tests at cool temperatures (4° C). RBCs must be separated from the EDTA plasma at 37° C to avoid binding of cold agglutinins to RBCs. Cold agglutinins are normally found in dogs and people. In humans, the titer should be greater than 1:64 to be considered abnormal. A diagnostic titer for cold agglutinins in sera of dogs and cats is unknown. One cat with cold hemagglutinin disease had a titer of 1:52,000.²⁰ RBC agglutination occurring at 4° C should disperse when blood is warmed to body temperature.

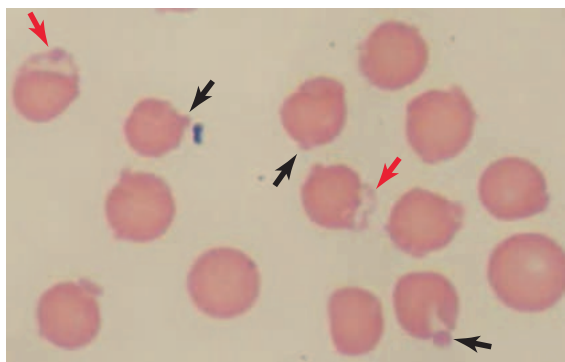


FIGURE 3-10. Eccentrocytes are RBCs in which part of the cell membrane has fused together, which squeezes out hemoglobin from that area, leaving a clear rim of membrane on one side of the RBC (red arrows). The displaced hemoglobin is forced into a spherical shape in the remaining part of the cell, which is not a true spherocyte. Cells resulting from this process that have no obvious tag of clear membrane are called pyknoocytes and not spherocytes. Onion poisoning in this dog also caused Heinz bodies, which appear as small paler staining round bodies at the edges of some RBCs (black arrows).

Additionally, rare dogs may have cryoglobulins or cryofibrinogen in their plasma or serum that can precipitate out of blood samples at cooler temperatures, such as in refrigerated centrifuges. Blood samples must similarly be held at body temperature until a test for cryofibrinogen or cryoglobulin can be performed, and these tests are so rarely performed that laboratories are not prepared to perform them.

Heinz Body Anemia

Many toxic substances oxidize hemoglobin, causing it to precipitate and form Heinz bodies, methemoglobin, or both. Heinz bodies stain like normal hemoglobin on Wright-stained blood smears, but they may appear as lighter-colored round bodies within RBCs or may bulge from the cell surface (Figure 3-10). Heinz bodies are more obvious in lysed RBC ghost cells (see Figure 3-6A). Heinz bodies stain prominently on smears stained with NMB or brilliant cresol green via the same method used for preparing reticulocyte smears (see Figure 3-6B). Heinz bodies stain a lighter blue than granules in reticulocytes. Heinz bodies in the dog can be small and more granular in size and shape, which can confuse identification. RBCs with small granular Heinz bodies may be misclassified as reticulocytes.

NOTE: Heinz body anemias are typically regenerative anemias (after a few days) and are characterized by Heinz bodies, eccentrocytes, pyknoocytes and even intravascular hemolysis, after exposure to oxidative toxins. Methemoglobinemia is often caused by the same toxins.

Eccentrocytes • Eccentrocytes, like Heinz bodies, result from oxidative injury to RBCs (see Figure 3-10). Onion poisoning and less commonly vitamin K₃ toxicity or diabetes mellitus causes eccentrocytes in dogs. Onion-eating

dogs may have symptoms ranging from what appears to be no evidence of anemia to life-threatening intravascular hemolysis. Eccentrocytes are RBCs in which part of the cell membrane is damaged and fuses together. This fusion of the internal layers of cell membrane squeezes hemoglobin out of that area, leaving a sharp line of demarcation between the areas that contain hemoglobin and those that do not contain hemoglobin (see Figure 3-10). Pyknoocytes are RBCs formed by the same process but in which no lip of membrane is seen. These are often misidentified as spherocytes, which may lead to an incorrect diagnosis of IMHA instead of an oxidative toxin poisoning.

Canine Heinz Body Anemia • Heinz bodies are not normally found in canine blood; therefore finding any Heinz bodies is abnormal. Canine Heinz bodies are often small, irregular, and multiple (see Figure 3-6B). Thus canine Heinz bodies may be overlooked and on smears stained for reticulocyte evaluation may be misidentified as reticulocytes. Heinz bodies stain a lighter blue color than reticulocytes and vary in size; therefore they can be differentiated from true reticulocytes when stained with reticulocyte stains. Causes of canine Heinz body anemia include exposure to onions, vitamin K₃, naphthalene, propylene glycol, benzocaine, methylene blue, copper, zinc, phenylhydrazine, and acetaminophen. Ingestion of cooked, uncooked, or dehydrated onions is the most common cause of canine Heinz body anemia. Vitamin K₃ is more toxic (and less effective) than K₁; therefore vitamin K₃ should not be used therapeutically.

Feline Heinz Body Anemia • Both healthy and sick cats often have Heinz bodies. Feline hemoglobin is more susceptible to oxidative damage, and the feline spleen is inefficient in removing Heinz bodies. Heinz body numbers are affected by diet (increased in cats eating fish diets and diets containing propylene glycol). Heinz bodies within RBCs are usually round, large (i.e., 0.5 to 3 μ m in diameter), and singular. One must consider number and mass of Heinz bodies, PCV, reticulocyte response, and likelihood of exposure to oxidative toxins to determine their significance in causing a hemolytic anemia. The number of Heinz bodies in feline blood may be categorized as occasional if less than 10%, moderate if 10% to 50%, and marked if greater than 50%.

NOTE: Feline hemoglobin is more susceptible to oxidative damage, and the feline spleen is inefficient in removing Heinz bodies. As a result, healthy cats can have up to 10% Heinz bodies in their blood. Cats with increased numbers of Heinz bodies may not have hemolytic anemia.

Oxidative toxins reported to cause Heinz body formation in cats include methylene blue, acetaminophen, phenacetin, phenazopyridine, propofol, propylene glycol, salmon-based diets, canned meat-based baby foods containing onion powder, and diseases including diabetes mellitus, hyperthyroidism, renal failure, and lymphoma. Increased Heinz bodies in cats eating semi-moist diets were the result of propylene glycol; therefore the U.S.

Food and Drug Administration prohibited addition of propylene glycol to cat food in 1994.⁵ Increased numbers of Heinz bodies in cats are not always associated with signs of a hemolytic anemia.

Methemoglobinemia

Oxyhemoglobin is the normal, oxygen-carrying form of hemoglobin in which iron is in the reduced (ferrous) state. Methemoglobin is a nonfunctional form of hemoglobin in which ferrous iron is oxidized to ferric iron. Methemoglobin is nonfunctional because it cannot bind oxygen. In healthy animals, approximately 1% of oxyhemoglobin is converted to methemoglobin daily. Methemoglobin reductase reduces methemoglobin back to oxyhemoglobin. Rarely, methemoglobin reductase deficiency occurs as a congenital defect in dogs and produces significant methemoglobinemia and cyanosis. The oxyhemoglobin method for hemoglobin concentration measures only oxyhemoglobin, whereas the usual cyanmethemoglobin method measures all types of hemoglobin, including methemoglobin.

NOTE: Methemoglobin is a nonfunctional form of hemoglobin. Methemoglobinemia may cause cyanosis or signs of anemia even in animals with normal PCV (physiologic anemia).

Methemoglobinemia can be detected by observing cyanosis of mucous membranes or brownish discoloration of blood, as well as specific testing for methemoglobin. Test is available at many reference laboratories. Methemoglobinemia should be suspected if Heinz bodies or eccentrocytes are seen. The animal may appear more hypoxic than the PCV suggests. The gross appearance of blood is frequently the first suggestion of methemoglobinemia. Blood with methemoglobinemia is darker and browner than normal and may not turn red when exposed to air. Placing a drop of a patient's blood and a drop of normal blood on white filter paper allows one to detect the darker color more easily. The animal's mucous membranes may appear darker or cyanotic if more than 30% of hemoglobin is affected. Causes of canine methemoglobinemia include benzocaine and acetaminophen administration. Causes of feline methemoglobinemia include benzocaine (Cetacaine), acetaminophen, phenacetin, methylene blue, DL-methionine, and phenazopyridine toxicity. Toxic substances (e.g., benzocaine, acetaminophen, methylene blue) may cause Heinz body anemia, methemoglobinemia, or both.

Blood Parasites

Hemoplasmas • *M. haemofelis* and *Candidatus M. haemominutum* (e.g., feline infectious anemia, hemobartonellosis) infection can be diagnosed by blood smear evaluation or by PCR testing. PCR is much more sensitive for detection of parasitemia and can indicate if the parasite is *M. haemofelis*, *Candidatus M. haemominutum*, or another hemoplasma. Blood smear evaluation is insensitive because parasite numbers are often below the concentration needed to see them microscopically. Stain precipitate is similar in size, shape, and color to

hemoplasmas and so is easily confused with the organisms, resulting in false-positive diagnosis. To differentiate *M. haemofelis* from stain precipitate, distinct ring forms should be identified (see Figure 3-4). A ring form looks like a lower-case letter *o* with a dark exterior ring and a pale interior. It may be harder to be certain of the diagnosis of *Candidatus M. haemominutum* if the organism is present in small numbers.

NOTE: The PCR test is a more sensitive test for detection of feline *Mycoplasma* sp. infection of erythrocytes than blood smear analysis and also appears to detect the canine organism.

Parasitemic episodes are often transient (i.e., 1 to 2 days) and separated by periods when few or no organisms are found. Therefore smears should be evaluated on several consecutive days to improve the likelihood of diagnosis. Obtaining fresh, nonanticoagulated, capillary blood by pricking the ear with a lancet and immediately making a thin blood smear provides a higher concentration of parasitized RBCs. The parasites may fall off RBCs in EDTA blood. Splenomegaly occurs frequently in feline infectious anemia, because splenic macrophages remove damaged RBCs and organisms. More severe anemia is expected with *M. haemofelis* than with *Candidatus M. haemominutum*. Concurrent infection with FeLV or feline immunodeficiency virus (FIV) can predispose a cat to hemoplasma infection and make the anemia more severe. FeLV and FIV testing is recommended for cats with anemia or other hematologic problems.

Mycoplasma haemocanis • *Mycoplasma haemocanis* principally causes anemia in dogs that have undergone splenectomy. It may occur in carrier dogs after splenectomy or in dogs that have previously undergone splenectomy after they contract infection via blood transfusion or tick bite. Glucocorticoid therapy may produce a functional splenectomy and thereby predispose to *M. haemocanis* infection. *M. haemocanis* appears typically as distinct chains of cocci on RBCs and less often with ring forms. The linearly arranged cocci must be differentiated from stain precipitate.

Babesia spp. • Several species of *Babesia canis* have been described in dogs that vary in virulence. All strains produce varying degrees of hemolytic anemia and thrombocytopenia. Both acute intravascular hemolysis and extravascular hemolysis are seen. Hemoglobinuria is common. Transmitted by ticks, *B. canis* is frequently accompanied by *Ehrlichia canis* and other infections. *B. canis* infection is seen most often in racing greyhound dogs. The diagnosis is made by demonstrating intraerythrocytic piriform (i.e., pear-shaped or teardrop-shaped) organisms on blood smears or by serology. Capillary blood has a higher concentration of parasitized RBCs, so examination of fresh non-anticoagulated ear prick blood samples is recommended.

Babesia gibsoni is smaller than *B. canis*; however, both organisms are large enough to be readily seen microscopically (Figure 3-11). *B. gibsoni* may be transmitted by dog

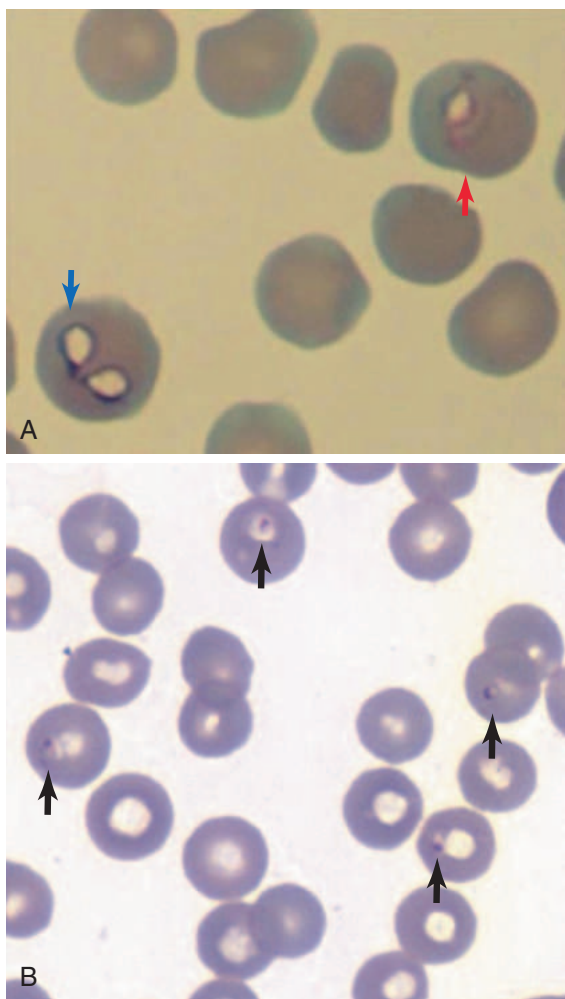


FIGURE 3-11. *Babesia canis* and *Babesia gibsoni* are recognized in blood smears. *B. canis* is larger (A) at 2.5 to 5 µm than *B. gibsoni* (B), which is about 1 to 3 µm. A canine RBC is about 7 µm in diameter. The classic double-teardrop form is seen with one RBC containing *B. canis* (blue arrow in A) but other shapes are common (red arrow). *B. gibsoni* is more round to oval (black arrows in B).

bites and occurs frequently in pit bull terriers, so this breed should not be used as blood donors. *B. gibsoni* causes hemolytic anemia and thrombocytopenia, but once infected, dogs become subclinical carriers.

Cytauxzoon felis • *Cytauxzoon felis* is a fatal, tick-transmitted disease of cats. It is diagnosed most consistently at necropsy by finding large schizonts in endothelial cells in lungs, liver, spleen, and lymph nodes. Endemic areas include Missouri, Oklahoma, Arkansas, Mississippi, Georgia, Florida, and Louisiana. Small piriform or safety pin-shaped organisms in RBCs are found on blood smears in less than 50% of affected cats. These bodies measure 1 to 5 µm and have a small, peripherally located nucleus. Clinical signs include icterus, depression, anorexia, fever, and dehydration.

Zinc or Copper Toxicity

Zinc-induced hemolytic anemia results from ingestion of zinc-containing objects (e.g., zinc nuts from portable kennels or pennies [U.S. coin minted after 1982]) by dogs. Zinc forms soluble salts in gastric acid and is absorbed. Zinc toxicity causes intravascular hemolysis. Clinical signs include severe anemia, icterus, leukocytosis, vomiting, and diarrhea. Diagnosis is confirmed by detection of zinc-containing objects in the stomach by radiographic examination and increased serum zinc concentration (normal canine serum zinc = 0.6 to 2 mg/kg) (see Chapter 17). An increase in Heinz bodies and also in spherocytic RBCs has been reported in zinc toxicity. It is unclear whether these are spherocytes, indicating immune damage, or pyknotocytes resulting from oxidative damage. Treatment of canine RBCs with zinc chloride induces IgG binding; therefore zinc-induced hemolysis may be a secondary form of IMHA.⁴ Copper toxicity may also induce a hemolytic anemia. One example is in copper storage disease in the liver of Bedlington terriers where the excessive copper may suddenly be released from hepatocytes and induce hemolytic anemia and even hemoglobinuria.

Hypophosphatemia

Hypophosphatemia causes hemolytic anemia in dogs and cats. In dogs, hemolysis can occur when the serum phosphorus concentration is less than 1.0 mg/dl. However, in cats, hemolysis may occur with less severe hypophosphatemia (i.e., <2.5 mg/dl).¹² Hypophosphatemia may be anticipated when refeeding cats with hepatic lipidosis. Hypophosphatemia may be a complication of insulin therapy. Diagnosis of hypophosphatemia (see Chapter 8) in hemolytic anemia is complicated by a common methodology error. Conjugated bilirubin in icteric samples interferes with some direct inorganic phosphorus (PO₄) assays.¹ The PO₄ concentration may appear falsely decreased or even too low to measure in very icteric samples. If in doubt, the clinician can verify the PO₄ concentration in icteric samples using inductively coupled plasma emission spectrometry methods modified for serum, which are typically used in toxicology laboratories.

NOTE: Hypophosphatemia-associated hemolytic anemia may result from refeeding cats with hepatic lipidosis or as a complication of insulin therapy.

Pyruvate Kinase Deficiency

Pyruvate kinase (PK) deficiency in dogs is an autosomal recessive genetic disease causing severe and persistent extravascular hemolysis. Characteristic features include moderate to severe anemia (i.e., PCV 18% to 25%) with marked reticulocytosis (i.e., 25% to 45%), and splenomegaly. It occurs in young basenji, West Highland white terrier, and beagle dogs. Affected dogs die by 3 years of age, and many dogs have terminal myelofibrosis and osteosclerosis. PK-deficient RBCs have inefficient energy production via the glycolytic pathway and thus have a shortened life span.

Phosphofructokinase Deficiency

Phosphofructokinase (PFK) deficiency is due to a single missense mutation in the M-PFK gene in English springer and American cocker spaniels, whippets, and mixed breed dogs. PFK-deficient Wachtelhund dogs do not have the same PFK mutation and thus the DNA test for this hereditary disease in those other breeds is negative in the Wachtelhund patients. Periodic intravascular hemolysis and hemoglobinuria associated with strenuous exercise or stress-induced hyperventilation causing respiratory alkalosis is a characteristic sign. Residual PFK activity in affected dogs is inhibited by alkalotic conditions. Splenomegaly, icterus, or both may be present. Diagnosis is suggested by excessive reticulocytosis compared to severity of the anemia and by ruling out common causes of hemolytic anemia. Dogs with PFK deficiency have decreased 2,3-diphosphoglyceride (DPG) concentration, which can be an initial test prior to firm diagnosis by analysis of RBC phosphofructokinase activity.¹¹

Other Hereditary Hemolytic Anemias

Familial nonspherocytic hemolytic anemia has been described in poodles and beagles. In poodles the cause was not determined, but PK activity was not evaluated. It was fatal by as early as 3 years of age. Myelofibrosis, osteosclerosis, and widespread hemosiderosis were noted at necropsy, as in dogs with PK deficiency. Hereditary nonspherocytic hemolytic anemia in beagles was studied extensively without identifying a cause. The anemia was not severe or fatal and lacked hemosiderosis, myelofibrosis, or osteosclerosis.

Dwarfism in Alaskan malamutes has been associated with stomatocytosis. Affected dogs have a well-compensated hemolytic anemia characterized by decreased RBC osmotic fragility, decreased RBC survival, and erythroid hyperplasia in bone marrow. Stomatocytes are RBCs with mouth-shaped central pallor. RBCs are macrocytic and hypochromic. The MCHC was consistently less than 30 g/dl. Compared with age-matched normal Alaskan malamutes, the dwarfs were not anemic.

Miniature schnauzers have stomatocytosis as an autosomal recessive trait but lack clinical signs of anemia. A miniature schnauzer-beagle dog had inherited stomatocytosis and prominent macrocytosis, apparently resulting from RBC swelling (see Figure 3-5). The dog had a PCV of 48% but had a mild reduction in RBC numbers (i.e., $5.01 \times 10^6/\mu\text{l}$) and hemoglobin concentration (12.5 g/dl).³ RBC survival was only slightly shortened. Stomatocytosis also occurs in the Drentse patrijshond breed as familial stomatocytosis-hypertrophic gastritis.

Hereditary elliptocytosis in dogs is associated with a deficiency of membrane protein 4.1. RBC morphology was characterized by macrocytosis, elliptocytosis, poikilocytosis, and RBC fragmentation, but anemia was not present.

Hemolytic anemia with increased osmotic fragility has been described in Abyssinian and Somali cats. Affected cats have a recurrent moderate to severe direct Coombs-negative hemolytic anemia with a few stomatocytes present. Splenomegaly, hemosiderosis, and lymphoid hyperplasia are also present. RBC osmotic fragility is markedly increased.

NONREGENERATIVE ANEMIA

Nonregenerative anemia is usually normocytic normochromic and lacks diagnostic changes in RBC morphology. Many nonregenerative anemias are only mild to moderate and occur as secondary complications of systemic diseases, especially inflammatory and neoplastic diseases. Diagnostic efforts in these cases should be aimed at the primary disease. An exception is anemia of renal failure that can become severe in late stages of disease. Severe nonregenerative anemia may exist separately or be part of generalized bone marrow suppression that results in bicytopenia or pancytopenia. Potential causes are listed in Box 3-1.

NOTE: Nonregenerative anemias can occur secondary to non-hematologic problems like inflammation and organ failure or as a primary bone hematopoietic system disorder.

Diagnostic Approach

One should first determine if leukopenia or thrombocytopenia is present in addition to nonregenerative anemia (see Box 3-2). Pancytopenia (i.e., decreased RBCs, leukocytes, platelets) or bicytopenia (i.e., two of the three cell types depleted) frequently indicates disease in the bone marrow, which is best diagnosed by a bone marrow biopsy and aspiration (see Chapter 2; also discussed later).²⁶

If only anemia is present, one should check the RBC indices, RBC cytograms and histograms, and blood smear to classify it as normocytic normochromic, microcytic hypochromic, or macrocytic normochromic. Normocytic normochromic anemia is the most common. If the anemia is mild to moderate, causes of secondary bone marrow suppression should be evaluated. Severe nonregenerative anemias are often associated with nonregenerative IMHAs or pure RBC aplasia. Microcytic hypochromic anemia occurs most frequently with iron deficiency (discussed later). Macrocytic normochromic anemia (discussed earlier) without reticulocytosis in cats is often associated with FeLV-induced myeloproliferative disorders. If a cause for the anemia cannot be identified, a bone marrow evaluation is indicated.

Secondary Anemias

Anemia of Inflammatory Diseases

Anemia of inflammatory or neoplastic diseases is the most common cause of nonregenerative anemia.⁷ Mild to moderate normocytic normochromic nonregenerative anemia that does not become severe, low serum iron, and increased iron in bone marrow and other tissues is typical of anemia of inflammatory diseases. Inflammatory changes in the CBC are discussed in Chapter 4. During inflammation, macrophages release inflammatory cytokines, including interleukin-1, interleukin-6, and tumor necrosis factor- α , that initiate several inflammatory

processes, including fever. Inflammatory cytokines cause iron to be sequestered in macrophages, which reduces serum iron and restricts the availability of iron to developing rubricytes, thus limiting erythropoiesis. RBC life span is also reduced. Other causes of anemia associated with cancer include blood loss, RBC fragmentation, neoplastic infiltration of bone marrow, and immune-mediated RBC destruction. Additionally, many chemotherapeutic agents suppress erythropoiesis.

NOTE: Anemia of inflammatory diseases is characterized by mild to moderate normocytic normochromic nonregenerative anemia that does not become severe, low serum iron, and increased iron in bone marrow and other tissues.

Anemia of Chronic Renal Disease

The diagnosis of anemia of chronic renal disease is confirmed by finding nonregenerative anemia associated with renal failure (see Chapter 7). The mechanism of the anemia is more complex than a relative deficiency of erythropoietin. Ineffective erythropoiesis, a shortened RBC life span, and blood loss may contribute to the anemia. However, this type of anemia responds well to erythropoietin therapy.

Anemia of Chronic Hepatic Disease

Anemia of chronic hepatic disease may have multiple causes (see Chapter 9). Abnormal lipid metabolism may cause altered RBC shapes (i.e., acanthocytes) and a shortened RBC life span. Marked poikilocytosis is a frequent finding in cats with hepatic lipidosis and inflammatory liver disease. Coagulation defects, caused by reduced hepatic synthesis of coagulation and anticoagulant factors, may cause hemorrhage particularly in cats. Decreased hepatic function can lead to deficiencies of nutrients needed for hematopoiesis. Microcytosis is a frequent finding in dogs with a PSS. These dogs often have a “functional iron deficiency” with increased hepatic iron but low serum iron, normal total iron-binding capacity (TIBC), and low percentage saturation of iron (Table 3-3).¹⁵

Hypothyroidism and Hypoadrenocorticism

Anemia secondary to hypothyroidism and hypoadrenocorticism usually is mild and clinically insignificant. Evaluation of endocrine disorders is discussed in Chapter 8.

Severe Nonregenerative Anemia

Causes of severe nonregenerative anemia without accompanying leukopenia or thrombocytopenia include nonregenerative immune-mediated hemolytic anemias (NRIMHAs), pure red blood cell aplasia (PRCA), iron deficiency anemia, late-stage chronic renal failure, FeLV and FIV infection, and some types of myelodysplastic syndrome.

Nonregenerative Immune-Mediated Hemolytic Anemia

NRIMHA is a form of IMHA in which erythropoiesis is inhibited.²⁶ This is thought to be caused by autoantibodies that destroy relatively mature erythroid precursor cells in bone marrow. Other concurrent pathologic changes that may be present in bone marrow include dyserythropoiesis, secondary hemophagocytic syndromes, myelofibrosis, and bone marrow necrosis. In many dogs, a maturation arrest is present in the erythroid series. A maturation arrest is a situation in which immature erythroid cells are present in normal or increased numbers but later stages of development are decreased or absent, presumably due to antibody-mediated destruction.

NOTE: NRIMHA and PRCA are forms of IMHA in which erythropoiesis is inhibited in the bone marrow, resulting in nonregenerative anemias.

Pure Red Cell Aplasia

PRCA is a severe nonregenerative anemia characterized by bone marrow that has few or no erythroid precursors, yet normal-appearing myeloid and megakaryocytic lineages.²⁶ Affected dogs and cats frequently have PCVs of less than 8% when initially examined. The cause is

TABLE 3-3. CANINE IRON PROFILES IN IRON DEFICIENCY ANEMIA, ANEMIA OF INFLAMMATORY DISEASE, AND PORTOSYSTEMIC SHUNTS

	NORMAL* MEAN (RANGE)	IRON DEFICIENT* MEAN (RANGE)	AID† MEAN ± SD	PSS‡ MEAN (RANGE)
Serum iron (µg/dl)	149 (84-233)	30 (8-60)	62 ± 14	77 (24-163)
TIBC (µg/dl)	391 (284-572)	387 (234-659)	193 ± 28	377 (302-452)
UIBC (µg/dl)	243 (142-393)	357 (216-633)		300 (184-379)
Saturation (%)	39 (20-59)	8 (2-19)		21 (6-45)

AID, Anemia of inflammatory disease; PSS, portosystemic shunt; TIBC, total iron-binding capacity; UIBC, unbound iron-binding capacity.

*Data from Harvey, French, and Meyer (1982).¹⁰

†Data from Feldman, Kaneko, and Farver (1981).⁷

‡Data from Meyer and Harvey (1994).¹⁵

believed to be immune-mediated destruction of early erythroid precursor cells. Antibodies against erythroid precursor cells were found in four of eight dogs with PRCA. The Coombs test is positive in some affected dogs. Most dogs respond to steroidal or nonsteroidal immunosuppressive therapy. Parvovirus infection has also been associated with PRCA.

Myelodysplastic Syndromes

Myelodysplastic syndromes (MDSs) are a group of hematologic disorders that are caused by an acquired genetic defect in hematopoietic stem cells and result in cytopenias in blood and dysplastic changes in blood or bone marrow.²⁸ In refractory anemia (RA) in dogs, anemia is moderate to severe, normocytic normochromic, and nonregenerative. The disorder results in production of abnormal erythroid precursor cells that have atypical morphologic features and die before being released into the blood. Bone marrow is usually normally cellular or hypercellular and has erythroid hyperplasia. Morphologic alterations that may be observed in bone marrow erythroid precursor cells include binucleated RBC precursors, asynchronous maturation of cytoplasm and nucleus, macrocytes, microcytes, megaloblasts, and Howell-Jolly bodies. Among the limited number of affected dogs described, the anemia appears to respond to erythropoietin therapy and affected dogs have prolonged survival.

NOTE: Myelodysplastic syndromes (MDSs) are a group of hematologic disorders that are caused by an acquired genetic defect in hematopoietic stem cells and result in cytopenias in blood and dysplastic changes in blood or bone marrow.

Congenital Dyserythropoiesis Syndromes

Congenital causes of dysmyelopoiesis include congenital dyserythropoiesis, polymyopathy, and cardiac disease in English springer spaniels; vitamin B₁₂ malabsorption in giant schnauzers; and congenital dyserythropoiesis of poodles (described earlier in this chapter). Congenital dyserythropoiesis, polymyopathy, and cardiac disease in English springer spaniels is characterized by moderate anemia with spherocytes, schistocytes, dacryocytes, codocytes, and vacuolated RBCs seen in blood smears. Bone marrow is characterized by erythroid hyperplasia with dyserythropoiesis (i.e., binucleation and abnormal mitotic figures).

Vitamin B₁₂ malabsorption in giant schnauzers is an autosomal recessive disorder characterized by chronic nonregenerative anemia and neutropenia. There is increased anisocytosis (increased RDW) but the MCV remains normal. Dysplastic features in the blood include macrocytes, schistocytes, acanthocytes, elliptocytes, keratocytes, hypersegmented neutrophils, and giant platelets. Dysplastic features in bone marrow include giant band neutrophils and asynchronous maturation in the erythroid series. Clinical signs resolve with parenteral administration of vitamin B₁₂.

Congenital dyserythropoiesis in poodles is a condition of toy and miniature poodles that is characterized

by macrocytosis in the blood and marked dyserythropoiesis in bone marrow. Dysplastic features in bone marrow include megaloblasts, binucleated erythroid cells, nuclear fragmentation, multiple Howell-Jolly bodies, and atypical mitotic figures. Despite the very abnormal morphology, the dogs are not anemic and have no clinical signs.

Sideroblastic Anemia

Sideroblastic anemia is a moderate to severe microcytic or hypochromic nonregenerative anemia in dogs that may be confused with iron deficiency anemia.²⁵ A variety of dysplastic features are present in blood and bone marrow. The role of pyridoxine deficiency (a known cause in people) as a cause of sideroblastic anemia in dogs has not been investigated.

Iron Deficiency Anemia

Iron deficiency anemia is not uncommon in tropical and semitropical areas. Harvey, French, and Meyer documented iron deficiency in 11% of anemic dogs in Florida.¹⁰ Reticulocytosis and polychromasia occurred in half of affected dogs. The most frequent cause of iron deficiency is persistent blood loss (e.g., hookworms, fleas, bleeding intestinal neoplasms). Blood loss anemias are initially strongly regenerative, then progress to nonregenerative as iron deficiency worsens. Anemia varies from mild to severe. Poikilocytosis with RBC fragmentation and hypochromasia is typical. Thrombocytosis can be marked, with many large platelets.

Hypoproteinemia, when present, supports recent and severe external bleeding; however, PP losses are replaced much faster than RBCs in the body, so PPs may be normal in chronic bleeding. Puppies normally have lower PP concentrations than adults do; therefore clinicians should use age-matched reference intervals when evaluating anemic puppies. Puppies also have lower PCV and higher reticulocyte values than adults. Puppies become iron deficient more rapidly than adults, because of their rapid growth and maximal erythropoiesis.

A CBC often identifies iron deficiency anemia (Figure 3-12). In the dog, microcytic (i.e., MCV < 60 fl), hypochromic (i.e., MCHC < 32 g/dl) RBCs are consistent with a diagnosis of iron deficiency. Newer automated hematology cell counters are more sensitive in detecting small numbers of microcytic hypochromic RBCs (see Figure 2-4) than are the MCV and MCHC.²² Exceptions include Japanese Akitas, Shibas, and chow chows, dogs that normally have small RBCs. Microcytosis and decreased serum iron often occur in dogs with PSSs and resemble iron deficiency (discussed earlier).

The serum iron profile is the best proof of iron deficiency and can provide confidence in the diagnosis. The owner and veterinarian may become discouraged at how slowly the PCV returns to normal. Expected iron profile values in iron deficiency are listed in Table 3-3, and are compared with iron values in dogs with anemia of inflammatory disease in Table 3-4.⁷

TIBC reflects the concentration of transferrin, the serum iron transport protein. Serum iron measures the iron bound to transferrin. Unsaturated iron-binding capacity (UIBC) measures the additional amount of iron that transferrin can bind. TIBC is the sum of serum iron

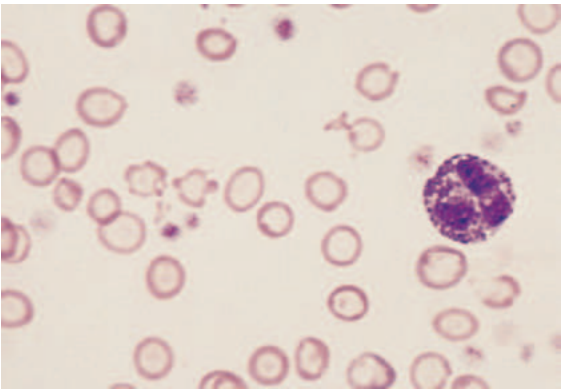


FIGURE 3-12. Canine iron deficiency anemia is characterized by hypochromasia. True hypochromasia has thin rims of hemoglobin in the RBCs. Leptocytes are cells with increased central pallor and may be misclassified as hypochromasia. Iron deficiency often also has poikilocytosis and thrombocytosis.

TABLE 3-4. COMPARISON OF SELECTED PARAMETERS IN ANEMIA OF INFLAMMATORY DISEASE AND IRON DEFICIENCY ANEMIA

	ANEMIA OF INFLAMMATORY DISEASE	IRON DEFICIENCY ANEMIA
RBC indices	Normocytic normochromic	Microcytic hypochromic
Serum iron	Low	Low
Total iron-binding capacity	Usually decreased	Usually normal
Bone marrow iron	Abundant to increased	Absent
Serum ferritin	High	Low
Inflammation	Present	Need not be present

RBC, Red blood cell.

plus UIBC. Percent saturation is serum iron divided by TIBC (normal = 20% to 60%). Percent saturation indicates availability of iron for erythropoiesis. Hemoglobin synthesis is impaired if percent saturation is less than 20%. Hemosiderin is not detectable in bone marrow samples of iron-deficient dogs. Serum ferritin correlates with tissue iron stores; therefore it is a good indicator of iron deficiency. Further, it is the best test to differentiate iron deficiency anemia from anemia of inflammatory diseases.

NOTE: Iron deficiency anemia is typically characterized by microcytosis, hypochromasia, variable reticulocytosis, poikilocytosis, thrombocytosis, low serum iron concentration, normal TIBC, and decreased serum ferritin.

Iron deficiency anemia associated with microcytosis occurs often (i.e., 70%) in 5-week-old kittens.²³ By 7 weeks of age when they start eating solid food, kittens stop producing microcytic cells. The iron deficiency is probably the result of the low iron content of milk diets.

Feline Leukemia and Feline Immunodeficiency Virus Infection

FeLV and FIV infections are major causes of feline nonregenerative anemia. Macrocytosis (i.e., MCV > 52 fl) without increased reticulocytes is an indication of FeLV infection.

Pancytopenia or Bicytopenia

Moderate to severe nonregenerative anemia combined with leukopenia or thrombocytopenia or both usually reflects a primary bone marrow disorder. If bone marrow cell production (i.e., hematopoiesis) is abruptly suppressed, leukocytes and platelets decrease in the blood before RBCs because RBCs have a longer life span.

Diagnoses associated with primary bone marrow disorders include both etiologic diagnoses and pathologic (i.e., descriptive) diagnoses. Pathologic diagnoses provide an understanding of the general mechanism of marrow injury but usually do not provide a specific diagnosis. Pathologic diagnoses include aplastic anemia, myelofibrosis, bone marrow necrosis, and bone marrow inflammation.

Aplastic Anemia

Diagnosis of aplastic anemia is confirmed by histopathologic examination of bone marrow core biopsies (see Chapter 2). Bone marrow aspirates in aplastic anemia are poorly cellular, perhaps only retrieving fat, so the aspirate may seem to be a poor sample. Bone marrow core biopsies permit evaluation of marrow architecture necessary to confirm marrow hypocellularity and replacement by fat. Possible causes of aplastic anemia in dogs include drug toxicities (e.g., estrogen, phenylbutazone, chemotherapeutic agents, sulfadiazine, quinidine, thiacetarsemide), *E. canis* and parvovirus infections, and immune-mediated processes.² In cats, causes of aplastic anemia include drug toxicities (propylthiouracil, methimazole, griseofulvin, chemotherapeutic agents), anorexia/starvation, and FeLV infection.

NOTE: Aplastic anemia is a pathologic diagnosis characterized by bicytopenia or pancytopenia in the blood and poorly cellular bone marrow replaced by fat.

Bone Marrow Necrosis/Inflammation

Ischemic and toxic injury to bone marrow results in marrow necrosis and may result in an inflammatory response. Bone marrow necrosis has been associated with a variety of conditions, including septicemia/endotoxemia, drug and toxin exposure, parvovirus and monocytic ehrlichiosis infections, disseminated intravascular coagulation, and malignancies. Necrotic lesions may resolve over time or may progress to myelofibrosis.

Myelofibrosis

Myelofibrosis is characterized by replacement of hemic tissue by fibrous connective tissue, collagen, or reticulin fibers. Myelofibrosis can be primary or secondary. Secondary myelofibrosis may occur after toxic injury to bone marrow and perhaps can result from chronic overstimulation of marrow by erythropoietin or thrombopoietin. Therefore myelofibrosis tends to be associated with bone marrow necrosis, immune-mediated anemias and immune-mediated thrombocytopenias, and congenital hemolytic anemias. Primary myelofibrosis is a chronic myeloproliferative disorder of erythroid, myeloid, and megakaryocytic cell lines. Growth factors released by abnormal megakaryocytes are implicated as the cause of fibroblast proliferation.

Drug-Induced Hematologic Dyscrasia

A large number of drugs have been reported to produce bone marrow dyscrasias in dogs or cats. In dogs, these drugs include estrogens, phenylbutazone, meclofenamic acid, sulfonamides, quinidine, chloramphenicol, cephalosporins, chemotherapeutic agents, captopril, tranquilizers, trimeprazine tartrate, mitoxantrone, thiacetarsemide, albendazole, phenobarbital, and phenothiazine. Drugs reported to cause bone marrow dyscrasia in cats include chemotherapeutic agents, chloramphenicol, griseofulvin, propylthiouracil, and methimazole.

Estrogen Toxicity • Canine estrogen toxicity may cause bone marrow destruction, resulting in aplastic anemia. During the first 3 weeks, however, thrombocytopenia, leukocytosis, and mild but progressive anemia typically characterize the CBC. Total leukocyte counts may exceed 100,000 WBCs/ μ l (Figure 3-13). The cause of the leukocytosis is uncertain. After 3 weeks, pancytopenia may occur. Testicular tumors, usually Sertoli cell tumors, may also cause pancytopenia or bicytopenia. Increased serum estradiol concentrations cannot be consistently documented. The PCVs in anemias associated with testicular

tumors have ranged from 6% to 38%. The reticulocytes were decreased in number or only slightly increased. Platelet counts varied from 3000 to 93,000/ μ l.

Sulfadiazine Toxicity • Doberman pinschers appear to be predisposed to sulfadiazine-induced hematologic dyscrasia. Affected dogs have anemia, leukopenia, thrombocytopenia, or a combination thereof with variable polyarthritis, lymphadenopathy, polymyositis, glomerulonephritis, and retinitis. Affected Doberman pinschers and other affected dogs usually recover after discontinuation of the drug.

Phenylbutazone Toxicity • Hematologic dyscrasias associated with phenylbutazone toxicity include severe aplastic anemia; transient neutropenia, with or without thrombocytopenia; thrombocytopenia; PRCA; and hemolytic anemia. Dogs with phenylbutazone-induced aplastic anemia rarely recover.

Ehrlichiosis

Canine monocytic ehrlichiosis is caused by *E. canis* (see Chapter 15 for serologic diagnosis). Acute infection consistently causes thrombocytopenia. Antiplatelet antibodies are consistently present during *E. canis* infection, and some dogs are direct Coombs-positive. Chronic ehrlichiosis can cause nonregenerative anemia, thrombocytopenia, and leukopenia. Infected dogs become persistently infected, and some dogs develop a severe chronic phase with pancytopenia in blood and aplastic anemia in bone marrow.

NOTE: *Ehrlichia canis* can cause both acute hematologic alterations and a severe chronic phase infection that results in aplastic anemia. The severe chronic phase does not occur in granulocytic ehrlichiosis.

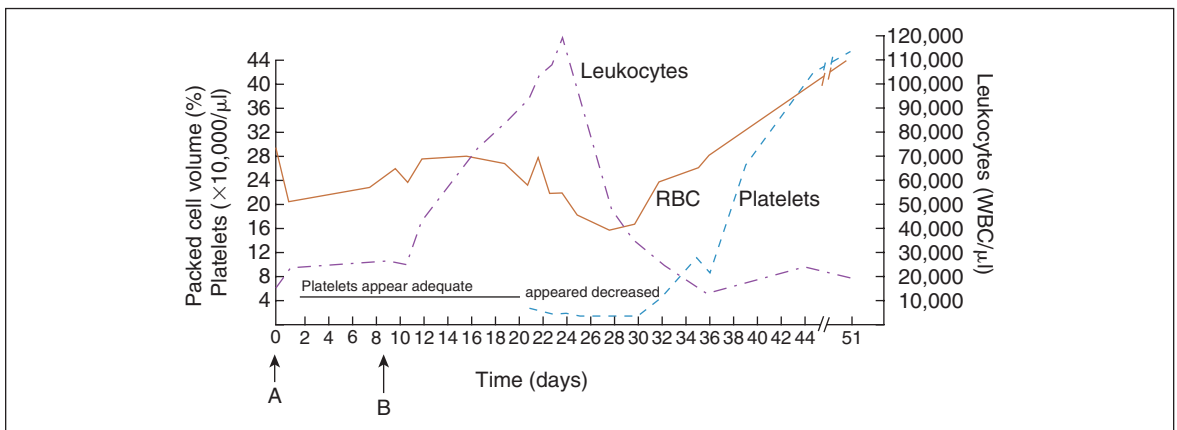


FIGURE 3-13. Estrogen toxicity in a dog. The transient, time-related hematologic changes in a dog with an excessive dose of estradiol included a bicytopenia consisting of thrombocytopenia and nonregenerative anemia and a profound leukocytosis. A, A bleeding episode occurred. B, Estradiol was given. RBC, Red blood cell; WBC, white blood cell.

Granulocytic ehrlichiosis is caused by *Ehrlichia ewingii* and *Anaplasma phagocytophila* in the United States. *Anaplasma phagocytophila* is common in Sweden. *Anaplasma* inclusions can be readily detected in neutrophils from infected dogs 4 to 14 days after infection and persist for 4 to 8 days.¹⁴ Hematologic changes are maximal during parasitemia, are transient, and include mild nonregenerative anemia, moderate thrombocytopenia, lymphopenia, neutropenia, and eosinopenia. Serum iron and TIBC decrease, suggesting anemia of inflammatory disease. A severe chronic phase does not occur.

Feline Leukemia Virus

In most (90%) FeLV-infected cats, RBC parameters are altered. FeLV infection is frequently associated with nonregenerative anemia. One pattern suggestive of FeLV infection is macrocytosis (i.e., MCV > 52 fl) without increased reticulocytes.

Feline Immunodeficiency Virus

Cats infected with FIV frequently have nonregenerative anemia. Neutropenia may be concurrent in some patients.⁸ Rarely, hemolytic anemia, possibly caused by the virus, occurs.

Parvovirus

Parvovirus infection in both dogs and cats (feline panleukopenia) destroys bone marrow precursor cells. Infectious panleukopenia causes severe, absolute granulocytopenia and lymphopenia. Anemia is mild and may not be detected, because PCV may not decrease below the reference interval. The mild anemia may be masked by dehydration. Anemia, when present, is usually nonregenerative, but an increase in reticulocytes may occur during the recovery period.

Myelodysplastic Syndromes and Leukemias

Although some types of MDS result in nonregenerative anemias (refractory anemia [RA] and refractory anemia with ringed sideroblasts [RARS]), other types (refractory cytopenias with multilineage dysplasia [RCMD] and refractory anemia with excess blasts [RAEB]) result in bicytopenia or pancytopenia.²⁷ Dysplastic features are seen in two or more cell lines in bone marrow and myeloblasts are increased (i.e., 5% to 20%) in RAEB. Dogs and cats with RAEB tend to have relatively short survival times and have a higher rate of progression to acute myelogenous leukemia than animals with other types of MDS. Acute leukemias are discussed in Chapter 4.

NOTE: Some types of myelodysplastic syndromes (refractory anemia, refractory anemia with ringed sideroblasts) cause nonregenerative anemias while other types (refractory cytopenias with multilineage dysplasia, refractory anemia with excess blasts) cause bicytopenias or pancytopenias.

Macrophage Proliferative Disorders

Macrophage and histiocyte proliferative disorders include a spectrum of disorders in dogs, two of which (malignant histiocytosis, hemophagocytic syndrome) cause significant hematologic alterations.²⁵ Malignant histiocytosis (disseminated histiocytic sarcoma) is an aggressive malignant proliferation of atypical cells in a variety of tissues that is associated with pancytopenia. Hemophagocytic syndrome is a benign condition characterized by proliferation of hemophagocytic macrophages, which results in cytopenias in the blood. Hemophagocytic syndromes can be idiopathic or occur secondary to IMHA, immune-mediated thrombocytopenia, infectious diseases, MDS, or neoplasia.

BLOOD TRANSFUSION AND BLOOD TYPING

Blood transfusion may be a life-saving treatment, but it is best avoided unless the anemia is severe or causing clinical signs. Blood contains many cell types and proteins that are highly immunogenic and may sensitize the patient to reactions to later transfusions. Indications for transfusion include when the PCV is less than 11% in cats and less than 13% in dogs and the presence of clinical signs including tachycardia, poor-quality pulse, lethargy, and weakness. In general, clinical signs are more important than is PCV in determining the need for transfusion. Donors' blood should be crossmatched to find the blood least likely to cause transfusion reactions. Acute transfusion reactions include fever, hypersensitivity reactions, shock, hemolysis, and disseminated intravascular coagulation. Chronic transfusion reactions include decreased life span of transfused cells or acquired immune reactions to the recipient's own cells, such as post-transfusion purpura. In post-transfusion purpura, repeated transfusions can lead to a sudden destruction of the recipient's own platelets.

Ideally, the blood types of the patient and donor animal should be determined. Alternatively, the clinician should use donor dogs that are negative for dog erythrocyte antigens (DEAs) 1.1, 1.2, and 7 (the RBC antigens most likely to cause transfusion reactions). Crossmatches should always be performed before transfusion, because at least eight blood groups have been described for dogs. Three feline blood groups have been described and designated A, B, and AB. As little as 5 ml of type-A blood transfused into a type-B cat can cause a fatal transfusion reaction. The incidence of type B is low in the United States, but some purebred cats (e.g., Abyssinian, Himalayan, British shorthair) have a higher incidence of type B. In-office kits (RapidVet-H; DMS Laboratories Inc., Flemington, NJ) to identify DEA 1.1 and feline A, B, and AB are available (see Figure 2-12).

POLYCYTHEMIA

Polycythemia is an increase in PCV, hemoglobin, or RBC count. Polycythemia can result from absolute polycythemia (i.e., increased RBC mass associated with increased

bone marrow production of RBCs) or relative polycythemia (i.e., increased PCV because of decreased plasma volume or splenic contraction). Neurologic signs, such as seizures and collapse, may occur when the PCV exceeds 70% (probably because of hyperviscosity of blood causing poor perfusion of the brain).

NOTE: Polycythemia can result from absolute polycythemia (i.e., increased RBC mass) or relative polycythemia (i.e., decreased plasma volume or splenic contraction).

Relative Polycythemia

Relative polycythemia results from decreased vascular fluid (i.e., dehydration, hemoconcentration, hypovolemia). Another cause is splenic contraction, which releases a concentrated bolus of RBCs from the spleen into the peripheral blood. In relative polycythemia, caused by hemoconcentration or dehydration, the PCV should return to normal after fluid replacement (Figure 3-14). Hemoconcentration is often accompanied by an increase in PP concentration, clinical syndromes that cause fluid loss (e.g., diarrhea, vomiting), and clinical evidence of hypovolemia. The PCV and PP concentration are

clinically useful indicators of hemoconcentration but are insensitive indicators of dehydration because of wide reference intervals. Changes in PCV and PP concentration from the mean reference intervals can show a trend toward hemoconcentration.

Relative polycythemia of splenic contraction may be more difficult to recognize, because one cannot consistently predict the degree of splenic contraction or relaxation. Splenic contraction is expected after exercise, excitement, or fear. The PCV should return to normal within 1 hour after the animal calms down.

Absolute Polycythemia

Absolute polycythemia is subdivided into primary and secondary conditions. The reader should not confuse secondary polycythemia with relative polycythemia discussed above.

Primary Absolute Polycythemia

Primary absolute polycythemia is called polycythemia vera or primary erythrocytosis, which is an uncommon chronic myeloproliferative disorder. The disorder is caused by uncontrolled proliferation of a defective clone of erythroid cells. Primary absolute polycythemia is usually diagnosed by excluding other causes of

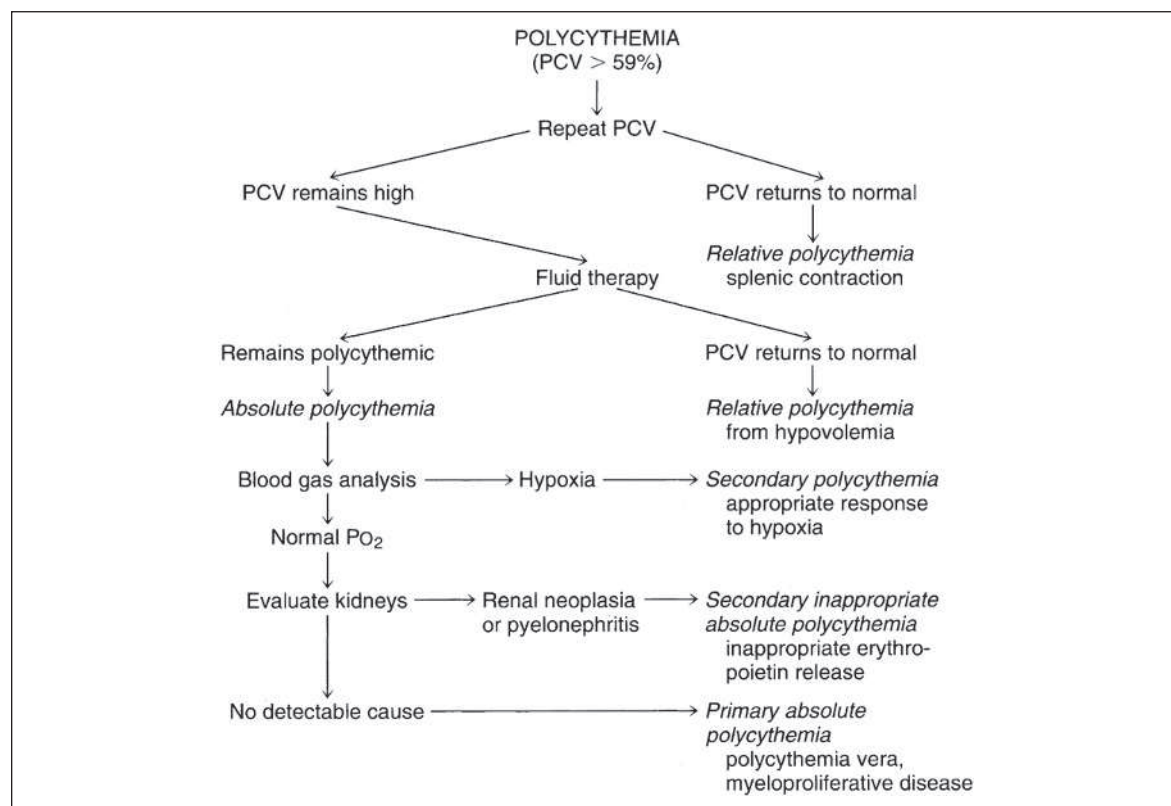


FIGURE 3-14. Diagnostic approach to polycythemia. The italicized common conclusions are made using the various procedures listed. Primary absolute polycythemia is diagnosed by exclusion of causes listed above it (i.e., when splenic contraction is unlikely, the hydration status is normal; no hypoxia is found from pulmonary, cardiac, or hemoglobin disorders; and the kidneys are normal). PCV, Packed cell volume; PO₂, partial pressure of oxygen.

polycythemia. The PCV usually remains at 70% to 80% despite fluid therapy. Serum erythropoietin concentration is not increased. Primary polycythemia is complicated by hyperviscosity that becomes increasingly more severe as the PCV exceeds 60%.

Secondary Absolute Polycythemia

Secondary polycythemia is divided into appropriate and inappropriate forms. Appropriate secondary polycythemia is caused by increased production of erythropoietin as the result of hypoxia (e.g., pulmonary or cardiac disease, living at high altitude). Arterial blood gas analysis documents hypoxemia (i.e., low partial pressure of oxygen). Serum erythropoietin concentrations should be increased. Additional evidence of secondary absolute polycythemia may include documentation of erythroid hyperplasia in bone marrow samples or slight polychromasia in blood samples despite polycythemia.

Inappropriate secondary polycythemia may result from renal diseases in which excessive erythropoietin secretion occurs. Polycythemia occurs in dogs with renal cell carcinoma, renal lymphoma, and chronic pyelonephritis. These cases have had PCVs that ranged from 64% to 81%. Serum erythropoietin concentration is usually increased. However, considerable overlap in serum erythropoietin activity has been found between normal dogs and dogs with primary and secondary polycythemia. In cases of secondary polycythemia caused by renal neoplasia, removal of the diseased kidney results in return of the PCV to normal.

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Leukocyte Disorders

Harold Tvedten and Rose E. Raskin

BASIC LEUKOCYTE CONCEPTS

Leukogram

Leukocyte responses in the patient are evaluated by the leukogram. The leukogram is the leukocyte portion of the complete blood count (CBC) and includes the total leukocyte (white blood cell [WBC]) count, differential leukocyte count (Diff), and description of WBC morphology. The relative leukocyte differential count (relative Diff) is the percentages of various leukocyte types (i.e., segmented neutrophils [segs], nonsegmented neutrophils [nonsegs], lymphocytes, monocytes, eosinophils, basophils). The absolute differential leukocyte count (absolute Diff) is the number of each type of leukocyte per volume (microliter or liter) of blood. Examination of leukocyte morphology on the stained blood smear is used to determine a relative Diff and detect various abnormalities. Selected hematologic techniques are described in Chapter 2. This chapter is intended to give an overview of understanding the leukocyte response and to answer common questions in diagnosis. Supplemental information regarding leukogram interpretation may be found in other textbooks.^{18,20,25,35,46}

Leukocytes are inflammatory cells, and changes in the leukogram are mainly used to identify the presence of inflammatory disease and characterize inflammation as to severity and type. The leukogram is not highly sensitive in detecting mild, focal, or chronic inflammation; therefore, a normal WBC count and Diff does not exclude inflammatory disease from the diagnosis. Acute phase protein concentrations, such as C-reactive protein in dogs and serum amyloid A in cats, are much more sensitive tests to document the presence of inflammation. The manual Diff is imprecise; therefore, the clinician should use prominent changes in the number of types of WBCs for diagnosis.

The leukogram usually does not confirm infection, but some patterns, such as a very severe left shift together with very toxic neutrophils, strongly suggest severe infection. Infection is more consistently confirmed by finding the organism and associated inflammatory reaction with cytology (Chapter 16) and culture of tissues that look abnormal on ultrasound, radiographs, or physical exam. Similarly, the leukogram often does not confirm

hematologic neoplasia such as malignant lymphoma, while cytology, histopathology, and histochemical and immunologic evaluation of hematopoietic tissues that appear abnormal on ultrasound, radiographs, or physical exam more consistently give a correct diagnosis (see later discussion of hematopoietic neoplasia under Leukemia).

Automated Versus Manual Differential Leukocyte Counts

The automated total leukocyte count (WBC) from most veterinary hematology instruments is accurate and precise. The automated differential leukocyte count (AutoDiff) of human hematology instruments has to a great extent replaced the manual Diff (ManDiff) in human medicine because this eliminates the cost of hiring a well-trained person to evaluate the smears and because of the greater precision with the AutoDiff. The AutoDiff has several uses in the dog and cat, but these uses vary with the instrument and the patient's problem. Current veterinary instruments fail to identify all types of canine and feline leukocytes. No automated instrument enumerates the number of bands and younger neutrophils, and most instruments fail to identify nonsegs or basophils. Many instruments detect the total neutrophil and lymphocyte counts, and often eosinophil counts, well. A proper indication for use of an AutoDiff is to monitor treatment response of these types of leukocytes over days. This takes advantage of the instrument's greater precision and cost savings in labor time.

Some instruments have sporadic errors in detection of eosinophils and monocytes. Some instruments offer only a three-cell Diff, which actually only indicates the number of total neutrophils and lymphocytes. Since a major use of the CBC and Diff is to document the presence and severity of inflammatory disease, it is necessary to use a manual Diff to document a left shift and toxic change in neutrophils. A ManDiff is often required to validate a monocytosis, eosinophilia, or basophilia recorded by an AutoDiff. The combination of precise data from hematology instruments and subjective observations from the blood smear give the greatest chance to make a proper diagnosis and prognosis during initial evaluation of an ill patient. Then, based on the

instrument being used and its ability to correctly measure the hematologic abnormality documented by a full CBC, including WBC, AutoDiff, and manual Diff, the clinician may subsequently monitor that change over time and treatment using only automated results.

NOTE: The combination of precise data from hematology instruments and subjective but correct observations from the blood smear give the greatest chance to make a proper diagnosis and prognosis during initial evaluation of an ill patient.

Absolute Versus Relative Differential Leukocyte Counts

Use of absolute WBC numbers allows more consistent evaluation of leukogram responses than use of relative percentages (Figure 4-1). For example, a WBC count of 10,000 leukocytes/ μl with 65% segs has 6500 segs/ μl . The 6500 segs/ μl is normal, but 65% segs are not always normal. A WBC count of 1000 leukocytes/ μl with 65%

segs indicates severe neutropenia (i.e., 650 segs/ μl). A WBC count of 50,000 leukocytes/ μl with 65% segs indicates neutrophilia (i.e., 32,500 segs/ μl).

Interpretation of the leukogram should begin with interpretation of the absolute cell count for each type of leukocyte. Then the changes are summarized by appropriate hematologic terms indicating an increase or decrease in a given leukocyte type. For example, “mild to moderate leukocytosis with mature neutrophilia, lymphopenia, and monocytosis” indicates that there was an increased total WBC count, an increase in mature segmented neutrophils but no increase in band neutrophils, a decrease in lymphocytes, and an increased numbers of monocytes, respectively. This particular description is typical for a recent glucocorticoid treatment or stress-type response.

NOTE: Absolute cell numbers rather than percentages should be used to evaluate the leukogram.

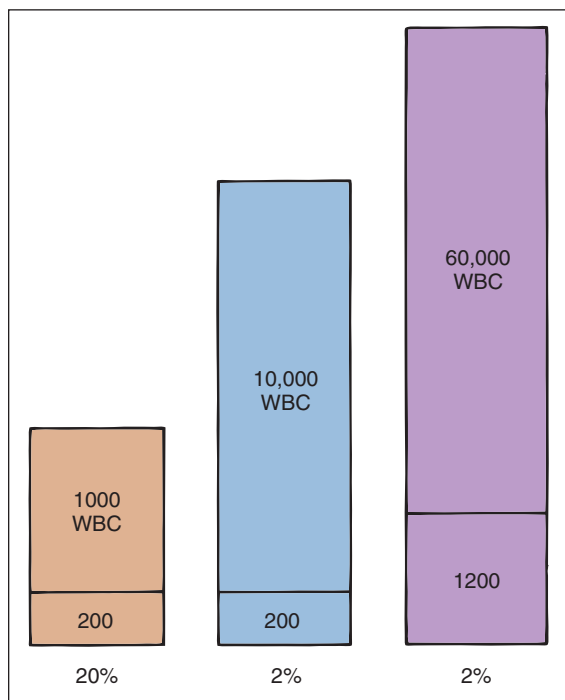


FIGURE 4-1 Relative and absolute leukocyte counts. The bottom chamber of each bar indicates the absolute number of band neutrophils, and the percentage below the bars indicates the relative percentage of band neutrophils. The relative change between the first and second bar (i.e., 20% to 2%) seems great; however, no change exists in the absolute number of band neutrophils in the blood (i.e., 200 bands/ μl of blood). The relative percentage of band neutrophils between the second and third bars seems identical. However, a normal number of band neutrophils exists in the dog with 200 bands/ μl of blood, but a true increase (left shift) in band neutrophils occurs in the dog with 1200 bands/ μl in the third bar. WBC, White blood cell.

Leukocyte Production, Circulation, and Emigration

To interpret the concentration of leukocytes in the blood, one must consider the rate of leukocyte production in bone marrow and release into blood, the distribution and circulating half-life of leukocytes in the vascular system, and the rate of emigration of leukocytes from blood into tissues. Understanding of normal hematopoiesis is also needed to understand classification of the leukemias (neoplasia of hematopoietic cells, discussed later).

Leukocyte Production

Granulocytes (i.e., neutrophils, eosinophils, basophils) and monocytes are produced in the bone marrow. Although the bone marrow produces some lymphocytes, most lymphocytes are produced by the peripheral lymphoid tissues (i.e., thymus, lymph nodes, spleen, tonsils, bronchial-associated lymphoid tissue, gut-associated lymphoid tissue). Leukocytes develop in the bone marrow from pluripotent and committed stem cells influenced by interleukins and colony-stimulating factors (Figure 4-2).²⁶

Neutrophils compose the majority of leukocytes in blood, and usually leukocytosis is caused by neutrophilia. Kinetics of neutrophils has been well studied and is best understood. Thus the initial discussion here focuses on neutrophils. Cellular “pools” are used to conceptualize and describe the “location” of neutrophils within the bone marrow and blood and to simplify interpretation of bone marrow and CBC data (Figure 4-3). Bone marrow is divided into two pools. The first, the mitotic pool of myeloblasts, promyelocytes, and myelocytes, provides a steady supply of neutrophils to meet tissue demand for these cells. The second pool is maturation and storage, which consists of metamyelocytes, bands, and segs that lack mitotic ability. Precursor cells undergo progressive maturation and provide a reserve pool of segs to meet sudden increased tissue demands for neutrophils until the mitotic pool increases neutrophil production. It is uncommon in the dog and cat to have leukopenia during

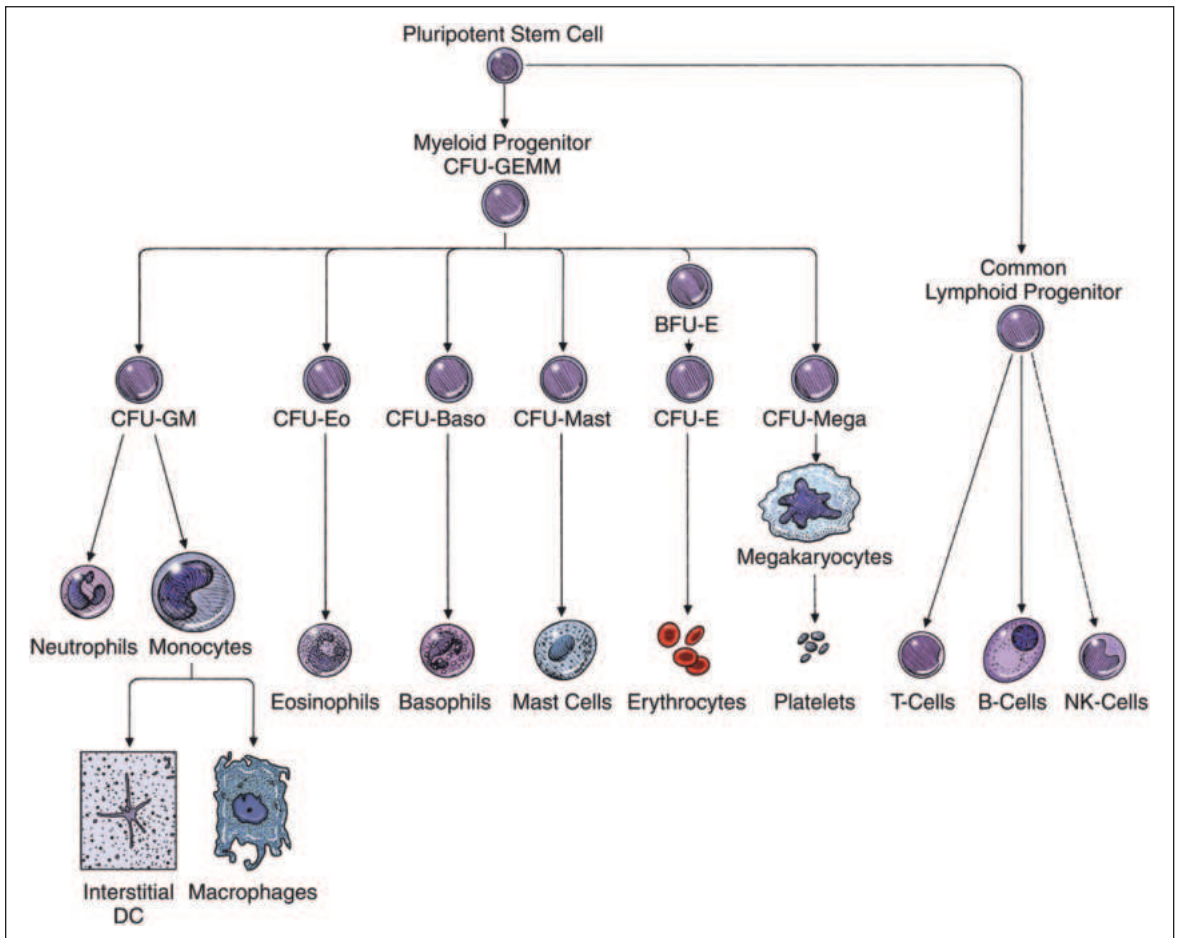


FIGURE 4-2 Differentiation of the bone marrow pluripotent stem cells. Baso, Basophil; BFU, burst-forming unit; CFU, colony-forming unit; DC, dendritic cell; E, erythroid; Eo, eosinophil; GEMM, granulocyte-erythroid-monocyte-megakaryocyte; GM, granulocyte-macrophage; Mega, megakaryocyte; NK, natural killer.

inflammatory diseases because this large storage pool is available for rapid release of neutrophils. The maturation and storage pools are combined in Figure 4-3. The most mature stages of neutrophil are preferentially released from the bone marrow into the blood first (segs, bands, metamyelocytes, myelocytes, and finally promyelocytes,

in that order). As bone marrow stores of segs are depleted, nonsegs (e.g., bands, metamyelocytes, younger neutrophils) are released into the blood, and a left shift occurs: A left shift is a specific indicator of inflammation, and the severity of the left shift reflects the severity of inflammation.

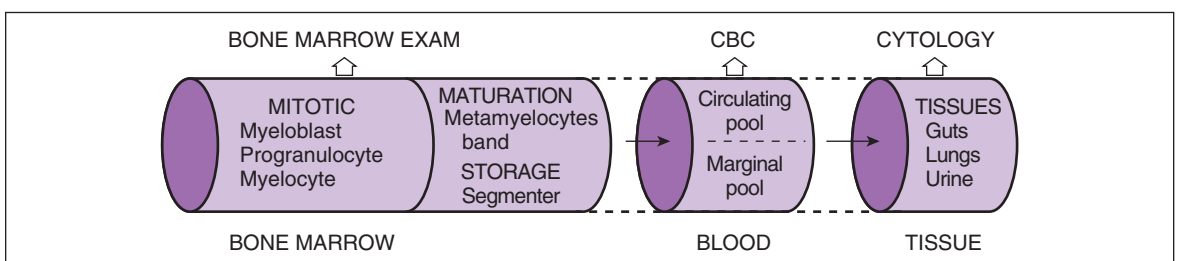


FIGURE 4-3 Neutrophil compartments in the body. Neutrophils in various areas of the body are grouped into pools for evaluation. The bone marrow cells are divided into the mitotic, maturation, and storage pools (see text). Neutrophils in blood are either in the circulating pool, which is sampled by a complete blood count (CBC), or the marginal pool, which is hidden from sampling via the CBC. Neutrophils move in one direction into the tissues, where they can be evaluated by cytology or histopathology. (Modified from Boggs DR, Winkelstein A: *White cell manual*, ed 3, Philadelphia, 1975, FA Davis.)

The maturation and storage pool constitutes 80% of the myeloid cell population, whereas the mitotic pool usually accounts for 20% of the myeloid series. In contrast with neutrophils, promonocytes and monocytes are released into blood at a relatively young age. This lack of monocyte maturation and storage in the bone marrow explains why monocytes are observed infrequently in most bone marrow aspirates, unless severe neutropenia is present.

Leukocyte numbers and morphology within the bone marrow can be evaluated by bone marrow aspiration for cytology and core biopsy for histopathology. Aspirate smears of marrow allow qualitative and quantitative observations regarding cell morphology and maturation. Core biopsy provides the best estimation of bone marrow cellularity and detects stromal reactions (e.g., myelofibrosis, granulomatous osteomyelitis). Chapter 2 discusses the interpretation of a bone marrow examination.

NOTE: The production of segs from bone marrow myeloblasts takes approximately 6 days in the dog and cat, but rapid release of neutrophils from the normally large storage pool can produce a neutrophilia within minutes to hours. Leukopenia is therefore uncommon in dogs and cats during even early stages of inflammatory disease. This is unlike the delay in release of reticulocytes, which peak in numbers in peripheral blood 4 to 6 days after onset of a regenerative anemia.

Neutrophil Circulation

When neutrophils are released into the blood, about half hesitatively stick and roll along the endothelial cells (i.e., in the marginal pool) and are not in the central axial flow of blood within vessels from which blood is taken during a venipuncture (i.e., in the circulating pool). Those neutrophils that are in the central axial flow of blood within vessels and are taken into a blood sample (and counted in the WBC count) are said to be in the circulating pool. The marginal neutrophil pool is a “hidden” population associated with the endothelial lining of capillaries, especially the lungs and spleen. The circulating and marginal cell pools make up the total blood neutrophil pool (TBNP). Neutrophils distribute between circulating and marginal cell pools, circulate for a brief period of time (half-life of 7.4 hours), and emigrate from blood vessels into tissues. Shifts between these pools can affect the WBC count, especially in cats. In dogs, circulating and marginal pools are about equal. In cats, the marginal pool is two to three times the size of the circulating pool (Table 4-1). Therefore, if neutrophils are mobilized from the marginal pool to the circulating pool in response to fear, excitement, or strenuous exercise, the neutrophil count can potentially double in dogs and triple in cats. This effect is called *physiologic leukocytosis* and is seen mainly in young healthy cats.

Neutrophil Emigration into Tissues

Neutrophils normally spend about 10 hours in the vascular system before emigrating from the blood vessels into the tissues. Emigration is a random (i.e., non-age ordered)

TABLE 4-1. TOTAL BLOOD NEUTROPHIL POOL, CIRCULATING NEUTROPHIL POOL, AND MARGINAL NEUTROPHIL POOL IN DOGS AND CATS

	DOG	CAT
TBNP × 10 ⁸ /kg	10.2	28.9
CNP × 10 ⁸ /kg	5.4	7.8
MNP × 10 ⁸ /kg	4.8	21.0

Total blood neutrophil pool (TBNP) in cats is larger than in dogs because of a very large marginal neutrophil pool. The relatively large feline marginal neutrophil pool (MNP), compared with that of the dog, allows a larger potential shift of neutrophils into the circulating neutrophil pool (CNP) with more dramatic leukocytosis during fear, excitement, or strenuous exercise.

and unidirectional (these cells do not return to the circulation) event. In health, neutrophils primarily migrate into the respiratory, digestive, and urinary tracts at a low rate in response to bacteria and other stimuli. Neutrophils lyse quickly in the septic environment of the lumen of the bowel. In inflammation, excessive tissue neutrophils may be visible as exudate or pus (see Chapter 16). In diseases such as enteritis, tissue neutrophils may be hidden from cytologic or gross observation; however, increased tissue demand for neutrophils usually is reflected in the leukogram.

NOTE: The CBC allows quantitative and morphologic observations about leukocytes in peripheral blood. The leukogram represents the balance of leukocyte production in bone marrow, distribution in the vascular system, and emigration from blood vessels into tissues. Cytologic examination of abnormal tissues, organs, or fluids can document where and what type of inflammation or infection exists.

LEUKOCYTOSIS AND NEUTROPHILIA

Leukocytosis is usually synonymous with neutrophilia. For example, among 232 CBCs with a leukocytosis of greater than 17,000 WBCs/μl, 226 (97%) had neutrophilia.

Differential Diagnosis of Neutrophilic Leukocytosis

The three most common causes of neutrophilia and leukocytosis are (1) inflammation, (2) stress and corticosteroids, and (3) exercise and epinephrine. Leukemia is relatively uncommon (discussed later) but may cause massive to no increase in the WBC count. Inflammation is most specifically identified by the presence of a left shift, or an absolute increase in nonsegs (Figure 4-4; see later discussion of left shift and hyposegmentation). Inflammation is also suggested by leukocytosis greater than that usually expected with corticosteroid- or

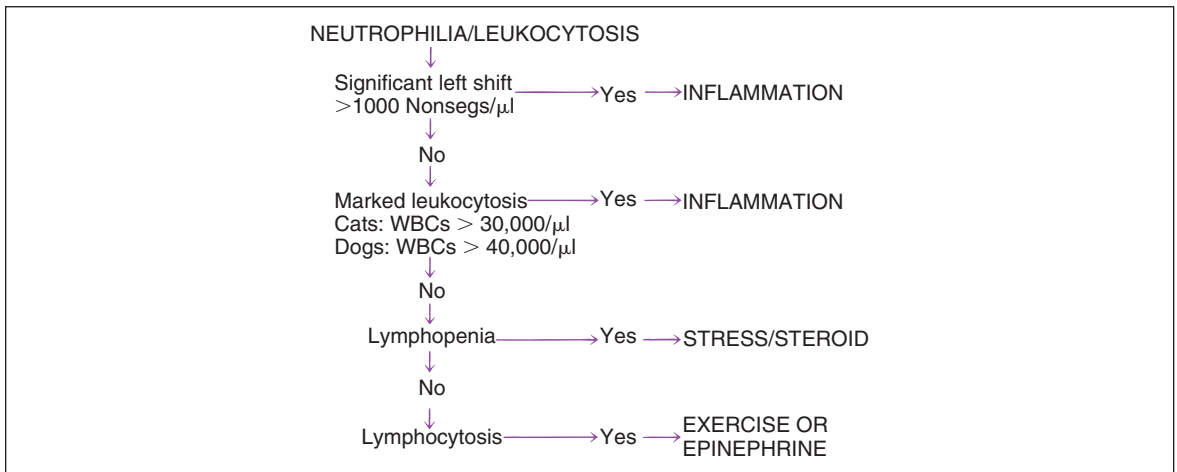


FIGURE 4-4 Evaluation of leukocytosis and neutrophilia. The common causes of neutrophilia and subsequent leukocytosis usually may be differentiated based on the immaturity of the neutrophils, the magnitude of the neutrophilia, and the tendency of the lymphocytes to increase or decrease. When the laboratory finding is present (Yes), the conclusion to the right is made. When the laboratory finding is absent (No), one moves down to the next differentiating feature. The values given are guidelines for more certain diagnosis, and lesser values may still be interpreted to give certain conclusions. For example, dogs with inflammation often have fewer than 40,000 white blood cells (WBCs)/ μl and fewer than 1000 nonsegs/ μl . Cases with mild neutrophilia may lack criteria to make a definite diagnosis. Granulocytic leukemia is rare and not considered here.

epinephrine-associated changes. When mild neutrophilia is present without a left shift, the specific cause of leukocytosis may be unclear. In such instances, the absolute lymphocyte count may be useful. Lymphopenia is commonly associated with an endogenous stress-associated or exogenous glucocorticoid treatment effect. Transient lymphocytosis may be caused by epinephrine-induced splenic contraction or an exercise-associated effect. Persistent lymphocytosis is often caused by chronic immune stimulus. Concurrent processes occur often. For example, inflammation usually causes stress-related lymphopenia. Corticosteroid treatment is very common.

NOTE: Leukocytosis and neutrophilia are not only caused by inflammation. Leukocytosis may be a response to glucocorticoid treatment or may result from acute stress, an epinephrine-type response to fear or exercise, and occasionally leukemia.

Inflammation

Inflammation is a common and important laboratory diagnosis. Inflammation usually causes neutrophilia and is the major rule out for neutrophilic leukocytosis. Neutrophils predominate in tissues during acute phases of many inflammatory diseases (e.g., peritonitis, arthritis). Proliferation of macrophages and lymphocytes occurs in more subacute to chronic inflammation (especially within tissues other than blood). But many chronic inflammatory diseases (suppurative or exudative diseases) may still have primarily neutrophils. Other inflammatory cells, such as monocytes-macrophages,

eosinophils, lymphocytes, plasma cells, and even basophils, increase in blood and other tissues in various types of inflammatory disease (see later discussions). Neutrophils are specialists at killing bacteria, and bacterial infection (i.e., sepsis) commonly causes neutrophilic types of inflammation (e.g., exudative, purulent, suppurative, abscess) as well as pyogranulomatous inflammation. However, neutrophilia occurs with other infections such as certain mycotic, protozoal, and viral infections (e.g., feline infectious peritonitis). Inflammation also may occur from nonseptic processes such as necrosis (e.g., pancreatitis, pansteatitis, immune-mediated hemolytic anemia, [IMHA]), chemical exposure (e.g., turpentine is an experimental method of abscess formation), immune-mediated diseases (e.g., systemic lupus erythematosus, IMHA), and toxins (e.g., endotoxin, snake bite). Neoplasms may cause inflammation in several ways, such as causing ulceration, causing necrosis in normal tissues, outgrowing or damaging the blood supply with subsequent necrosis in the tumor, predisposing the patient to infection, or producing a paraneoplastic effect wherein tumor products stimulate the bone marrow to produce neutrophils or eosinophils.

NOTE: Neutrophilia with a left shift greater than 1000 nonsegs/ μl ($1.0 \text{ nonsegs} \times 10^9/\text{L}$) specifically indicates inflammatory disease.

Left Shift

A left shift (increased absolute numbers of immature neutrophils) indicates inflammatory disease in the patient (see Figure 4-4). Identification of immature neutrophils

requires blood smear evaluation. Immature neutrophils such as bands, metamyelocytes, myelocytes, and younger neutrophils are termed *nonsegmented neutrophils (nonsegs)* in general. Identification of nonsegs is subjective and varies greatly among microscopists. Most experienced microscopists, who examine blood smears daily, clearly recognize increased immaturity and toxic changes in neutrophils during inflammation. However, the number of bands, metamyelocytes, and myelocytes reported will vary among observers. Exact numbers of these cells are often impossible to determine when severe toxic change is present, in which case a subjective description should be used; for example, "The blood had a severe degenerative left shift with severe toxic change in neutrophils, though exact numbers of each cell type could not be determined." may be the most honest description. Even in nontoxic reactions the division between segs and nonsegs may be difficult, and the term *hyposegmentation* is then used to indicate that the neutrophils looked more immature than normal though the number of reported nonsegs was not increased.

Because of the effect of the observer's impression in classification of nonsegs, one should use a definite increase in nonsegs for a certain conclusion of inflammation. A finding of greater than 1000 nonsegs/ μl is a reasonable threshold for a left shift. (Note that 1000 nonsegs/ μl equals $1.0 \text{ nonsegs} \times 10^9/\text{L}$.) Small changes from day to day or slight changes from the reference values should be interpreted with caution because manual differential leukocyte counts are especially imprecise with nonsegs and cells found in low numbers, such as basophils. Mild left shifts (i.e., 300 to 1000 nonsegs/ μl) occur in hemorrhagic, chronic, or granulomatous diseases. No left shift may be noted in many patients with inflammatory disease, thus the absence of a left shift or a very mild left shift does not exclude inflammation from the diagnosis.

The absolute number of nonsegs and their state of immaturity indicate the severity of the left shift. Immature neutrophils (nonsegs) observed in blood include bands (stabs), metamyelocytes (juveniles), myelocytes, and promyelocytes. Bands usually constitute most of the left shift because the more mature neutrophils are released from bone marrow first. Neutrophils younger than bands indicate an increasingly severe left shift associated with increasingly intense inflammation. If several myelocytes and metamyelocytes are found, the number of metamyelocytes, myelocytes, and promyelocytes should be reported individually (not simply grouped as nonsegs) to indicate severity of the left shift. Detectable numbers of blast cells (i.e., myeloblasts) or irregular maturation patterns may suggest granulocytic leukemia. More nonsegs than segs indicates a degenerative left shift and a poor prognosis.

NOTE: The severity of a left shift is reflected by the absolute number of nonsegs and individual numbers of the more immature stages (e.g., metamyelocytes and myelocytes) present. Increasing severity of the left shift indicates increasing severity of the inflammatory disease.

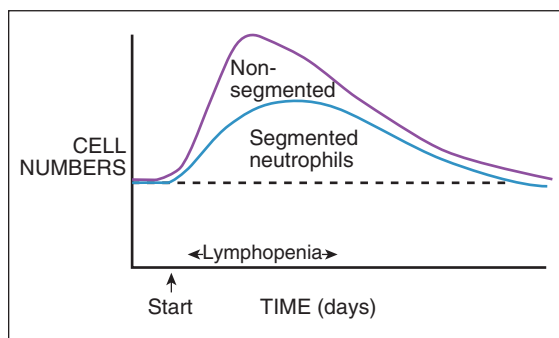


FIGURE 4-5 Expected leukocyte changes with resolving inflammation. The greatest leukocytosis and left shift are expected early in acute inflammation. This period is also accompanied by the lymphopenia of stress. During later phases of inflammation, a more mature form of neutrophilia is expected, because bone marrow hyperplasia and marrow production of neutrophils should be adequate to allow maturation of neutrophils before release into blood. Tissue demand for neutrophils also tends to decrease during recovery.

Leukogram Changes in Inflammation

Figure 4-5 shows a likely pattern of leukocyte changes through a typical inflammatory response, with a distinct onset of inflammation and then progressing uniformly to resolution. Changes in individual patients may vary from this typical pattern due to treatment, rupture of an abscess, or the like. The greatest left shift is expected in early stages of the disease process, because as the preexisting bone marrow storage pool is depleted of segs, then more bands and metamyelocytes are released to meet early intense demand. With time, myeloid hyperplasia within the bone marrow expands neutrophil production. When neutrophil production and maturation time are sufficient, mainly mature segs are released into the blood and the severity of the left shift should diminish or disappear. If tissue inflammation stabilizes at a persistent low to moderate level, the bone marrow should reach a production rate sufficient for most neutrophils to mature before release. Thus chronic inflammation may be characterized by little to no left shift and minimal to no leukocytosis. Therefore, chronic or mild inflammation may be difficult to document by leukogram data alone. Acute phase proteins, such as C-reactive protein or serum amyloid A, are sensitive indicators of inflammation and may be used to complement the leukogram. Increased rouleaux (see Chapter 2) in canine blood smears or fever in the patient suggests inflammation. Increased rouleaux formation usually is associated with production of acute phase proteins such as fibrinogen. A normal leukogram appearance does not exclude inflammatory diseases, especially if inflammation is mild or chronic, or only involves a surface (e.g., cystitis).

NOTE: Chronic inflammation may have mild (slight, mature neutrophilia) or no changes in the leukogram because, with time, increased bone marrow production of neutrophils matches tissue consumption.

Bone Marrow Response During Inflammation

Myeloid hyperplasia of the bone marrow is expected with inflammation of greater than 2 to 3 days' duration. Bone marrow sampling is seldom indicated in patients with inflammatory diseases because a leukocytosis and left shift are usually present, and these indicate the marrow is active. Persistent, moderate to severe leukopenia in a dog or cat with inflammatory disease is unexpected and thus may be an indication to examine the bone marrow for the cause (see Chapter 2).

Prognosis

Obviously factors other than neutrophil counts affect prognosis, such as the cause of the disease and site of inflammation. However, the neutrophil counts reflect the current balance of effective bone marrow production and tissue demand. If the bone marrow is responding typically to an inflammatory process with a mild to moderate neutrophilia and mild to moderate left shift, the prognosis is relatively good. Leukocytosis in dogs is usually less than 40,000 WBCs/ μ l. In 182 canine CBC exams with leukocytosis, 151 (83%) had 17,500 to 39,990 WBCs/ μ l. Leukocytosis in this range is thus mild to moderate and suggests a favorable prognosis. Only 5% had marked to extreme leukocytosis of 61,050 to 127,500 cells/ μ l. Leukemoid reactions of over 50,000 to 60,000 WBCs indicate a poor prognosis because, even in the presence of excessive numbers of leukocytes (usually neutrophils), the cause of the inflammatory response is not corrected. The magnitude of feline leukocytosis is usually less than that in dogs (i.e., 70% of cases are < 30,000 WBCs/ μ l). The severity of eosinophilia in eosinophilic inflammation is less than neutrophilia during neutrophil inflammation. Therefore, a leukemoid reaction of eosinophils (due to strong eosinophil inflammation, not leukemia or hypereosinophilic syndrome) is diagnosed when there are more than 25,000 to 30,000 eosinophils/ μ l.

Leukocyte count criteria, suggesting a poor prognosis, are summarized in Table 4-2 (see also later discussion of

Toxic Neutrophils). A degenerative left shift, leukopenia, neutropenia, lymphopenia, leukemoid reaction, or a combination thereof is an atypical, unexpected response to inflammatory disease indicating severe disease, toxemia, severe stress, inadequate bone marrow production, problems interfering with an effective response, or a combination of these. A degenerative left shift has more nonsegs than segs, regardless of total leukocyte count. Finding more immature than mature neutrophils indicates that the bone marrow cannot produce neutrophils at a rate sufficient for them to mature completely prior to release. Either tissue demand for neutrophils has escalated dramatically, cell production is decreased, or both. Severity of change and trend over daily CBCs are important in assessing prognosis. A degenerative left shift, severe neutropenia and leukopenia, lymphopenia, and marked toxic change in most neutrophils often suggest gram-negative sepsis, such as a ruptured intestine or parvovirus enteritis.

Both leukopenia and neutropenia are unfavorable prognostic signs. These findings suggest that bone marrow is incapable of producing sufficient numbers of neutrophils, that tissue consumption of neutrophils is overwhelming, or both. Neutropenia, whether primary (bone marrow disease) or secondary (excessive tissue consumption), severely predisposes the patient to infection and septicemia. Leukopenia is usually caused by neutropenia, but lymphopenia may also cause leukopenia despite normal neutrophil numbers. Lymphopenia usually indicates stress. Severe or persistent lymphopenia indicates severe or persistent stress. In severe leukopenia (e.g., <1000 WBCs/ μ l), a left shift is not concluded even if all neutrophils are nonsegs, because an increase in the absolute number of immature neutrophils greater than 1000 WBCs/ μ l is not possible. (Trying to perform a manual differential WBC count is also not necessary because the total WBCs already indicates a neutropenia and lymphopenia, and imprecision of a 25 to 50 ManDiff count with abnormally appearing cells is very great. Automated instrument differential counts are more accurate in severe leukopenia.)

A leukemoid reaction is a marked leukocytosis (>50,000 to 100,000 WBCs/ μ l) due to inflammatory disease and not leukemia. *Leukemoid* means leukemia-like because of the magnitude of leukocytosis. A leukemoid reaction indicates a poor prognosis because, despite abundant (and actually excessive numbers of) neutrophils, the inflammatory disease is not being corrected. Causes of leukemoid reactions are often severe localized infections (e.g., pyometra, abscess). Additional causes are IMHA, paraneoplastic syndromes with bone marrow stimulation (e.g., metastatic fibrosarcoma, renal carcinoma, rectal adenoma), rare parasitism (e.g., *Hepatozoon canis* infection), and neutrophil functional defects (canine leukocyte adhesion protein deficiency [CLAD] of Irish setters).³⁹ With pyometra and some walled-off abscesses, there is an anatomic problem preventing healing. Pus and the infectious agent or other cause cannot drain out of the body, and antibiotics may not penetrate the lesion. With CLAD, dysfunctional neutrophils are incapable of correcting common infections even in high numbers. The leukemoid reaction in IMHA seems an exception, but acute massive destruction of erythrocytes and

TABLE 4-2. LEUKOGRAM FINDINGS INDICATING A POOR PROGNOSIS

FINDING	REASON FOR POOR PROGNOSIS
Degenerative left shift	Tissue demand exceeds bone marrow's production of neutrophils or causes inadequate time for maturation of neutrophils
Leukopenia	Tissue demand exceeds bone marrow's production of neutrophils
Leukemoid reaction	Even excessive numbers of neutrophils cannot correct the problem
Toxic neutrophils	Moderate to many, moderately to severely toxic neutrophils are associated with longer hospitalization, higher treatment costs, and increased fatality
Severe or persistent lymphopenia	Indicates severe and persistent stress

phagocytosis of debris by macrophages is a strong stimulus for an inflammatory reaction.

Differentiation of a leukemoid reaction from chronic granulocytic leukemia (CGL) is difficult. CGL is rare, so the odds favor diagnosis of a leukemoid reaction when massive neutrophilia exists. Both leukemoid reactions and CGL lack the blast cells or atypia seen in acute granulocytic leukemia (see Myeloid Neoplasms later in this chapter). Left shifts in both leukemoid reactions and CGL often involve mainly bands, and both can have a few more immature forms. Cytologic examination of lymphadenopathy is often a key in diagnosis of CGL. Lymph node aspirates (and even liver or spleen aspirates) with CGL look like bone marrow in having mixed hematopoiesis with a few to moderate number of immature myeloid cells, including myeloblasts. The number of blast cells is not obviously increased, so bone marrow aspirates in CGL are often not diagnostic. The leukocytosis of a leukemoid reaction should resolve after appropriate treatment (e.g., removal of pyometra uterus). Clinical signs of illness, toxic neutrophils, and evidence of diseases that cause leukemoid reactions (e.g., pyometra) suggest diagnosis of a leukemoid reaction. Dogs with CGL have persistent leukocytosis and often do not look sick.

NOTE: A guarded to poor prognosis is indicated by the presence of a degenerative left shift, leukopenia, leukemoid reaction, or moderate to marked neutrophil toxicity.

Toxic Neutrophils

Toxic neutrophils were associated with increased fatality, length of hospitalization, and treatment costs in dogs.³ The prevalence of pyometra, parvovirus infection, acute renal failure, peritonitis, IMHA, disseminated intravascular coagulation (DIC), pancreatitis, septicemia, and neoplastic disorders was significantly higher among dogs with toxic neutrophils. Bacterial infections cause severe toxic changes in neutrophils, such as in secondary bacterial enteritis in parvovirus enteritis. However, toxic neutrophils occur in disease without infection (e.g., IMHA, pancreatitis, chemotherapeutic agents, renal failure). The

presence of toxic neutrophils in cats was associated with a significantly higher prevalence of shock, sepsis, panleukopenia, peritonitis, pneumonia, and upper respiratory tract diseases, as were infectious (viral and bacterial) and metabolic disorders.³⁶ Negative findings in cats with toxic neutrophils were milder than with dogs, suggesting that cats form toxic neutrophils with milder diseases than dogs.

Classification of toxemia may be quite detailed³ or more simplified (see Chapter 2). The number of neutrophils that appeared toxic (percentage; or few, moderate, many) and severity of morphologic change should be reported. A few (1+) toxic neutrophils are of minimal importance. However, moderate to many (2+ to 4+) toxic neutrophils should not be ignored (see Figure 2-9). Toxic change in neutrophils, though subjective, is sometimes the only indicator of disease because numerical results of the leukogram appear normal in many cases. A blood smear evaluation by a competent observer should always be included during initial evaluation of a sick patient.

Stress and Corticosteroid Response

Corticosteroid treatment and stress (endogenous cortisone release) are very common and cause prominent changes in the leukogram (Table 4-3). The classic leukogram pattern from recent corticosteroid treatment or acute stress is moderate leukocytosis with mature neutrophilia, lymphopenia, and eosinopenia. In dogs, mild to moderate monocytosis also may occur (e.g., 2500/ μ l). Leukocytosis from corticosteroid treatment in dogs may reach a maximum of 30,000 to 40,000 cells/ μ l, with a predominance of neutrophils, but more commonly there are 15,000 to 25,000 WBCs/ μ l. Neutrophilia develops in 4 to 12 hours after treatment with a glucocorticoid and returns to baseline values in less than 24 hours. In cats, leukocytosis after corticosteroid treatment is usually a little weaker (e.g., 22,000 WBCs/ μ l with 18,000 neutrophils/ μ l), without monocytosis.

Lymphopenia without neutrophilia is the most common change in the leukogram and usually indicates stress. Chronic stress (e.g., chronic renal failure), long-term corticosteroid treatment, or hyperadrenocorticism is suggested by lymphopenia. The leukogram in chronic stress may seem normal. Stress causes eosinopenia, but normal animals may have few eosinophils so that

TABLE 4-3. LEUKOCYTE CHANGES IN A DOG TREATED WITH DEXAMETHASONE

	FRIDAY	SATURDAY	MONDAY	TUESDAY	WEDNESDAY
WBCs/ μ l	12,200	22,200	19,600	31,100	29,300
Segs/ μ l	9525	18,648	10,976	26,433	25,491
Bands/ μ l	0	0	196	0	0
Lymphocytes/ μ l	1905	1998	5096	1866	879
Monocytes/ μ l	635	1554	2156	2799	3132
Eosinophils/ μ l	635	0	1176	0	0

WBCs, White blood cells.
Hematologic data are from an apparently normal dog treated daily with dexamethasone (except on Sunday) to illustrate the corticosteroid and stress response. Data for Friday, the day before treatment began, should be used for baseline (reference) values. Exceptions to the classic pattern occurred every day except Wednesday.

TABLE 4-4. EFFECTS OF CORTISONE ON CANINE GRANULOCYTES

	GRANULOCYTE COUNT (cells/ μ l)	TBGP ($\times 10^7$ /kg)	T $\frac{1}{2}$ (hours)	GRANULOCYTE TURNOVER RATE ($\times 10^7$ cells/kg/day)
Control	5600 (3.0-8.4 $\times 10^3$)	88 (53-112)	5.3 (3.8-6.3)	301 (157-468)
Treated	13,800 (8.7-30.1 $\times 10^3$)	162 (67-269)	7.6 (6.0-9.2)	352 (136-438)

Values are given as mean (range). Neutrophilia in the cortisone-treated dogs is reflected by an increased granulocyte count, which is approximately the absolute neutrophil count. The total blood granulocyte pool (TBGP) is the total number of granulocytes (mainly neutrophils) in the body and is based on body weight. It illustrates a true increase in the neutrophils in the blood of dogs treated with glucocorticoids. The circulating half-life (T $\frac{1}{2}$) of neutrophils illustrates that neutrophils accumulate in the vascular system because of a longer life span. The granulocyte turnover rate documents an increased release of neutrophils from the bone marrow.

From Boggs DR, Winkelstein A: *White cell manual*, ed 3, Philadelphia, 1975, FA Davis.

eosinopenia is often overlooked. Stress or corticosteroid treatment can help explain the lack of eosinophilia in patients with eosinophilic inflammatory diseases.

NOTE: Glucocorticoids (endogenous stress or treatment) have prominent effects on the leukogram. Lymphopenia is the most common alteration in the leukogram of dogs and cats and reflects chronic stress or long-term corticosteroid treatment. The acute glucocorticoid effect is classically a mature neutrophilia, lymphopenia, eosinopenia, and in dogs a monocytosis peaking at 4 to 12 hours post-treatment.

After corticosteroid exposure, the total blood neutrophil pool (TBNP) expands because of decreased emigration of neutrophils from the blood into the tissues and increased release of neutrophils from the bone marrow into the blood (Table 4-4). In addition, neutrophils are shifted from the marginal pool (hidden) to the circulating pool (where they are included in blood collected by venipuncture). A left shift is not expected with stress or corticosteroid treatment because neutrophil release from the bone marrow is usually too mild to stimulate release of bands and metamyelocytes in the presence of a normal bone marrow storage pool of neutrophils.

Nuclear hypersegmentation of neutrophils (called a *right shift*) is more likely because corticosteroids decrease emigration of and prolong the circulating half-life of neutrophils in the blood. As neutrophils age, progressive nuclear hypersegmentation or lobulation develops. Hypersegmented neutrophils have five or more nuclear lobes.

Acute corticosteroid-induced changes are transient; therefore, one or more of the expected changes may not be seen depending on how long after treatment the sample was taken. Maximal leukocyte changes occur at 4 to 12 hours and may be normalized by 24 hours. For example, Table 4-3 presents hematologic data from a healthy dog treated daily with dexamethasone (except Sunday). One day after initial treatment (Saturday), five of the expected steroid-stress features occurred (i.e., leukocytosis, neutrophilia, no left shift, eosinopenia, monocytosis). Lymphopenia was not present. On day 3 (Monday with no dexamethasone treatment on Sunday) there was lymphocytosis, monocytosis, and eosinophilia most resembling physiologic leukocytosis (described next). Only on day 5 (Wednesday) was the full classic corticosteroid or stress pattern observed.

Exercise and Epinephrine Response

Transient physiologic leukocytosis is noted mainly in young, healthy cats during epinephrine release from fear or after strenuous exercise (e.g., struggling during venipuncture). This response is unlike the steroid-stress response described previously. It is incorrect (at least hematologically incorrect) to call this epinephrine-mediated response “stress.” The TBNP remains unchanged, but a sudden shift of cells occurs from the marginal to the circulating neutrophil pool. This tends to cause a neutrophilic leukocytosis. Contraction of the spleen tends to cause lymphocytosis and polycythemia. There is no increased release of neutrophils from the bone marrow, nor decreased emigration of neutrophils from the capillary beds. Physiologic leukocytosis in cats is greater in magnitude than in dogs because cats have a larger marginal neutrophil pool (three neutrophils in the marginal pool for every neutrophil in the circulating pool; see Table 4-1). Physiologic leukocytosis in cats may be significant; the WBC count often reaches 20,000/ μ l, and neutrophilia may be overshadowed by lymphocytosis (6000 to 15,000/ μ l).²⁰ Dogs have such weak physiologic leukocytosis that it is seldom recognized clinically. Physiologic leukocytosis has been noted in research dogs that are bled routinely by the same person and an individual animal’s reference values are available from previous hematology testing. Then, if they are suddenly exercised or frightened (e.g., new blood taker), the mild increases in WBCs, neutrophils, lymphocytes, and packed cell volume (PCV) can be detected.

NOTE: Fear, through the fight-or-flight response, causes an epinephrine-induced leukocytosis, most obvious in healthy young cats. It is an immediate, mild to moderate increase in neutrophils and lymphocytes that subsides gradually over 30 to 60 minutes. Lymphocytosis differentiates this physiologic leukocytosis from a steroid-stress response, which typically has lymphopenia.

LEUKOPENIA AND NEUTROPENIA

Leukopenia and neutropenia occur infrequently in dogs and cats because they have a large bone marrow storage pool of neutrophils. Leukopenia indicates a poor prognosis. Neutropenia is usually caused by excessive tissue

TABLE 4-5. MAJOR CAUSES OF NEUTROPENIA

	Animals Affected	
	DOGS	CATS
Consumption of Neutrophils		
Overwhelming sepsis/ endotoxemia (important)	✓	✓
Parvovirus enteritis (important)	✓	✓
Salmonellosis	✓	✓
Immune-mediated destruction (rare)	✓	
Bone Marrow Suppression		
Feline leukemia virus (FeLV) (important)		✓
Feline immunodeficiency virus (FIV)		✓
Parvovirus (important)	✓	✓
Ehrlichiosis	✓	
Bone marrow toxicity	✓	✓
Estrogen (endogenous/ exogenous)	✓	
Phenylbutazone*	✓	✓
Phenobarbital	✓	
Cancer chemotherapy	✓	✓
Irradiation	✓	✓
Leukemia (important)	✓	✓
Myelophthisis/myelonecrosis	✓	✓
Immune-mediated destruction of neutrophil precursors (rare)	✓	✓

*Incomplete list of other drugs is in text.
(Courtesy of Dr. M.D. Willard.)

consumption of neutrophils during severe inflammation and/or reduced bone marrow production (Table 4-5). (See also the discussion of bone marrow in Chapter 2 and the discussed of myeloid hypoplasia in the section on Bone Marrow Problems Causing Neutropenia later.) A third cause of neutropenia, which is rare and more theoretical, is a temporary shift of neutrophils from the circulating to the marginal pool, where they cannot be counted. Endotoxin can cause this change. This transient form of neutropenia is actually “pseudoneutropenia,” because the TBNP is unchanged. Rarely, immune-mediated or “steroid-responsive” neutropenia occurs with lupus or some drug treatments.

During excessive tissue utilization of neutrophils, neutropenia may be severe; this stimulates release of very immature neutrophils, even myelocytes and promyelocytes, from the bone marrow. The left shift is degenerative when nonsegs outnumber segs. An inflammatory process involving a large surface area, such as septic peritonitis, enteritis, or septicemia, tends to cause severe neutropenia and leukopenia. In contrast, localized infections (abscess or pyometra) with pyogenic bacteria usually cause leukocytosis. Gram-negative bacterial infections are often severe and cause consumptive neutropenia. When a

degenerative left shift, marked toxic change, and leukopenia occur, gram-negative sepsis should be suspected.

Neutropenia due to overwhelming infection may be difficult to distinguish from that due to bone marrow depression. Neutrophils have a short life span in blood (e.g., 10 hours), so neutropenia develops before anemia or thrombocytopenia. Thus neutropenia without thrombocytopenia or anemia suggests severe inflammation. Bicytopenia or pancytopenia (decreases in cells of two or three cell lines, respectively) suggests primary bone marrow disease. However, overwhelming infection often causes DIC, which causes thrombocytopenia (bicytopenia). Inflammation causes anemia that may exist prior to a severe exacerbation of the disease. Erythrocytes have long life spans, so anemia is often not apparent early in primary bone marrow disease. Primary bone marrow disease may develop insidiously and lack clinical signs, whereas consumptive neutropenia develops rapidly in a very ill animal. A left shift, toxic changes in neutrophils, and rouleaux formation suggest neutropenia as the result of severe inflammation or infection.

Severe, primary neutropenia predisposes the animal to septicemia. Thus neutropenia due to bone marrow disease may first be noted when a secondary infection develops. Neutropenia and leukopenia are side effects of cancer chemotherapy. Neutrophil counts are often lowest 5 to 7 days after initiation of treatment. Neutrophil counts of less than 1000 to 2000 cells/ μ l require monitoring the patient for sepsis. Sepsis (probably from enteric bacteria) is presumed to be present if the patient has fewer than 500 to 1000 neutrophils/ μ l and is febrile. It is recommended to suspend chemotherapy with myelosuppressive agents if the neutrophil count drops below 2500 cells/ μ l or the platelet count is less than 50,000/ μ l.

Bone Marrow Problems Causing Neutropenia

Diagnostic testing of patients with persistent, undiagnosed neutropenia includes bone marrow aspiration biopsy, core biopsy, or both. This may detect myeloid hypoplasia, ineffective granulopoiesis, bone marrow necrosis, myelofibrosis, disseminated granulomatous inflammation, leukemia, and other diseases (see Chapter 2). A proper history may reveal treatments or toxins that can affect granulopoiesis (e.g., estrogen or phenylbutazone). Ineffective granulopoiesis confuses many clinicians. The leukopenia is accompanied by normal to increased numbers of myeloid cells (i.e., myeloid hyperplasia). Neutropenia due to myeloid hypoplasia seems more logical. In ineffective granulopoiesis, neutrophils are destroyed in the bone marrow by apoptosis before they mature and are released into the blood. Apoptosis (programmed cell death) occurs in healthy dogs as part of the mechanism to regulate effective neutrophil production. Apoptosis is affected by various mediators, and there is no morphologic change to allow diagnosis of a specific cause. Death of cells within the marrow may be excessive in various infections and drug treatments. Approximately half of feline leukemia virus (FeLV)-positive, neutropenic cats have marked granulocytic hyperplasia indicating excessive apoptosis, whereas half have myeloid hypoplasia suggesting viral destruction of hematopoietic tissue as

the cause. Ineffective granulopoiesis may also be an idiosyncratic drug reaction (e.g., phenobarbital).

Damage to or depletion of myeloid cells may cause myeloid hypoplasia (see Chapter 2). Causes of myeloid hypoplasia include parvovirus infection, endogenous and exogenous estrogen toxicosis in dogs, *Ehrlichia canis* infection, cancer chemotherapy, irradiation, and idiosyncratic reactions to drugs such as phenylbutazone, trimethoprim-sulfadiazine, or chloramphenicol (see Chapter 3). Immune-mediated neutropenia has not been well proven in dogs and cats, but steroid-responsive neutropenias have been described.

Neutropenia may occur from laboratory error. The AutoDiff may be checked by inspection of the instrument's leukocyte graphics (see Chapter 2) or by screening the blood smear. When in doubt, a full ManDiff should be performed. The total WBC count is seldom wrong, but instrument flags should alert the operator to a problem. Prominent leukocyte aggregation is uncommon but indicates the WBCs were not evenly distributed in the EDTA sample and therefore the WBC count may be incorrect. In such cases new blood samples with different anticoagulants should be obtained and analyzed without delay. Blood specimens from intravenous fluid administration lines may have excessive dilution by the fluid.

Parvovirus Infection

Parvovirus enteritis may be associated with leukopenia, neutropenia with severe toxic changes, and lymphopenia. Parvovirus infects and destroys rapidly dividing cells (e.g., intestinal crypt epithelium, lymphoid tissue, hematopoietic cells). Massive neutrophil exudation through the damaged mucosa contributes to leukopenia, as does hematopoietic cell damage in the bone marrow. Leukopenia is transient and usually early in canine parvovirus disease, so it may be missed without multiple CBCs. Mast cells from the inflamed gut may be detected in blood smears. The mast cells come from damaged intestinal mucosa and do not indicate mast cell neoplasia. Neutrophilic leukocytosis is expected with recovery. During periods of intense granulopoiesis, a leukemoid reaction or other disturbances of normal cell maturation may occur. With complete clinical recovery, baseline leukogram values are regained. Diagnosis of parvovirus enteritis is discussed in Chapter 9. Panleukopenia in cats has a similar hematologic response.

Feline Leukemia Virus and Feline Immunodeficiency Virus Infections

FeLV infection diagnosis and associated disorders are described in Chapter 15. FeLV causes many different hematologic changes, including cytopenias, such as neutropenia.⁹ Feline immunodeficiency virus (FIV) infection is also associated with cytopenias (i.e., neutropenia, anemia, thrombocytopenia). Therefore, any hematologic abnormality in a cat indicates testing for FeLV and FIV.

Cyclic Hematopoiesis

Cyclic hematopoiesis (i.e., gray collie syndrome, cyclic neutropenia) is an autosomal recessive disease characterized primarily by cyclic neutropenia with 11- to 12-day cycles, described originally in silver-gray collie pups. Neutropenia as severe as 0 to 400/ μ l predisposes affected

collies to life-threatening bacterial infections. A stem cell defect causes cyclic decreases in production of platelets, other granulocytes and monocytes, and erythrocytes (i.e., reticulocytes). Because of a longer half-life of platelets and erythrocytes, numerical change in these cells is less noticeable than for neutrophils. Cyclic hematopoiesis, often with a more irregular periodicity, also has been observed in other breeds of dogs, in rare cats with FeLV infection, and after cyclophosphamide treatment of some dogs. Oscillations of neutrophils, other leukocytes, reticulocytes, and platelets occur at 8- to 29-day intervals. Hemograms obtained at 2- to 3-day intervals should document cyclic neutropenia, but daily CBCs may be required to document cycling of other cells and platelets.

MONOCYTOSIS AND MONOCYTOPENIA

Monocytosis occurs in about 30% of hospitalized dogs and 11% of cats. Blood monocytes mature into macrophages in tissues. Macrophages contribute to granulomatous and pyogranulomatous inflammation. Macrophages remove necrotic debris, kill fungi and some parasites, inactivate viruses, attempt to remove foreign bodies, remove senescent and abnormal red blood cells (RBCs), and destroy neoplastic cells. Monocytosis is expected in inflammatory diseases with a high need for macrophages. For example, monocytosis is common in IMHA, in which large numbers of RBCs are destroyed in macrophages. Necrotic cell debris also must be removed to allow tissue regeneration and healing. Monocytosis accompanies supuration, pyogranulomatous and granulomatous inflammation, necrosis, malignancy, hemolytic or hemorrhagic disease, and immune-mediated diseases.

Although macrophages are a "late" component of most inflammatory processes, monocytosis may occur in both acute and chronic disease processes and monocytosis alone should not be used to indicate chronicity of a process. (See earlier discussion on the canine corticosteroid response, which commonly has a monocytosis 8 to 12 hours after treatment.) If concurrent lymphopenia and eosinopenia are present, a stress or corticosteroid leukocyte response is likely. If lymphopenia and eosinopenia are not present, chronic inflammation or tissue destruction should be suspected. Monocytopenia is not significant. Low numbers of monocytes normally are present in the blood. Monocytes are also unevenly distributed on the blood smear, so imprecision is great with manual monocyte counts. Automated cell hematology analyzers often have difficulty in differentiating monocytes and large lymphocytes. With low numbers of monocytes normally and great imprecision in counting them, a monocytopenia cannot be reliably detected.

LYMPHOCYTOSIS

Persistent non-neoplastic lymphocytosis usually signifies strong immune stimulation from chronic infection, viremia, immune-mediated disease, or recent immunization. Supportive laboratory evidence of chronic infection

(in addition to history and physical findings) may include hyperproteinemia with polyclonal gammopathy; presence of “reactive” lymphocytes; CBC evidence of inflammation; or cytologic or histologic documentation of inflammation, lymph node hyperplasia, or both. Lymphocyte counts up to 17,000 to 30,000/ μ l have been reported with *Ehrlichia canis* infection (or 9000/ μ l in *Toxoplasma gondii* infection in cats).⁴ Milder lymphocytosis (e.g., 4500/ μ l) may be seen during later recovery phases of *Anaplasma phagocytophilum* infection in dogs. Lymphocytosis may not be present in dogs and cats with chronic infections. Lymphocytosis up to 20,000/ μ l was seen in feline but not canine IMHA. Lymphocytosis up to 13,000/ μ l and 9000/ μ l may be seen in canine and feline hypoadrenocorticism (Addison’s disease), respectively, although only in 5% to 10% of patients.⁴ Transient lymphocytosis occurs in physiologic leukocytosis (discussed earlier). Lymphocytosis in feline physiologic leukocytosis is usually under 20,000/ μ l but may exceed 36,000/ μ l.⁴⁰ The previous examples of lymphocytosis were often maximal values and not commonly expected values. Maximal values are useful in differential diagnosis of lymphoid leukemia but should not exclude diagnosis of other causes of lymphocytosis.

Reactive Lymphocytes and Blast-Transformed Lymphocytes

Reactive lymphocytes are large, immune-stimulated lymphocytes with dark-blue cytoplasm and irregular, scalloped, or cleaved nuclei. They are also called *immunocytes*, *virocytes*, and *variant lymphocytes*. In contrast, blast-transformed lymphocytes have a large nucleus with a light, dispersed chromatin pattern with prominent nucleoli or nucleolar rings. Rare reactive lymphocytes are visible in blood smears from healthy animals, whereas a few to several reactive lymphocytes may occur in blood smears from sick or recently vaccinated animals. Reactive lymphocytes are not of special diagnostic significance. The number of reactive lymphocytes does not consistently reflect the degree of immune stimulation, nor is it pathognomonic for any specific disease. Reactive blast-transformed lymphocytes may appear unusually numerous, large, or active (especially in young animals) and may be mistaken for lymphoid leukemia. Reactive lymphocytes may confuse staging or diagnosis of recurrence of disease in patients with lymphoma.

LYMPHOPENIA AND EOSINOPENIA

Severe stress or exogenous corticosteroid administration (see Stress and Corticosteroid Response earlier) usually causes lymphopenia and eosinopenia. Lymphopenia is also expected early in inflammation (see Figure 4-5), and the return of the lymphocyte count to normal is a good prognostic sign indicating recovery from the stress of inflammation. Lymphopenia occurs in some viral diseases by direct viral damage of lymphoid tissue and through lymphocyte redistribution from stress or antigen exposure. Canine viral diseases causing lymphopenia include distemper, infectious canine hepatitis, parvoviral

enteritis, and coronavirus enteritis. Cats may have lymphopenia in panleukopenia and FeLV infection.

Loss of lymphocyte-rich lymph has been stated to cause lymphopenia. Examples include chylothorax in dogs and cats, protein-losing enteropathy and lymphangiectasia in dogs, and disruption of normal lymphatic circulation by inflammatory, infectious, or neoplastic diseases in both species.¹⁸

Age affects the lymphocyte count. Younger animals usually have higher counts. For example, the minimal lymphocyte counts expected in dogs of various age categories are 2000/ μ l from 3 to 6 months of age; 1500/ μ l from 8 to 24 months of age; and 1000/ μ l over 24 months of age. Thus one uses different lymphocyte counts to indicate lymphopenia in young dogs.

Eosinopenia may be difficult to document by routine WBC counts. Eosinophils may be too few to be observed in leukocyte differential counts of normal animals, so reference values may go down to 0. Variation expected with a 100-cell, manual differential leukocyte count when 2% of a cell type (e.g., eosinophils) is present is 0% to 8% (95% confidence interval).³⁴ Eosinopenia in a sick animal may be likely if 0% eosinophils are seen and if concurrent lymphopenia is present. The most common causes of eosinopenia are severe stress associated with illness and response to corticosteroid administration.

Pseudoeosinopenia in many greyhounds, other sight hounds, and occasionally other breeds of dogs may result from misidentification of “vacuolated” or “gray” eosinophils. Instead of having red-orange granules, gray eosinophils have only clear-staining granules that look like empty vacuoles. Alternatively, vacuolated eosinophils may have mainly vacuoles with occasional typical red-orange granule or hints of red in mainly clear granules. It is often uncertain if there are empty vacuoles from degranulation or if the granules are unstained. The clear, unstained granules in the greyhound eosinophil may be seen as granules and not empty vacuoles on electron microscopy. Hematology instruments (e.g., Advia 2120) may occasionally fail to detect eosinophils in some samples.

EOSINOPHILIA

Eosinophilia usually indicates eosinophilic inflammation. However, eosinophilic inflammation in tissues is often unaccompanied by detectable eosinophilia in blood because eosinophils have a very short half-life in blood. Cytology or histology will document the eosinophilic inflammation in tissues (e.g., lung, skin, gut). Eosinophilia is an increase in eosinophils over the laboratory’s reference values, which may vary from less than 750 to less than 1300/ μ l eosinophils in dogs and less than 750 to less than 1500/ μ l in cats. Over 5000/ μ l eosinophils is a very strong response and may be termed *hyper eosinophilia*. A leukemoid reaction of eosinophils (due to strong eosinophil inflammation but not leukemia or hyper-eosinophilic syndrome) is concluded when there are more than 25,000 to 30,000 eosinophils/ μ l. The rottweiler and German shepherd breeds have greater reference values for eosinophils and more eosinophilic diseases.²² The rottweiler has a tendency to develop eosinophilic

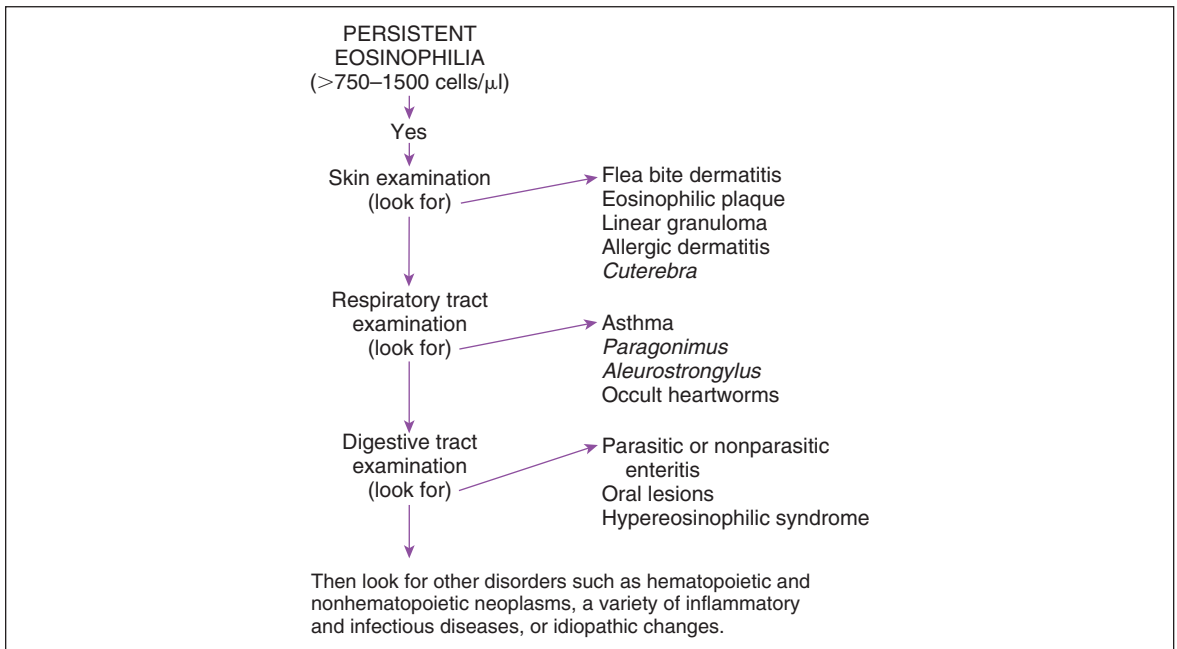


FIGURE 4-6 An algorithm to diagnose common causes of eosinophilia in cats.

diseases such as hypereosinophilic syndrome and eosinophilic meningoencephalitis. Siberian huskies and Alaskan malamutes also have increased frequency of pulmonary infiltrates with eosinophils (PIE; pulmonary bronchopneumopathy; eosinophilic pneumonia), eosinophilic pulmonary granulomatosis, and oral eosinophilic granuloma. Cavalier King Charles spaniels are predisposed to eosinophilic granuloma and eosinophilic stomatitis.

Eosinophils kill parasites, regulate the intensity of hypersensitivity reactions mediated by immunoglobulin E (IgE) antibodies, and may promote inflammation and tissue damage.¹⁰ Eosinophils kill parasites by attaching to them and forming a digestive vacuole between the eosinophil and parasite. Eosinophil granules contain potent molecules such as major basic protein and eosinophil peroxidase, which degranulate into the digestive vacuole and damage the wall of the parasite or ovum and kill it. A more intense eosinophilic response occurs with parasites within tissues (e.g., heartworms, strongyles, migrating lung flukes such as *Paragonimus kellicotti*). Endoparasites such as tapeworms or *Giardia*, which do not invade tissue, generally do not incite eosinophilia. Some parasites may not stimulate eosinophilia until they die and expose previously hidden antigens. Production of eosinophilia is an immune response. The first exposure to a parasite produces a modest, delayed eosinophilia. The second exposure to the same parasite results in an intense, dramatic eosinophilia.

An eosinophilic inflammatory response occurs to certain allergens. Lymphocytes respond to the allergen by producing an IgE-type immune response. IgE binds to mast cells, which triggers an inflammatory response. Eosinophil inflammation unfortunately often damages normal tissue locally. For example, eosinophilic

inflammation from inhaled allergens in feline asthma may cause damage to respiratory epithelium. Eosinophils are attracted to inflamed tissue by mast cell products and lymphokines. Eosinophilic inflammation is strongly coupled to mast cells and basophils. Eosinophils may be abundant in mast cell tumors and lymphoma masses because of production of mediators from mast cells or lymphocytes that attract eosinophils.

Diagnostic Approach

Parasitic and allergic processes are considered first when identifying the cause of eosinophilia (Figures 4-6 and 4-7). However, eosinophilia is often not associated with parasite infestations or allergy. Eosinophilia may accompany other inflammatory reactions; fungal (e.g., cryptococcosis), viral (e.g., FeLV), or bacterial (e.g., *Streptococcus* or *Staphylococcus* species) infection; and neoplasia (e.g., mast cell tumor, lymphoma, mucinous carcinomas, fibrosarcomas, eosinophilic leukemia). Hypereosinophilic syndromes can produce severe, persistent circulating and tissue eosinophilia.

Mast cells are sentinel cells, and are numerous near surfaces such as the skin, digestive tract, respiratory tract, and genitourinary tract. Therefore, the cause of eosinophilia can also be evaluated via a body systems approach. The integumentary system is examined for ectoparasites, dermatitis, or masses. Flea bite allergy is a common cause of eosinophilia in cats. The respiratory tract may be evaluated cytologically by transtracheal wash, bronchial brushings, swabs, or collecting material adherent to endotracheal tubes. Heartworm disease and PIE (pulmonary bronchopneumopathy; eosinophilic pneumonia) are common causes of eosinophilia in dogs (disease incidence varies

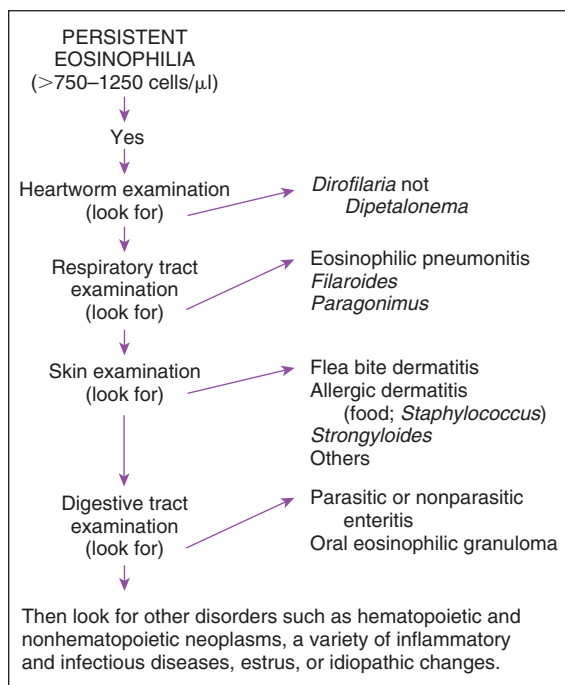


FIGURE 4-7 An algorithm to diagnose common causes of persistent eosinophilia in dogs.

depending on location). Fine-needle aspiration or surgical biopsy may aid in evaluating dermal or pulmonary masses. Examination of the upper alimentary tract should include visual inspection of the oral cavity for eosinophilic granulomas, especially the eosinophilic granuloma complex of cats and Siberian husky dogs.

Fecal ova examination is inexpensive, though the presence or absence of ova is usually unrelated to the primary cause of eosinophilia. If eosinophilic gastroenterocolitis is suspected, the clinician should consider endoscopic examination, surgical biopsy, and cytologic evaluation of any lesions. Estrus in dogs occasionally causes eosinophilia. Differential diagnosis of very strong eosinophilic inflammatory diseases, hypereosinophilic syndromes, and eosinophilic myeloproliferative diseases may be difficult in some cases without an obvious cause.

BASOPHILIA

Basophils are considered rare in normal dogs and cats. However, basophilia (defined as finding 1 or more basophils during a manual Diff and reported as $\geq 100/\mu$ l or $0.1 \times 10^9/L$) was seen in 8% of Swedish canine patients. Extreme canine basophilia of greater than or equal to $2000/\mu$ l ($2.0 \times 10^9/L$) without eosinophilia is rare and can suggest chronic myeloproliferative disease if persistent and combined with prominent thrombocytosis. Automated instruments (i.e., Advia 2120, Cell Dyn 3500, Sysmex XT-2000iV, and LaserCyte) fail to detect canine and feline basophils and thus never detect basophilia. Basophils may be counted as large unstained cells (LUCs)

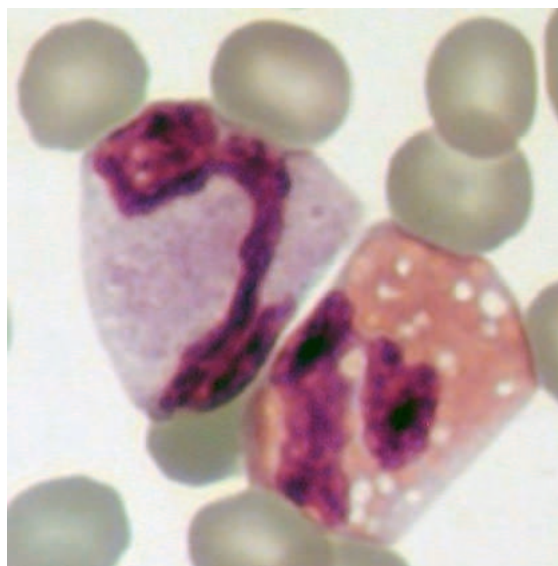


FIGURE 4-8 A canine basophil and eosinophil are shown. The canine basophil often does not have the distinct, dark-blue granules seen in many other species, but is identified by a long thin granulocyte nucleus and lavender-colored cytoplasm. Granules may stain well with some stains. The canine basophil is often mistaken for a toxic neutrophil or monocyte. The eosinophil has various-sized round granules.

by the Advia 2120. Basophilia of 2% may be missed in manual differential counts because of imprecision of the method. Basophilia must be 3% to 6% to be consistently detected.

Basophilia may not be detected because basophils are difficult to identify. Canine basophils have few, widely scattered purple (i.e., metachromatic) or poorly stained granules, especially with water-based stains. These cells may be misidentified as monocytes or toxic neutrophils (Figure 4-8). Feline basophil granules are numerous, moderately sized, round to oval, and grayish beige to light lavender (Figure 4-9). This appearance of feline basophils is unique, but feline basophils may be mistaken for eosinophils with faded granules or monocytes. Feline eosinophils have red-orange, rod-shaped granules, whereas monocytes have blue-gray cytoplasm that is devoid of distinct granules. Infrequently, feline basophils have one or two dark-staining purple granules that facilitate cell identification.

Basophilia is usually accompanied by eosinophilia, and diagnosis is mainly directed toward identifying the cause of the eosinophilia. Basophils are an integral component of hypersensitivity reactions, as are mast cells and eosinophils. Rottweiler dogs have an increased frequency of basophilia but also eosinophilia. Basophils are involved in hemostasis, lipid metabolism, rejection of ticks, and tumor cell killing.¹⁸ Basophilia is often caused by parasitism (especially *Dirofilaria immitis* infection) and hypersensitivity reactions. Additionally, basophilia is associated with lipemia or with canine and feline mast cell tumors, or occurs as a form of chronic granulocytic leukemia.¹⁸

ABNORMAL NUCLEAR MORPHOLOGY AND CYTOPLASMIC INCLUSIONS

Hyposegmentation is decreased nuclear segmentation of granulocytes, which usually indicates immaturity and a response to inflammation. Hypersegmentation presents with excessive lobulation, defined as more than five lobes. This nuclear change reflects more mature neutrophils. Hypersegmentation may be the result of prolonged neutrophil time in circulation, which is commonly associated with corticosteroid treatment. However, genetic developmental abnormalities, nutritional problems, preleukemic or dysplastic conditions, and leukemia may be responsible for nuclear changes. Dysplasia and leukemia are discussed later in this chapter.

Cytoplasmic inclusions, such as Döhle bodies and toxic granulation, may be associated with toxic change in neutrophils. Circulating neutrophils may contain particles phagocytized in blood, such as Heinz bodies, cell debris, opsonized erythrocytes (or hemosiderin), medications, or mast cell granules. Infectious agents such as bacteria (i.e., *Anaplasma*, bacteremia), protozoa (i.e., *Hepatozoon canis*, *Leishmania* spp.), or yeast (i.e., *Histoplasma capsulatum*) may be found within the cytoplasm of circulating leukocytes (Figures 4-10, 4-11, and 4-12). Rarely, genetic diseases are responsible for a variety of cytoplasmic inclusions. Staining artifacts and precipitate may confuse the observer.

Pelger-Huët Anomaly

Pelger-Huët anomaly (PHA) is an inherited, lifelong disorder of leukocyte development characterized by nuclear hyposegmentation of neutrophils, other granulocytes, and monocytes. Almost all neutrophils resemble bands or metamyelocytes (i.e., absolute leukocyte results look

like a severe degenerative left shift) (Figures 4-13 and 4-14). The characteristic WBC appearance in PHA should be recognized and these neutrophils should be reported as Pelger-Huët cells and not nonsegs to avoid misdiagnosis by clinicians reading the CBC, who may falsely conclude very serious inflammation. The nuclear chromatin pattern of PHA granulocytes appears coarse and mature, and the cytoplasm is clear and devoid of toxic changes. Neutrophils function normally, and a predisposition to infection has not been demonstrated. PHA occurs in both dogs and cats and is presumed to be inherited in an

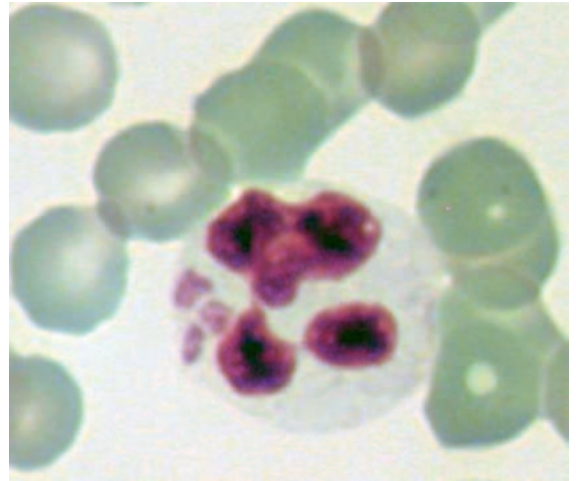


FIGURE 4-10 A canine neutrophil with *Anaplasma phagocytophilum*. There are three morula in the cytoplasm, but otherwise the nuclear and cytoplasmic morphology is normal.

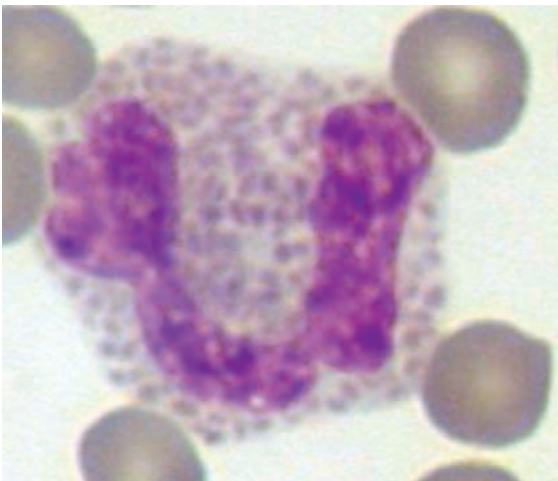


FIGURE 4-9 A feline basophil has round lavender-colored granules and the not bright-orange rod-shaped granules of the eosinophil. The feline basophil is often mistaken for an eosinophil.

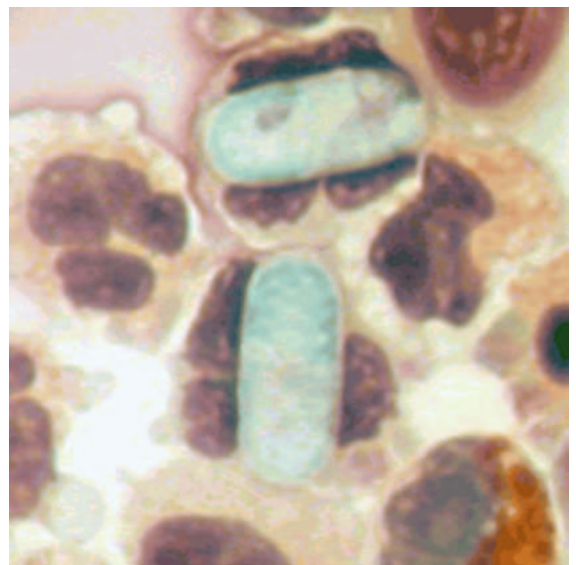


FIGURE 4-11 Two canine neutrophils containing *Hepatozoon canis*. This was a buffy coat smear to concentrate the number of leukocytes.

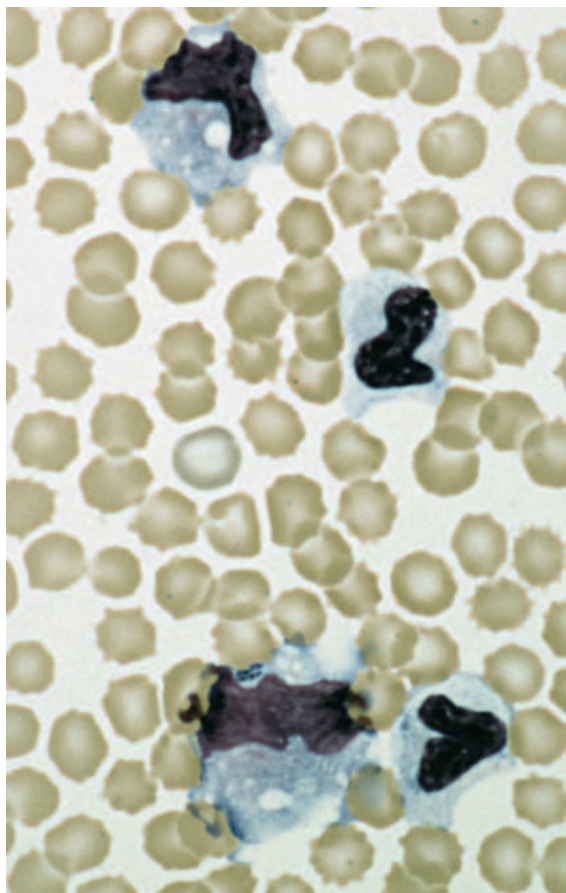


FIGURE 4-12 Two monocytes (left) and two toxic, young band neutrophils (right side of photo) in a 16-week-old Doberman puppy with probable chronic granulomatous disease. The lower monocyte contains two rod-shaped bacteria. The dog had a degenerative left shift with more bands than segs and a moderate monocytosis. The monocytes had many vacuoles and looked activated (blue cytoplasm). It is difficult to differentiate toxic immature neutrophils from monocytes.

autosomal dominant manner.⁸ However, PHA may be inherited in an autosomal incompletely dominant pattern in Australian shepherds, suggesting that the expression of the anomaly is governed by two or more alleles.¹⁹

Bacterial infection, drug treatment, developing granulocytic leukemia, or FeLV infection may cause acquired nuclear hyposegmentation of neutrophils (i.e., pseudo-PHA). True PHA can be confirmed by finding the anomaly in blood smears of parents, siblings, or other relatives or by proving inheritance of the trait by prospective breeding trials. True PHA has persistent nuclear changes on repetitive blood smear analysis and no clinical signs.

Genetic Diseases with Cytoplasmic Inclusions *Chédiak-Higashi Syndrome*

Chédiak-Higashi syndrome (CHS) occurs as an autosomal recessive trait in Persian cats with yellow-green eyes and a diluted smoke-blue haircoat.⁴⁶ CHS is associated

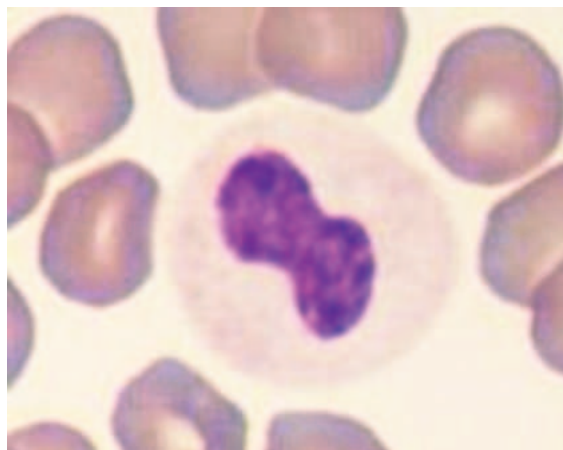


FIGURE 4-13 A canine Pelger-Huët neutrophil has a nuclear shape like a metamyelocyte but a very mature dense chromatin pattern of a mature neutrophil. The cytoplasm is clear and not toxic. A Pelger-Huët neutrophil should be recognized as such and reported as a Pelger-Huët neutrophil and not a band or metamyelocyte, which would mislead the person reading the report.

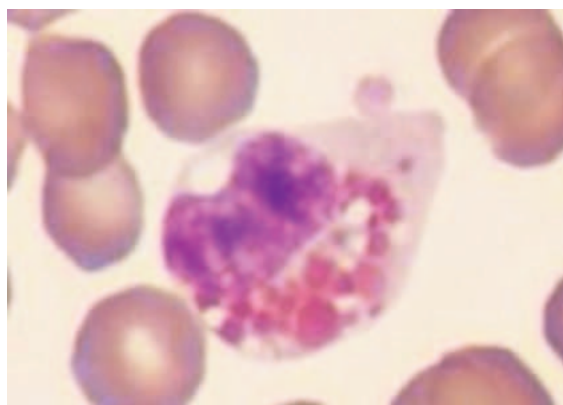


FIGURE 4-14 A canine Pelger-Huët eosinophil also has a metamyelocyte-shaped nucleus but with a dense clumped chromatin pattern. Various-sized granules are typical of the canine eosinophil.

with fusion of lysosomal granules into large pink cytoplasmic inclusions in neutrophils, eosinophils, and other cells. Coarse clumping of melanin granules is associated with color dilution of the haircoat and irises. In addition, decreased choroidal pigment causes a red fundic reflex and photophobia in bright light. Blood smear examination and the cat's gross appearance confirm diagnosis of CHS. Neutrophil inclusions are positive on peroxidase and Sudan black B stains. In contrast with other animal species, cats with CHS have no predisposition to infection. Cats with CHS have an increased bleeding time secondary to mild platelet dysfunction, however. Hemostasis may be prolonged slightly after trauma, venipuncture, or elective surgery.

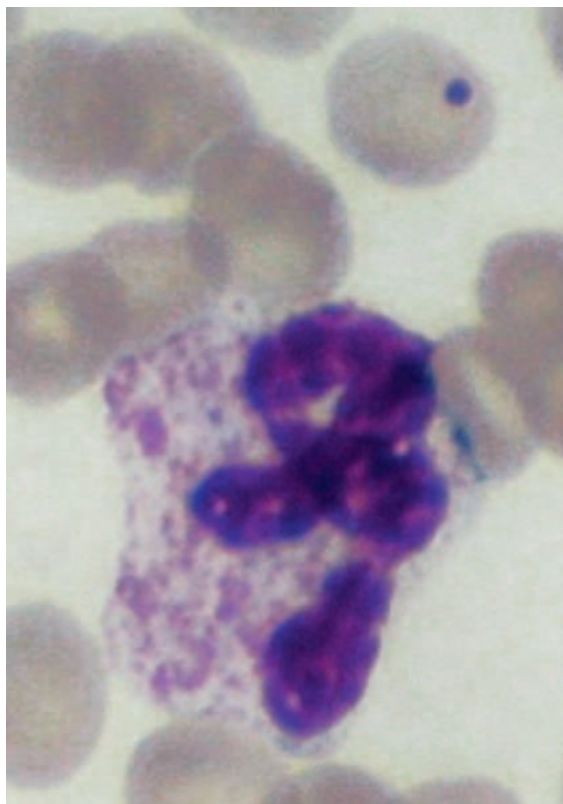


FIGURE 4-15 A Birman cat neutrophil granulation anomaly with prominent granules resembling the staining of granules in progranulocytes in the bone marrow.

Birman Cat Neutrophil Granulation Anomaly

A staining anomaly of neutrophils stained with Romanowsky-type stains is an autosomal recessive trait in Birman cats.⁴⁶ The usually neutral-staining granules of neutrophils instead stain prominently, similar to granules in progranulocytes (Figure 4-15). The defect is asymptomatic but needs to be differentiated from toxic granulation and mucopolysaccharidosis (MPS) types VI and VII.

Lysosomal Storage Diseases

Storage diseases are rare inherited enzyme defects that cause accumulation of intermediate metabolites of complex molecules within cellular lysosomes. Depending on the nature of the metabolite and its affinity for Romanowsky-type stains, cellular inclusions may appear purple and stringy, as in MPS VI (i.e., Maroteaux-Lamy syndrome) of cats or MPS VII of dogs, or may appear as clear vacuoles in leukocytes in cats with lysosomal acid lipase deficiency. The characteristic purple granules of MPS can be differentiated from toxic granulation, because cytoplasmic basophilia is absent and toxic granulation is uncommon in dogs and cats. Lysosomal storage diseases often are associated with progressive central nervous system or skeletal disease but may be identified on a CBC by observing characteristic inclusions or vacuoles in circulating leukocytes.

LEUKOCYTE FUNCTION DEFECTS

Neutrophil function disorders have been reviewed by Weiss.⁴⁶ Primary neutrophil dysfunctional errors are rarely documented. They may be suspected in animals with a history of recurrent infections in association with a normal to extremely elevated neutrophil count and lack of neutrophil migration into sites of infection as determined by cytology. Diagnosis of a primary neutrophil dysfunction requires excluding common causes of immune suppression or secondary neutrophil dysfunction such as stress, glucocorticoid treatment, nutritional deficits, endocrine changes (e.g., late luteal phase of estrus in bitches), or various infections. Neutrophil function tests include neutrophil adherence, chemotaxis, phagocytosis, bactericidal activity, or a combination thereof. Tests of neutrophil function are labor intensive, take a long time to perform and are thus expensive, and are available only through a few specialists or research laboratories to which the animal must be referred for diagnosis.

Canine CD11/CD18 Adhesion Protein Deficiency

Canine adhesion protein deficiency (i.e., canine leukocyte adhesion molecule deficiency [CLAD], canine granulocytopeny syndrome) occurs infrequently in Irish setter and Irish setter-cross puppies. CLAD is an autosomal recessive disease. Affected puppies have recurrent bacterial infections before 12 weeks of age. Leukogram abnormalities often include a leukemoid reaction (e.g., up to 208,000 cells/ μ l). Fluids from sites of infection contain few neutrophils, because they are less able to emigrate from the microvasculature to the sites of infection. Neutrophils lack CD11/CD18 adhesion proteins on the plasma membrane surface, which normally facilitate phagocytosis of organisms and cell emigration from blood vessels.³⁹

Chronic Granulomatous Disease in Doberman Pinschers

Chronic respiratory disease in eight closely related Doberman pinschers was attributed to impaired neutrophil bactericidal activity.⁷ Neutrophils phagocytized bacteria normally but were unable to kill them. Immunoglobulin concentrations, complement concentrations, and mitogen-stimulated lymphocyte transformation were normal. Septicemia, monocytosis, and a degenerative left shift are shown in a 16-week-old Doberman puppy in a litter of Swedish puppies with likely chronic granulomatous disease of Doberman pinschers (see Figure 4-12).

Myeloperoxidase Deficiency

In addition to neutropenia in cyclic hematopoiesis of gray collies, a bactericidal defect due to myeloperoxidase deficiency has been described.⁴⁶ An acquired myeloperoxidase deficiency was noted in dogs with severe consumption of neutrophils in inflammatory diseases such as parvovirus enteritis, pyometra, pyothorax, and pneumonia.¹⁷

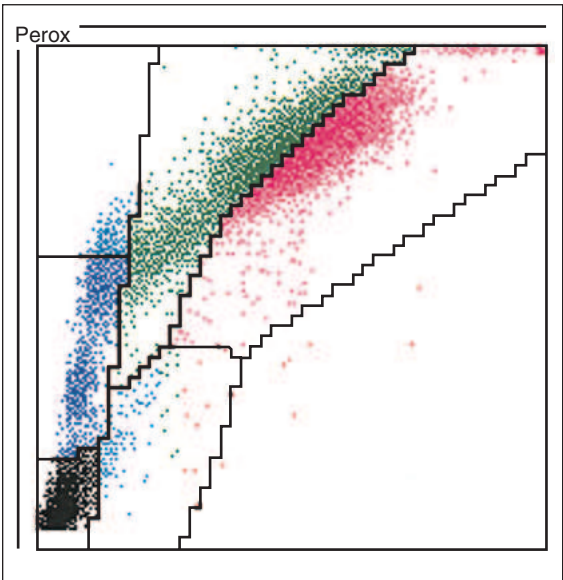


FIGURE 4-16 Acquired myeloperoxidase deficiency in dogs is seen with severe inflammatory diseases and may be detected and the severity graded by the magnitude of an error in the Advia 2120 classification of neutrophils. The Advia uses size (y axis) and peroxidase staining (x axis) of leukocytes to perform an automated differential leukocyte count. In this peroxidase dot plot, the red dots are neutrophils but about half of the neutrophil cluster (in red oval) was classified as monocyte (green dots) reflecting decreased peroxidase activity in the neutrophils. The number of neutrophils misclassified as monocytes reflects the severity of decrease in peroxidase.¹⁷ Over half the neutrophils being incorrectly classified as monocytes was used to indicate the worst grade of myeloperoxidase deficiency. The Advia monocyte count was 40% in this blood sample, but the manual monocyte count was only 11%.

This caused a misclassification of neutrophils as monocytes by the Advia 2120 (Figure 4-16). The Advia uses size of leukocytes and peroxidase staining for its AutoDiff. The percentage of neutrophils misclassified as monocytes was used to grade the deficiency of myeloperoxidase. Grade 3 (worst deficiency) had greater than 50% of neutrophils misclassified as monocytes (see Figure 4-16). Neutrophil function in these dogs was not evaluated.

Recurrent Infections in Weimaraners

Defective neutrophil function was identified in weimaraner puppies with recurrent fevers and infections. Their neutrophils had a decreased chemiluminescence response to phorbol esters.

Acquired Neutrophil Dysfunction

Acquired neutrophil dysfunction has been reported in a few dogs with poorly regulated diabetes mellitus, pyoderma, demodicosis, protothecosis, and lead toxicosis. A lack of pyuria (no neutrophils seen) is noted in some dogs with diabetes mellitus or hyperadrenocorticism in which bacteria may be cultured from cystocentesis samples. This suggests a defect in neutrophil migration in those diseases. In cats, neutrophil dysfunction has been documented infrequently in FeLV infection and feline infectious peritonitis.⁴⁶

DYSPLASTIC AND NEOPLASTIC CONDITIONS OF BLOOD CELLS

The proliferative responses of leukocytes previously discussed arise from the purposeful need to replace missing circulatory cells primarily related to increased utilization or consumption. The bone marrow in this condition demonstrates hyperplasia. Other hematopoietic cell proliferations may either be poorly regulated with abnormal development and morphology, known as *myelodysplasia*, or be nonpurposeful, unregulated new growth, recognized as *neoplasia*. Malignant neoplasia of blood cells is termed *leukemia*, literally meaning “white blood” from the observed large buffy coat. Leukemia reflects an abnormal population of hematopoietic cells found within the blood or bone marrow as opposed to solid tissues, such as lymph nodes, spleen, or liver.

Non-Neoplastic Myelodysplasia

Criteria used by some to designate significant dysplasia involve having greater than 10% dysplastic cells in one or more hematologic cell lines in the bone marrow and concurrent cytopenia in the blood.⁴⁴ Morphologic abnormalities of hematopoietic cells may occur under benign or neoplastic conditions (Table 4-6).

TABLE 4-6. FORMS OF MYELODYSPLASIA AND ASSOCIATED CONDITIONS

GENERAL CATEGORY	ASSOCIATED CONDITIONS
Congenital myelodysplasia	Toy and miniature poodles (erythrocytes and precursors) Cavalier King Charles spaniels (platelets) Giant schnauzers (neutrophilic precursors)
Secondary MDS	Drugs: azathioprine, cyclophosphamide, cytosine arabinoside, vincristine, chloramphenicol Infectious agents: FeLV, FIV, FIP Nutritional deficiencies: folate, cobalamin receptor defect in giant schnauzers Immune-mediated disease: immune-mediated anemia/thrombocytopenia Malignant diseases: lymphoma, plasma cell myeloma, lung adenocarcinoma
Primary MDS	Idiopathic clonal defects

FeLV, Feline leukemia virus; FIP, feline infectious peritonitis virus; FIV, feline immunodeficiency virus; MDS, myelodysplastic syndrome.

Congenital Myelodysplasia

Inherited myelodysplasia exists in breeds such as toy and miniature poodles with enlarged erythrocytes and their precursors, or in Cavalier King Charles spaniels with abnormally large platelets related to an autosomal recessive defect. These dogs have no clinical signs, and the dysplastic findings are usually incidental.

Secondary Myelodysplastic Syndrome

This form of myelodysplasia is not a neoplastic condition but one that is acquired secondary to another disease or condition and is the most common form of dysmyelopoiesis, accounting for nearly 5% of bone marrow disorders reported in a recent canine study.⁴⁵ This may be associated with drug-induced and nutritional problems that are reversible once the inciting cause is removed. Some myelodysplasia occurs with certain feline viral infections and is commonly noted with immune-mediated disease. For example, in IMHA, exuberant production of erythrocytes may occur related to the rapid and poorly regulated development, resulting in nucleated red cells with excessive cytoplasm and an eccentrically, not centrally placed, nucleus (Figure 4-17). A recent example of dyserythropoiesis was reported in a dog associated with bone marrow metastasis of pulmonary adenocarcinoma.²³

Leukemia

Leukemia may be suspected based on extreme increases in WBC concentration numbers or relative to morphology that is atypical or markedly immature for the cell type. Lymphoid or myeloid (nonlymphoid) cells may be involved in leukemia. Common causes of leukemia include (1) virus infections, such as FeLV, which may affect progenitor cell development; (2) genetic abnormalities, inherited or acquired, that lead to altered cell growth; (3) defective immune systems, such as with FIV infection, that do not permit normal defense mechanisms against leukemia; (4) chemicals (e.g., benzene, therapeutic drugs), which have rarely been associated

with leukemia; and (5) radiation, which may alter the health of progenitor cells.

Clinical signs of leukemia vary. Common signs include lethargy, pale mucous membranes, anorexia, weight loss, fever, frequent infections, icterus, and abnormal bleeding such as petechiae. Physical findings often include hepatosplenomegaly, lymphadenopathy, or tonsillar enlargement.

General Laboratory Findings

Strong laboratory indicators of leukemia include marked leukocytosis of a monomorphic cell type, the presence of many blast cells, or morphologic irregularities in shape or size of late-stage forms. A prominent buffy coat may be noted when measuring the PCV related to a marked leukocytosis or thrombocytosis. Blast cells have a large round nucleus, fine chromatin pattern, and one or more prominent nucleoli. Reactive and blast-transformed lymphocytes are often present in small numbers (e.g., 1 to 5/ blood smear) and are not diagnostic. Numerous reactive and blast-transformed lymphocytes may occur in ill, nonleukemic animals (e.g., 1% to 5% of WBCs) and may mimic leukemia. Rare mitotic figures do not indicate leukemia because mitotically active cells such as rubricytes, promyelocytes, myelocytes, monocytes, and reactive lymphocytes are encountered in nonleukemic animals. Recognition of the subtle morphologic irregularities may be difficult. Abnormalities include megaloblastic erythroid precursors that have an excessive amount of cytoplasm and unusual nuclear chromatin pattern, enlarged segmented neutrophils with many nuclear lobes (i.e., macropolycytes), enlarged highly vacuolated platelets, or dwarf megakaryocytes, and asynchronous maturation wherein the degree of maturity of nucleus and cytoplasm differ.

White cell counts in leukemia are variable, ranging from leukocytosis to leukopenia, or even within normal limits. Anemia is often nonregenerative, but rarely hemolysis is found in some forms of leukemia. Thrombocytopenia, or less commonly thrombocytosis, is associated with the other hematologic changes. Laboratory tests used to diagnose hematopoietic neoplasia or myelodysplasia and evaluate the extent of involvement include bone marrow evaluation, biochemical profiles, cytology, serology, and urinalysis, in addition to hematology. If changes in the blood are not diagnostic, bone marrow examination may confirm leukemia. Blast cells are often found in much higher numbers in marrow than in blood. Bone marrow evaluation should involve optimally an aspirate and core biopsy with concurrent peripheral blood evaluation. Cytology of effusions or solid tissue masses may help to determine a primary source. Serology is indicated for evaluation of leukemia-causing viruses. Protein evaluation of the serum and urine is helpful in determining the presence of gammopathies associated with lymphoproliferative conditions (see Chapter 12).

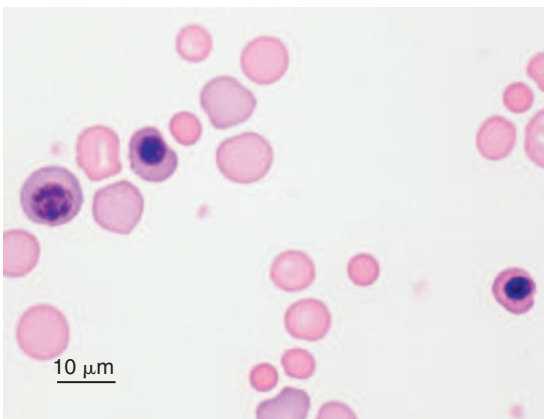


FIGURE 4-17 Secondary myelodysplasia of three nucleated erythroid precursors is depicted in a sample from a dog with a strongly regenerative spherocytic immune-mediated hemolytic anemia. Notice the faint basophilic stippling in the precursor (far right) and abundant cytoplasm in all three rubricytes.

NOTE: Leukemia is less common than other causes of alterations in leukocyte numbers and morphology. Stress, inflammation, and reactive lymphocytes are much more common.

Advanced Diagnostic Testing

The following information is for those choosing to treat hematopoietic neoplasia and who need a more specific diagnosis for treatment choices. Definitive diagnosis of specific leukemias usually requires a veterinary clinical pathologist. Slide review from routine CBC or bone marrow cytology with Wright-stained smears of EDTA-anticoagulated blood may allow morphologic differentiation of the cellular origin, such as lymphoid versus myeloid (i.e., nonlymphoid). If cell morphology in a leukemic patient is insufficient to determine the cell lineage, one may use specialized tests (i.e., cytochemistry, electron microscopy, immunocytochemistry, genetics testing) usually conducted at academic institutions or referral laboratories.³⁸ Cytochemistry involves nonimmunologic cytoplasmic markers for enzymes, lipids, or glycogen within the cell.³¹ Enzymes include peroxidase, found in certain granulocytes, and nonspecific esterases, found in monocytes or T lymphocytes (Table 4-7). Electron microscopy is helpful to study the ultrastructural features of the cytoplasm and nucleus.^{13,14} Immunocytochemistry (Table 4-8) involves staining of specific cell surface antigens by the use of antibodies against CD3 (T cells), CD4 (T-helper cells), CD8 (T-cytotoxic or suppressor cells), CD14 (monocyte progenitors), CD21 (B cells), and CD79 (B cells).²⁸ Blood and bone marrow specimens for these special procedures require specific instructions in handling, preparation, and fixation. Therefore, the clinical pathologist should be consulted to avoid delay in diagnosis caused by improper sampling or sample handling. Even more specialized diagnostic tests are available that involve genetic testing such as clonality (e.g., polymerase chain reaction for antigen receptor rearrangement [PARR]) for lymphoid neoplasms and detection of cytogenetic abnormalities for all hematopoietic neoplasms.^{6,42}

Classification Schemes

Classification schemes for hematopoietic neoplasms were developed and refined over many years, beginning with purely morphologic comparisons such as the French-American-British (FAB) classification system, which separates acute myeloid leukemia into eight subtypes, M0 through M7 (Figure 4-18) and its veterinary counterpart (Figure 4-19), both of which are based on the cell of origin and its degree of maturity as determined by examination of Romanowsky-type (e.g., Wright) stained blood and bone marrow smears. Various classification systems also have been used to classify lymphoid neoplasms. Other classification systems, such as the National Cancer Institute Working Formulation and updated Kiel System, rely on morphologic and immunologic features. Further distinction of leukemia may be determined by cytochemical and immunochemical reactions (Figure 4-20). The most recent World Health Organization (WHO) classification scheme for hematopoietic and lymphoid neoplasia identifies the different disease types by tumor location plus morphologic, immunophenotypic, and cytogenetic criteria to best determine the clinical prognosis for each disease (Box 4-1).³⁷ The lymphoid neoplasms involve lymphocytes and plasma cells; the myeloid neoplasms involve the remaining nonlymphoid leukocytes, including mast cells, erythroid cells, and megakaryocytes or

TABLE 4-7. SELECTED CYTOCHEMICAL STAINS FOR IDENTIFICATION OF CELL TYPES IN CANINE AND FELINE LEUKEMIAS

STAIN	CELLS THAT STAIN
Peroxidase	Neutrophils—all stages positive Monocytes—weakly positive when present Eosinophils—positive in dog, negative in cat
Sudan black B	Neutrophils—all stages positive Monocytes—weakly positive when present Eosinophils—positive in dog, negative in cat
Leukocyte alkaline phosphatase	Neutrophils—myeloblasts, strong Monocytes—monoblasts, weak or rare in dogs Lymphocytes—subset of B cells Eosinophils—between the granules in cat Basophils—weak or occasional staining
Chloroacetate esterase	Neutrophils—strongly positive Basophils—moderately positive Mast cells—variably positive Megakaryocytes—weakly positive when present
Alpha-naphthyl butyrate esterase	Monocytes—diffuse positive Lymphocytes—focal positive in T cells Megakaryocytes—diffuse positive
Nonspecific esterase + fluoride*	Lymphocytes—positive in dog, variable in cat Megakaryocytes—weakly positive in dog
Periodic acid-Schiff	Neutrophils—all stages positive Megakaryocytes—positive in dog, variable in cat Mast cells—variably positive Lymphocytes—plasma cells Eosinophils—weak or variable staining Basophils—weak or variable staining Monocytes—weak or variable staining
Acid phosphatase	Neutrophils—all stages positive Lymphocytes—focal positive in T cells Eosinophils—positive Basophils—positive in cat, variable in dog Monocytes—diffuse positive Megakaryocytes—diffuse positive Mast cells—positive

*Inhibition of nonspecific esterase with fluoride is used to inhibit or reduce the staining of monocytes.

platelets singly or in combination; and the histiocytic neoplasms involve macrophages and dendritic cells.

A system based on the WHO classification of human lymphoid tumors has been suggested for animals.⁴¹ This veterinary system separates lymphoid neoplasms by immunophenotype and tumor site location, primarily

TABLE 4-8. IMMUNOCHEMICAL ANTIBODIES USEFUL FOR THE RECOGNITION OF LEUKOCYTE SUBPOPULATIONS IN HEMATOPOIETIC NEOPLASIA

ANTIBODY	LEUKOCYTE SUBPOPULATION
CD1c	Dendritic cells
CD3	T lymphocytes
CD4	T-helper lymphocytes, canine neutrophils
CD5	T lymphocytes, B-lymphocyte subset
CD8	T-cytotoxic lymphocytes
CD11b	Granulocytes, monocytes, macrophages
CD11c	Monocytes, granulocytes, dendritic cells
CD11d	Macrophages, granular lymphocytes
CD14	Monocytes
CD18	Pan-leukocyte
CD20	B lymphocytes
CD21	Mature B lymphocytes
CD34	Hematopoietic stem cells
CD41/61	Platelets
CD45	Pan-leukocyte
CD61	Platelets
CD79a	B lymphocytes
Factor VIII-related antigen	Megakaryocytes/platelets
Mac387	Granulocytes, monocytes
MHC II	Macrophages, dendritic cells, lymphocytes
Myeloperoxidase	Granulocytes, monocytes
Neutrophil specific antigen	Granulocytes

supported by morphologic criteria, and may provide better prognostic information than previous classification schemes. Histopathology plays a key role in using this system. The lymphoid leukemic forms are classified as acute or chronic. An abundance of blast cells or immature cells identifies the leukemia as acute. Chronic leukemia

has a proliferation of differentiated cells that may be difficult to distinguish from hyperplasia (e.g., reactive response). Generally, chronic leukemias have a more favorable prognosis than acute leukemias and therefore patients survive for longer periods of time.

The morphologic criteria to classify acute myeloid leukemia (AML) in dogs and cats was developed previously by the American Society for Veterinary Clinical Pathology (ASVCP).¹⁵ The percentage of blast cells was suggested as equal to or greater than 30% of nonlymphoid and nonhistiocytic cells in the bone marrow for AML, whereas chronic myeloid leukemia, myelodysplastic syndrome, and leukemoid reaction would be expected to have less than 30% blast cells (see Figure 4-19). However, this percentage has shifted to 20% to reflect the recognition in humans and animals that the range between 20% and 30% is a gray zone with a strong tendency toward a more aggressive clinical course.³⁷ The AMLs are further separated and a subtype is assigned (M1 to M7) depending on the cell type (see Figure 4-19). Adjectives (e.g., granulocytic or myelogenous, monocytic, lymphocytic; see Figure 4-18) are used to designate the cell lineage. If the cell type cannot be determined, it is simply called an acute undifferentiated leukemia.

NOTE: In general, lymphoid neoplasms are more responsive to treatment than acute myeloid leukemia.

Lymphoid Neoplasia

These lymphoid conditions may originate from solid tissues, such as the lymph nodes (i.e., nodal), alimentary tract, thymus, spleen, eye, and skin, or from the bone marrow, such as plasma cell myeloma or some forms of leukemia. A persistent increase in differentiated lymphocytes over time without reaction to known infectious agents or antigenic stimulants suggests neoplasia in lieu of immune stimulation. Lymphoid neoplasms arranged in descending frequency of occurrence are lymphoma, plasma cell myeloma, chronic lymphocytic leukemia (CLL), and acute lymphoblastic leukemia (ALL). The

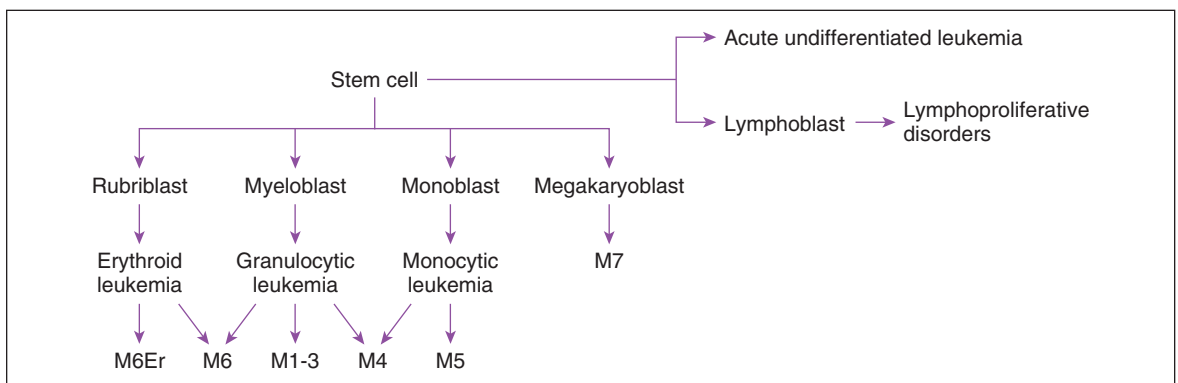


FIGURE 4-18 Diagram depicting origin of the most common neoplastic disorders of immature hematopoietic cells using the FAB classification. M1-3, Myeloblastic and promyelocytic leukemias; M4, myelomonocytic leukemia; M5, monocytic leukemia; M6Er, erythroleukemia with erythroid predominance; M6, erythroleukemia; M7, megakaryoblastic leukemia.

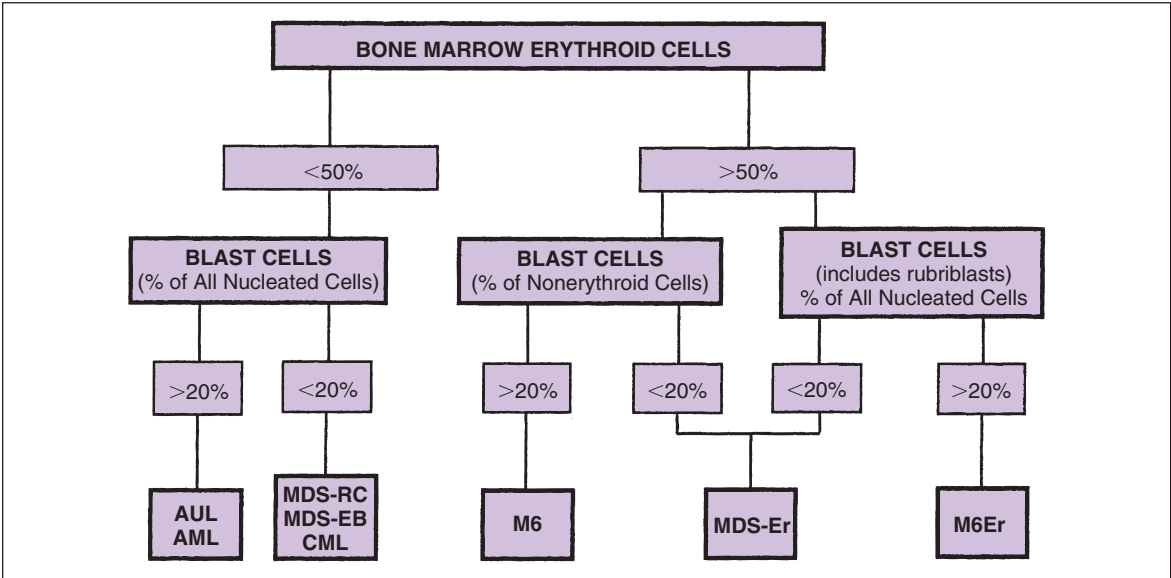


FIGURE 4-19 A scheme to classify immature myeloid leukemias in dogs and cats using the bone marrow erythroid cells from all nucleated cells (ANC). ANC is a group that excludes lymphocytes, plasma cells, macrophages, and mast cells. Nonerythroid cells are calculated as ANC minus erythroid cells. Blast cells include myeloblasts, monoblasts, and megakaryoblasts. AML, Acute myeloid leukemias M1–M5 and M7; AUL, acute undifferentiated leukemia; CML, chronic myeloid leukemias; MDS-EB, primary myelodysplastic syndrome with excess blasts; MDS-Er, myelodysplastic syndrome with erythroid predominance; MDS-RC, primary myelodysplastic syndrome with refractory cytopenia; M6, erythroleukemia; M6Er, erythroleukemia with erythroid predominance. (Reprinted with permission from Raskin RE: Myelopoiesis and myeloproliferative disorders. *Vet Clin North Am Small Anim Pract* 26:1023, 1996.)

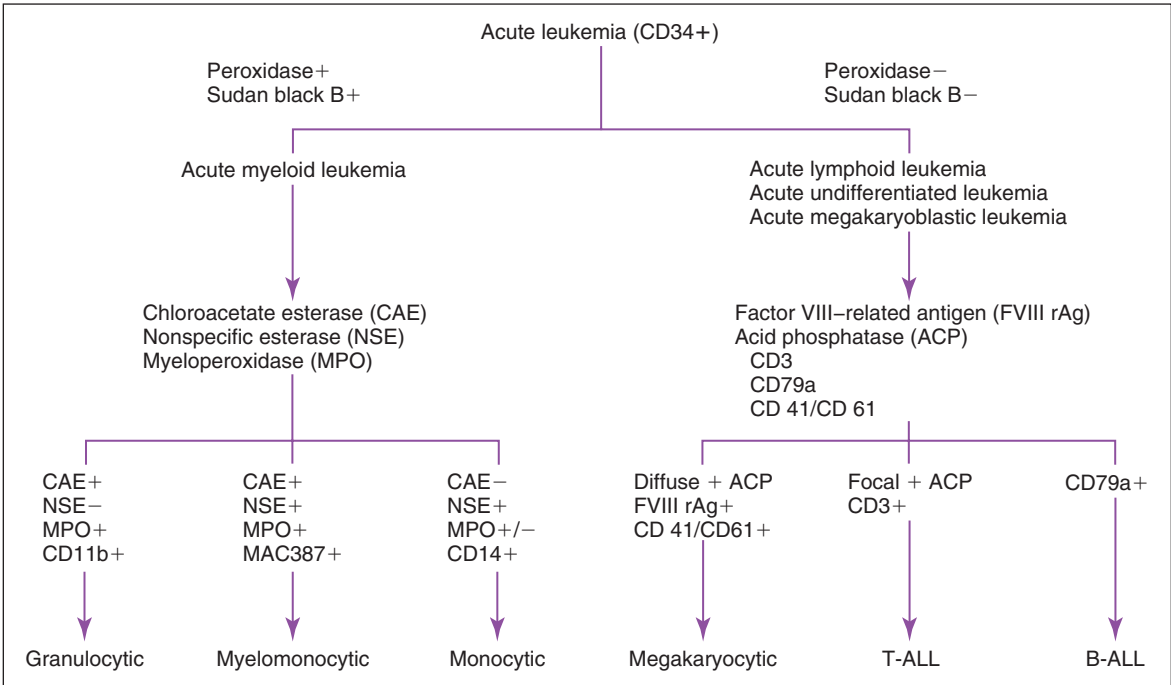


FIGURE 4-20 Simplified classification of acute leukemia using cytochemical stains and immunochemistry. B-ALL, B-cell acute lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia; +, positive; –, negative.

BOX 4-1. LIST OF WELL-DESCRIBED LYMPHOID AND MYELOID NEOPLASMS BASED ON THE 2008 WORLD HEALTH ORGANIZATION CLASSIFICATION SCHEME

LYMPHOID NEOPLASMS

Precursor:

Lymphoblastic Leukemia/Lymphoma (B-, T-, Null-Cell Types)

Mature:

Lymphocytic Leukemia/Lymphoma (B-, T-Cell Types)

Granular Lymphocyte Leukemia/Lymphoma (T-, NK-Cell Types)

Plasma Cell Neoplasia

Myeloma

Plasmacytoma (Extramedullary Sites)

Lymphoma (B- and T-Cell Types of Nodal and Extranodal Sites)

Mycosis Fungoides/Sézary Syndrome (T-Cell Type)

Marginal Zone (B-Cell Type)

MYELOID NEOPLASMS

Myelodysplastic Syndromes:

Myelodysplastic Syndrome—Refractory Cytopenia

Myelodysplastic Syndrome—Excess Blasts

Acute Myeloid Leukemias:

Acute Myeloid Leukemia (Myeloblastic, Monoblastic, Erythroid, Megakaryoblastic)

Myeloproliferative Neoplasms:

Chronic Myelogenous/Neutrophilic Leukemia

Chronic Eosinophilic Leukemia

Chronic Basophilic Leukemia

Polycythemia Vera

Essential Thrombocythemia

Primary Myelofibrosis

Mastocytosis (Cutaneous and Systemic)

Histiocytic Neoplasms

Histiocytic Sarcoma (Dendritic Cell)

Hemophagocytic Histiocytic Sarcoma (Macrophage)

Canine Cutaneous Histiocytoma (Dendritic Cell)

Feline Progressive Histiocytosis (Dendritic Cell)

approximate annual incidence rate for leukemias in dogs and cats is 31 and 224 cases per 100,000 animals, respectively. Lymphoid malignancies, including lymphoma, are responsible for over 85% of cat leukemias and over 65% of the dog leukemias. Cats have a 6-fold greater incidence of lymphoma and a 16-fold greater incidence of myeloid neoplasia than dogs, primarily the result of induction of neoplasia by FeLV infection.

Precursor Lymphoid Neoplasms

Acute Lymphoblastic Leukemia • ALL occurs less frequently than lymphoma. This rapidly progressive condition occurs mostly in middle-aged dogs (mean 6 years) and frequently in FeLV-infected cats. Because leukemia

originates in the bone marrow, ALL may be associated with variable nonregenerative anemia, neutropenia, and thrombocytopenia in any combination. As the condition advances, lymphoblasts are found in enlarged visceral organs such as the liver and spleen. In ALL, lymphocyte size on the stained blood smear varies, with many lymphoblasts. A CBC usually allows diagnosis of ALL; however, bone marrow is more consistently abnormal (i.e., dense infiltration by lymphoblasts). Lymphoblasts are difficult to distinguish from poorly differentiated myeloid precursors without cytochemical stains, cell surface markers, or PCR clonality tests (see Figure 4-20). The presence of CD34 surface antigen is particularly helpful in distinguishing metastasis from an immature solid tissue lymphoma from a blast cell originating from the bone marrow, and this in turn may influence response to therapy. Most feline ALL cases have the T-cell immunophenotype, but canine cases may be T-, B-, or null-cell phenotypes. Hypercalcemia is an infrequent laboratory finding.

Reactive or blast-transformed lymphoid cells on blood smears, especially in young animals undergoing strong immune stimulus (e.g., infections), may confuse the diagnosis of ALL or other leukemias. In many non-neoplastic conditions (e.g., immune stimulation by illness or vaccination), a few to several reactive lymphocytes and blast-transformed lymphocytes (perhaps five per blood smear) may be observed. In contrast, ALL has a predominance of lymphoblasts and perhaps five lymphoblasts per oil immersion field. The use of buffy coat preparations to concentrate the leukocytes to find a few lymphoblasts is not advised, because these preparations often have several blast-transformed lymphoid cells in normal animals.

Mature Lymphoid Neoplasms

Chronic Lymphocytic Leukemia • CLL has a relatively favorable prognosis of continued life for about 12 months after diagnosis. It occurs mostly in middle-aged to elderly dogs (mean 10 years), with a higher frequency in females. Cats rarely have this form of leukemia; most that do are FeLV negative. Differentiation of CLL from ALL or lymphoma with a leukemic blood profile is based on the relatively mature appearance of lymphocytes in CLL versus finding lymphoblasts in the other two conditions. Lymphocytes in CLL are larger than normal and more homogeneous in appearance than in normal animals.

Diagnosis of CLL is aided greatly by magnitude of the lymphocytosis, which may exceed 40,000 to 100,000 lymphocytes/ μ l. Maximum lymphocytosis in extreme immune reactions (e.g., chronic canine rickettsial infections) seldom exceeds 25,000 lymphocytes/ μ l and usually is less than 15,000 lymphocytes/ μ l. Lymphocytosis in cats with strong persistent immune stimulus in nonleukemic diseases may reach higher maximum lymphocyte counts (e.g., 40,000/ μ l). Reactive lymphocytosis in young, asymptomatic cynomolgus monkeys can reach 70,000 lymphocytes/ μ l for 1 or 2 weeks. These extreme changes may be called a lymphocytic leukemoid reaction. CLL is the first entity to rule out when lymphocytosis exceeds 25,000 to 40,000 lymphocytes/ μ l. The likelihood of CLL increases proportionally to lymphocytosis; counts greater

than 50,000 to 100,000/ μ l are particularly diagnostic. Mild normocytic normochromic, nonregenerative anemia is common, with variable thrombocytopenia.

Fine-needle aspirates of lymph nodes, spleen, and liver in CLL document lymphoid proliferation, but differentiation of hyperplasia from CLL may be difficult. CLL has a more homogeneous population, whereas hyperplasia has a more diverse population of lymphocytes, plasma cells, and lymphoblasts. Biopsy and histopathology may better document infiltration by neoplastic lymphocytes with loss of tissue architecture. In bone marrow aspirates, greater than 15% to 20% lymphocytes among the nucleated cells present suggests CLL.

Immunophenotyping has demonstrated many of these cases to be T cell in origin in the dog. Dysproteinemia may occur in dogs with B-cell CLL related to monoclonal gammopathy (most often immunoglobulin M [IgM], but immunoglobulin A [IgA] and immunoglobulin G [IgG] gammopathies also occur). Use of a diagnostic test to determine clonality by analysis of gene rearrangements may be helpful to decide whether lymphocyte proliferation is malignancy or hyperplasia.

Granular Lymphocyte Leukemia/Lymphoma • A subtype of lymphoid leukemia and lymphoma involves mostly medium-sized lymphoid cells having abundant light-blue to clear cytoplasm that contains several small red or purple granules, termed *azurophilic granules*. These cells are called *large granular lymphocytes* (LGLs) and are normally present in the blood of many animal species; specifically in the dog, LGLs are present in 0% to 5% of leukocytes or in 0% to 19% of lymphocytes. The LGL count may be increased as a result of immune stimulation such as seen in canine ehrlichiosis. However, neoplastic proliferations of LGLs are common. In the cat, granular lymphocytes associated with intestinal lymphoma have prominent large coarse purple cytoplasmic granules. In the dog, LGL malignancies may appear as leukemia in the blood or as lymphoma in tissues such as the lymph nodes and spleen. In these tissue forms, blood lymphocyte counts can be normal; however, neutropenia may be evident. The granules do not stain well with aqueous-based Wright stains, such as those found in commercial quick stains. However, the granules appear readily with Wright-type stains. Immunophenotyping of these cells indicate most are cytotoxic T cells expressing CD3 and CD8.

In the dog, LGL leukemia may be first recognized as a benign nontransitory proliferation of granular lymphocytes in the peripheral blood, termed *persistent lymphocytosis*. These cells are thought to arise from the red pulp of the spleen. As disease progresses, splenomegaly is commonly noted and cytologic aspirates demonstrate a marked increase in the LGL population. Typically, the bone marrow appears normal or contains minimal LGL involvement. Most dogs with LGL leukemia appear as though they have CLL, with a slow indolent course over several years, whereas a small group may have a more aggressive course, similar to ALL.²⁴

Plasma Cell Neoplasms • Plasma cell myeloma (PCM) is a disorder of bone marrow plasma cells. Extramedullary plasmacytoma occurs in soft tissues (e.g., liver, kidney,

spleen, skin). Four diagnostic features of PCM are (1) hyperproteinemia with a monoclonal gammopathy, (2) osteolytic lesions in the spine, (3) greater than 15% to 20% plasma cells in bone marrow aspirates, and (4) Bence Jones proteinuria (see Chapters 7 and 12). Bence Jones proteinuria is infrequent in dogs and cats, and these light chains of antibodies are best identified by electrophoresis of urine with detectable proteinuria. Ehrlichiosis may mimic plasma cell neoplasia in having strong plasmacytosis in the bone marrow and an oligoclonal gammopathy in serum resembling a monoclonal gammopathy.

Histologic or cytologic evaluation of osteolytic areas or soft tissue masses is most diagnostic of PCM (see Chapter 16). Neoplastic plasma cells may exhibit anisocytosis, anisokaryosis, and a finely dispersed chromatin pattern. Binucleate plasma cells occur frequently in PCM, but binucleated plasma cells are normally visible in hyperplastic lymphoid tissue (e.g., lymph node aspirate). Plasma cell leukemia (i.e., neoplastic plasma cells in blood) occurs infrequently.

Nodal Lymphoma • Lymphoma (formerly lymphosarcoma) is the most common lymphoid neoplasm of dogs and cats. Lymphoma usually originates in peripheral lymphoid tissues but may develop within extranodal sites (e.g., skin) or from bone marrow lymphocytes that populate solid tissues. Multicentric disease is more common in dogs and usually involves the peripheral lymph nodes, spleen, and liver. In descending frequency, other forms of canine lymphoma include alimentary, thymic, and cutaneous disease. Thymic lymphoma is most common in young cats, whereas alimentary lymphoma is more common in older cats. Cats may also develop multicentric, renal, and cutaneous lymphoma. About 60% to 80% of cats with the thymic or multicentric forms of lymphoma are positive for FeLV group-specific antigen, whereas only 30% of cats with alimentary lymphoma are positive.

The CBC is not sensitive in identifying canine lymphoma; 21% of affected dogs have lymphocytosis and 25% have lymphopenia. At the time of diagnosis, up to 57% of dogs may be leukemic.³² Approximately 30% of cats with lymphoma have a leukemic blood profile. The CBC may document concurrent cytopenias, including anemia, thrombocytopenia, and leukopenia. Bone marrow involvement is used in staging lymphoma and so helps to determine treatment. Bone marrow evaluation is needed, because CBC results may not reflect marrow involvement and vice versa. Bone marrow core biopsies are most sensitive in identifying leukemic involvement (e.g., 97% of canine cases compared with 50% by blood smear analysis alone or 60% using bone marrow aspirate biopsies alone).³² Hypercalcemia (i.e., >12 mg/dl), a factor most associated with T-cell lymphomas (see Chapter 8), is helpful in directing diagnostic efforts toward a lymphoid malignancy. Monoclonal gammopathy may occur infrequently with B-cell lymphoma.

Lymphoma is diagnosed daily by cytopathologists (see Chapter 16). Fine-needle aspirates of lymph nodes or other affected tissues and organs usually disclose a homogeneous population of lymphoblasts or larger lymphocytes. If cytology is equivocal in distinguishing

extreme lymphoid hyperplasia from early lymphoma, surgical biopsy with histopathology may provide a definitive diagnosis based on alterations of normal tissue architecture. Surgical or endoscopic biopsies of deep tissues also may be diagnostic. This is often helpful in cases of marginal zone lymphoma, a type of B-cell lymphoma with an indolent course that usually presents with a mixed lymphocyte population of cells that mimics the appearance of a reactive lymph node.

Immunophenotyping is useful for prognostic evaluation of lymphoma cases; dogs with T-cell lymphoma are at significantly higher risk of relapse and early death compared with B-cell lymphoma. Diagnostic immunocytochemistry of fine-needle aspirate material (or evaluation by flow cytometry) and immunohistochemistry of tissue sections are performed currently at certain academic institutions (e.g., University of California at Davis and Colorado State University). However, while generally “T is terrible and B is best,” this is not always the case, and prognostic indicators involve other parameters.

In contrast to the mediastinal or thymic lymphoma that is composed of neoplastic lymphocytes and most associated with young cats and dogs, a neoplasm of thymic epithelium, termed *thymoma*, contains a variable numbers of mature lymphocytes and is usually observed in older dogs and cats. Both neoplasms will present as a cranial mediastinal mass, often with evidence of coughing, dyspnea, or thoracic effusion, including chylothorax. Thymic tumors are associated with paraneoplastic hypercalcemia. Thymoma may involve a concurrent mature lymphocytosis exceeding 25,000/ul of blood and may be confused with CLL. Thymomas are potentially treatable. Surgical biopsy is the preferred method of diagnosis for thymoma, as cytology may be confusing because of secondary chylothorax or the presence of many mast cells.

Extranodal Lymphoma (Mycosis Fungoides) • Mycosis fungoides is an uncommon form of epitheliotrophic cutaneous lymphoma (ECL) usually beginning in the skin and progressing to involve lymph nodes, spleen, and bone marrow. A diagnostic feature of mycosis fungoides is focal accumulation of lymphoid cells within the epidermis, forming “Pautrier’s microabscesses.” “Sézary syndrome” is a rare variant of ECL associated with a leukemic blood profile characterized by large T cells with markedly convoluted nuclei.²¹ The neoplastic cells of ECL are cytotoxic T cells that express CD3 and CD8.

In comparison to the epitheliotrophic mycosis fungoides, nonepitheliotrophic lymphoma is less frequent. This form was previously believed to be of B-cell origin; however, recent findings indicate this is exclusively T-cell lymphoma, although the morphologic and immunologic characteristics differ from mycosis fungoides. Both forms are aggressive and respond sporadically or poorly to treatment.²⁹

Myeloid Neoplasms

Characteristics of selected myeloid neoplasms are described under the following headings (see [Box 4-1](#)). Myelodysplastic syndromes (MDS) involve a preleukemic condition as well as a neoplasia of mature and poorly differentiated nonlymphoid cells that display significant

dysplasia. Generally, AMLs are severe, rapidly progressive diseases, essentially unresponsive to treatment. In contrast, myeloproliferative neoplasms involve relatively mature cells that are released from the bone marrow and the clinical course may be long. This category also includes the proliferative conditions involving mast cells, such as cutaneous mastocytoma and systemic mastocytosis. In myeloid neoplasia, the leukemic cell type may vary over time in the same animal; therefore, the diagnosis may reflect a specific point in time.

Blast cells of various cell types often lack identifiable characteristics, and the initial impulse is to designate an undifferentiated blast cell as a lymphoblast. Blast cells in myeloid neoplasia are distinguished by sequential differentiation into more mature myeloid cells, if present, or with cytochemical staining of a poorly differentiated leukemia (see [Table 4-7](#)). Cytogenetic investigations in animals are beginning to determine chromosomal defects similar to those of people with the same disorders, and prognostic markers may be helpful in treating these conditions.^{6,12,16}

NOTE: Myeloid neoplasia occurs infrequently in contrast to lymphoid neoplasia, which is much more commonly observed.

Myelodysplastic Syndromes

Primary MDS is a clonal neoplastic disease, indicating it originates from a single transformed stem cell that affects multiple nonlymphoid lineages such as erythroid, granulocytic, and megakaryocytic precursors. Dysplastic changes may occur as preleukemia (i.e., before the onset of overt leukemia, which may take months to years to develop). This neoplastic condition is best considered as “leukemia *in situ*,” that is, the defect remains localized to the bone marrow and does not spread or metastasize through the blood to other parts of the body. Generally primary MDS presents as persistent peripheral cytopenia in one or more nonlymphoid lineages together with significant morphologic abnormalities. The bone marrow is typically hypercellular in the affected cell line, and the blast cell percentage is less than 20% of the nucleated cells using the most current human WHO classification scheme.³⁷ Two subtypes of MDS appear to have predictive value in cats, with significant differences in median survival time.⁴⁴ One subtype, MDS with refractory cytopenia, was defined as less than 6% myeloblasts in bone marrow specimens and demonstrated 11.7 months’ survival, while cats with the other subtype, MDS with excess blasts, defined as 6% to 30% myeloblasts in the bone marrow, survived less than 1 month.

Acute Myeloid Leukemia

AML refers to a collection of neoplastic disease affecting precursors of nonlymphoid cells. Subtypes M0 through M7 are defined by the cell of origin and the degree of differentiation.³⁰ Cytochemical staining (see [Figure 4-20](#) and [Table 4-7](#)), immunocytochemistry (see [Table 4-8](#)), or flow cytometry can be used to distinguish the subtypes.^{2,43}

For example, *acute myeloblastic (myelogenous) leukemia* (AML-M2) usually consists of neutrophils, but coproduction of basophils and eosinophils may occur. Total leukocyte counts are variable, but extreme leukocytosis may occur. Blast cells are often found in circulation. Severe anemia and thrombocytopenia may accompany AML. Myeloblasts, promyelocytes, and atypical cells denote subtypes M1 to M3. Myeloblasts may have a moderately basophilic cytoplasm with few small azurophilic granules. A disorderly maturation sequence also suggests neoplasia but may occur during repopulation of the bone marrow after cellular destruction (e.g., toxin, parvovirus).

Repopulation of the bone marrow begins with immature cells (i.e., myeloblasts, progranulocytes, myelocytes), without the expected predominance of mature bands and segmenters; this appearance initially resembles leukemia, but progressive maturation eventually restores a normal marrow cell population. Diagnosis may require reevaluation of the bone marrow after a few days.

Myelomonocytic leukemia (M4) is characterized by combined production of neutrophils and monocytes by the bipotential stem cell, resulting in myeloblasts and monoblasts that equal or exceed 20% of nonerythroid cells in the bone marrow. This is the most common nonlymphoid leukemia. Percentage of monocytes and neutrophils may change as the disease progresses. Moderately severe anemia is expected.

Monocytic leukemia (M5) occurs infrequently. Cytochemical staining may prevent misdiagnosis of monocytic leukemia as ALL. Monoblasts are increased to 20% or greater of the nonerythroid cells of the bone marrow. Monoblasts have basophilic cytoplasm that lacks any obvious granulation; nuclei are irregularly rounded, producing a folded or creased appearance.

Erythroleukemia (M6) involves proliferation of immature and atypical erythroid and granulocytic cells. The erythroid component exceeds 50%, with myeloblasts and monoblasts (together) equal to or greater than 20% of nonerythroid cells. A variant form, called M6E_r, consists predominantly of erythroid precursors; in this form rubriblasts exceed 20%. The M6 conditions are more common in cats than in dogs. Severe nonregenerative anemia and dysplastic changes are frequently prominent. Over time, this form of leukemia may change in appearance and progress to involve predominantly granulocytic precursors. It is often associated with FeLV infection in cats.

Megakaryoblastic leukemia (M7) has been reported in both dogs and cats.¹¹ Leukocyte and platelet counts vary, and circulating megakaryoblasts may be found. These cells have a round nucleus and scant basophilic cytoplasm with a ragged irregular cell surface. Platelet morphology is often bizarre, characterized by giantism and abnormal granulation. Diagnosis of this form of leukemia requires positive cytochemical reactions to periodic acid-Schiff, alpha naphthyl acetate esterase, acetylcholinesterase, factor VIII-related antigen, or immunologic markers. Electron microscopy may reveal characteristic alpha granules or early internal membrane demarcation systems. Some previously reported cases have been misdiagnosed as AML-M7 that were more correctly identified as myeloid hyperplasia with myelofibrosis.

Myeloproliferative Neoplasms

This group of neoplasms comprises most of the chronic myeloid leukemias, which appear with a marked increase in numbers of the affected specific mature cell type. Blast cell percentages are less than 20% in the bone marrow, and cytogenetic abnormalities have been documented in rare cases.^{6,12}

Chronic myelogenous leukemia (CML) and *chronic neutrophilic leukemia* (CNL) differ from acute granulocytic leukemia in that segmented and band neutrophils predominate. The two conditions are similar to each other except for the presence of the Philadelphia chromosome translocation in CML and not found in CNL. The left shift in CNL may extend back to promyelocytes, and bone marrow myeloid proliferation may involve orderly myeloid maturation with a marked increase in the myeloid:erythroid ratio. This ratio may fall between 4:1 and 25:1 or be as high as 36:1. The bone marrow contains less than 20% myeloblasts of nonlymphohistiocytic cells (see Figure 4-19). Diagnosis of CNL is often based on finding marked, persistent leukocytosis (40,000 to 200,000/ μ l) and exclusion of a leukemoid reaction, such as inflammatory leukocytosis (discussed earlier in the section on Inflammation). Anemia is mild to moderate and platelet counts are variable. Lymphadenopathy with lymph node aspirates that resemble marked extramedullary hematopoiesis may occur in CGL. The prognosis and response to treatment are better than for AML, with death occurring months after diagnosis because of a blast cell crisis.

Chronic eosinophilic leukemia is a variant form of myelogenous leukemia. This type of leukemia is rare but has been reported in the dog and documented in the cat associated with FeLV infection. It is characterized by a marked, persistent eosinophilia (often > 50,000/ μ l) and a shift toward immaturity. A moderate anemia may be present. It may be difficult to differentiate from hypereosinophilic conditions (e.g., hypereosinophilic syndrome, allergies, parasitism, eosinophilic inflammatory diseases, mast cell tumors, certain lymphomas). Leukemic eosinophils will leave the bone marrow and infiltrate solid tissues, such as the lymph nodes, liver, and spleen.

Chronic basophilic leukemia is another variant form of myelogenous leukemia that is uncommon and reported to occur mostly in dogs. This form of neoplasia can be distinguished from mast cell leukemia by subtle nuclear indentation, segmentation, or lobulation. In dogs, the cytoplasmic granules in neoplastic basophils may be coarser than those in mast cells. The disorder has been associated with thrombocytosis and anemia. Prognosis is good with treatment.

Chronic myelomonocytic leukemia (CMMoL) is an uncommon disorder in which blast cells of both granulocytic and monocytic lines involve less than 30% of nonlymphohistiocytic bone marrow cells. Cases often display peripheral monocytosis greater than 4000/ μ l, and dysplastic changes are significant. For this reason, this form of chronic myeloid leukemia is placed in a separate WHO category reflecting the myeloproliferation and myelodysplasia together. Diagnosis is suspected if marked monocytosis accompanies neutrophilia, without

indications of an inflammatory condition. CMMoL may progress over time to an AML.

Polycythemia vera (primary erythrocytosis) is a rare disease in dogs and cats involving the neoplastic production of mature, anucleated erythrocytes. Polycythemia is suggested by brick-red mucous membranes related to markedly increased hematocrits (i.e., PCV 65% to 82%). Splenomegaly, if present, is mild. Polyuria, polydipsia, hemorrhage, and neurologic disorders occur in 50% of canine cases. Definitive diagnosis of polycythemia vera requires ruling out other causes of erythrocytosis or absolute polycythemia (see Chapter 3). Diagnosis is based on demonstration of an absolute increased red cell mass, a normal arterial partial pressure of oxygen (PaO_2), and a decreased serum erythropoietin concentration that is measured at specialized laboratories. Renal cysts, pyelonephritis, and tumors must be excluded, because they can also produce absolute polycythemia (i.e., inappropriate secondary erythrocytosis) as a paraneoplastic syndrome with increased serum erythropoietin concentration.

Essential thrombocythemia (i.e., primary thrombocythemia) is a rare chronic myeloproliferative disease characterized by proliferation of megakaryocytes and unregulated platelet production reported in the dog and cat. It occurs unrelated to physiologic or responsive thrombocytosis. Clinical signs include splenomegaly and platelet function abnormalities, such as spontaneous bleeding and thromboembolism (see Chapter 5). Platelet counts are persistently above 600,000/ μl and often greater than 1 million/ μl , which predispose to microthrombosis and microvascular ischemia. Neutrophilia or basophilia may also be seen.

Primary myelofibrosis (previously idiopathic myelofibrosis) has been termed *agnogenic myeloid metaplasia* or *chronic megakaryocytic-granulocytic myelosis*. This uncommon condition results in intramedullary and extramedullary hematopoiesis that is accompanied by a reactive or secondary marrow fibrosis late in the course of the disease. The hematopoietic precursors most involved are granulocytic and megakaryocytic forms, which infiltrate the spleen and liver. Some cases may be mistaken for AML of megakaryocytic origin. The peripheral blood often has concurrent immature granulocytes and erythroid cells, termed a *leukoerythroblastic reaction*. Erythrocytes may display poikilocytosis with a teardrop formation. Bone marrow aspiration is often difficult, related to the presence of myelofibrosis; therefore, core biopsy is recommended to confirm the diagnosis. Survival varies from months to years, depending on the response to treatment for the nonregenerative anemia.

Mast cell neoplasia encompasses a variety of presentations from an isolated mass to dissemination. Mast cell leukemia may originate in the bone marrow of dogs and is rare. Mastocytemia or systemic mastocytosis may occur secondary to a solid mast cell tumor (see Chapter 16) or may suggest severe inflammatory disorders, particularly parvoviral enteritis. In general, the larger the number of mast cells in blood, the more likely that systemic mast cell neoplasia is present, especially in the absence of enteritis. Mast cells are not expected in blood smears from healthy dogs.⁵ Normal canine marrow has 0 to 1 mast cell/1000 nucleated cells, and greater than 10 mast cells/1000 nucleated cells in bone marrow smears is

considered increased and supportive of hemolymphatic involvement in disseminated mast cell neoplasia.

Mast cell leukemia can be distinguished from basophilic leukemia based on morphologic and cytochemical criteria. The total number of mast cells per blood smear in dogs with enteritis and mastocytemia usually ranges from 2 to 9, but 30 to 90 mast cells per smear can be found. It is rare to find circulating metastatic mast cells from mast cell tumors of the skin in dogs and cats. Circulating mast cells are more common in cats affected with the visceral form of mast cell neoplasia producing diffuse, moderate to marked splenomegaly. Erythrophagocytosis by the circulating mast cells is not unusual, and this may contribute to anemia. Typically, disseminated disease involves the spleen, liver, distant lymph nodes, or bone marrow. Cytology or surgical biopsy documents disseminated mast cell neoplasia best.

Histiocytic Neoplasms

Histiocytic neoplasms are a significant and important group of bone marrow and solid tissue neoplasms involving dendritic cells and macrophages. They range from benign to highly aggressive malignancies in domestic animals.

Presentation of marked anemia suggests a variant of histiocytic sarcoma, hemophagocytic histiocytic sarcoma, which involves a malignancy of macrophages. Immunologic markers are helpful in making this distinction. Both dogs and cats may present with the histiocytic sarcoma complex of diseases. Solid tissue forms of dendritic neoplasia are seen in the dog as cutaneous benign histiocytoma and in the cat as feline progressive histiocytosis.

Histiocytic Sarcoma

Histiocytic sarcoma is a localized neoplasm of myeloid dendritic cells that may become disseminated over time, at which point the condition is termed *disseminated histiocytic sarcoma*.¹ The disseminated form is very similar to a more aggressive disease known as malignant histiocytosis, which forms multisystem lesions simultaneously. That also arises from myeloid dendritic cells. Older animals are at greater risk, and certain breeds such as golden and flat-coated retrievers, rottweilers, and Bernese mountain dogs have increased incidence.³³ Primary sites affected include the periarticular subcutis, spleen, lungs, and bone marrow; secondary sites are the liver, lymph nodes, and kidneys. Anemia, thrombocytopenia, and hyperbilirubinemia are the most common laboratory abnormalities. Cytology demonstrates malignant histiocytes to be large, frequently markedly pleomorphic round or stellate cells with abundant (sometimes vacuolated) basophilic cytoplasm. Nuclei are oval to reniform with lacy chromatin and prominent multiple nucleoli. Multinucleate cells are common, and mitotic figures are often frequent. A prolonged survival may be possible with surgical excision of a localized histiocytic sarcoma.

Hemophagocytic Histiocytic Sarcoma

A hemophagocytic variant of histiocytic sarcoma is associated with a Coombs'-negative regenerative anemia related to marked erythrophagocytosis by the neoplastic splenic red pulp and bone marrow macrophages.²⁷ A similar

situation may occur with infectious or inflammatory disease. The more anaplastic appearance of the malignant cells and the lack of a history of concurrent infection may help distinguish malignant histiocytosis from the inflammatory condition. Differentiation of malignant histiocytosis from other neoplasms is confirmed by demonstration of positive histiocytic cytochemical and immunohistochemical markers with negative lymphoid or epithelial markers. Similar-appearing cells may actually be T-cell or B-cell lymphoma when immunophenotyping or gene rearrangement studies are performed. Due to the rapid progression of the disease, prognosis is poor. Other laboratory findings include hypoalbuminemia, thrombocytopenia, and hypocholesterolemia, with the two former findings as negative prognostic variables.

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5

Hemostatic Abnormalities

Harold Tvedten

GOALS OF HEMOSTASIS TESTING

Recently the goals of hemostasis testing have undergone a paradigm shift. Previously testing was primarily used to determine why the patient was bleeding or if it would be likely to bleed during surgery. Thus most testing had related to establishing platelet numbers and function, tests of defects in coagulation, and tests of fibrinolysis for disseminated intravascular coagulation (DIC). More recently there is emphasis in establishing if the animal is hypercoagulable and prone to thromboembolic problems. Thromboelastography (TEG) has become very popular, especially in Europe, to establish whether the net effect of cells and plasma factors in blood shows a hypercoagulable, normocoagulable, or hypocoagulable tendency. TEG testing has indicated that patients can be hypercoagulable even if they have thrombocytopenia and/or deficiencies of coagulation factors causing prolongation of coagulation tests such as activated partial thromboplastin time (aPTT). TEG is a more global test of hemostasis than tests that evaluate specific deficiencies.

Hemostasis and Diagnosis of Disorders

The objective of hemostasis is to maintain a balance of coagulation and fibrinolysis to preserve proper vascular structure and function and blood fluidity. When a vascular structure is damaged, it is necessary to have adequate platelet numbers and function (primary hemostasis), as well as sufficient factors and cofactors of the coagulation system (secondary hemostasis), to stop bleeding. An intact fibrinolytic system localizes clotting to the area of vascular damage and acts to reopen blood vessels while healing occurs. An imbalance of these factors leads either to hypercoagulation and thromboembolic disorders or to hypocoagulation and hemorrhage or both in DIC.

This chapter has three sections. The first section introduces the components of normal hemostasis, including vascular endothelium, platelets, coagulation factors, and fibrinolysis. The second section describes selected hemostatic tests and factors that affect how they may be used in diagnosis. The third section briefly describes selected inherited and acquired disorders of hemostasis and their laboratory diagnosis.

Evaluation of the cause of bleeding varies from handling of overt problems (e.g., severe epistaxis), to detection of suspected problems (e.g., von Willebrand's disease [vWD] in a Doberman pinscher), to investigation of unexpected problems found during laboratory examination (e.g., mild to moderate thrombocytopenia, evidence of hepatic disease). Signs of bleeding problems may include prolonged bleeding after parturition, estrus, or minor trauma (e.g., venipuncture, loss of deciduous teeth), or spontaneous hemorrhages (i.e., petechiae, ecchymoses, hematomas). Gastrointestinal tract bleeding may appear as fresh red blood or dark tarry stools and is often occult. Bleeding into joints and muscle may cause lameness. Bleeding into body cavities may be diagnosed from fluid cytology. Small hemorrhages such as petechiae and ecchymoses suggest a platelet or vascular defect (Figure 5-1). Epistaxis is often associated with platelet defects, perhaps because of the paucity of tissue between the vessels and the nasal mucosa. In contrast, coagulation defects are characterized by large, deep hemorrhages (e.g., hematomas, hemarthroses).

Evaluation of a patient with a suspected bleeding disorder requires not only selection of appropriate tests but, for the best diagnosis, requires a complete history and physical examination because of the high association of hemostatic disorders with breed, age, treatments, and poisonings. Some screening tests should be run in private practice. Point-of-care analyzers such as the VetScan VSpro Coagulation Analyzer (Abaxis North America, Union City, CA) are available for in-clinic analysis of aPTT and prothrombin time (PT). However, hemostasis testing by reference laboratories and especially hemostasis centers should have better control of methods and reagents and a more complete selection of tests that may be necessary to identify mild or uncommon problems (see later discussion of methodology).

A simple approach for test selection is shown in Figure 5-2. If physical examination shows no cause of the bleeding or bleeding seems disproportionate to the injury, the expense of hemostatic tests (relatively expensive testing) is warranted to document and localize the defect. Some situations require only a single test (e.g., von Willebrand's factor [vWF] assay or capillary bleeding time as a presurgical screen for elective surgery on a Doberman

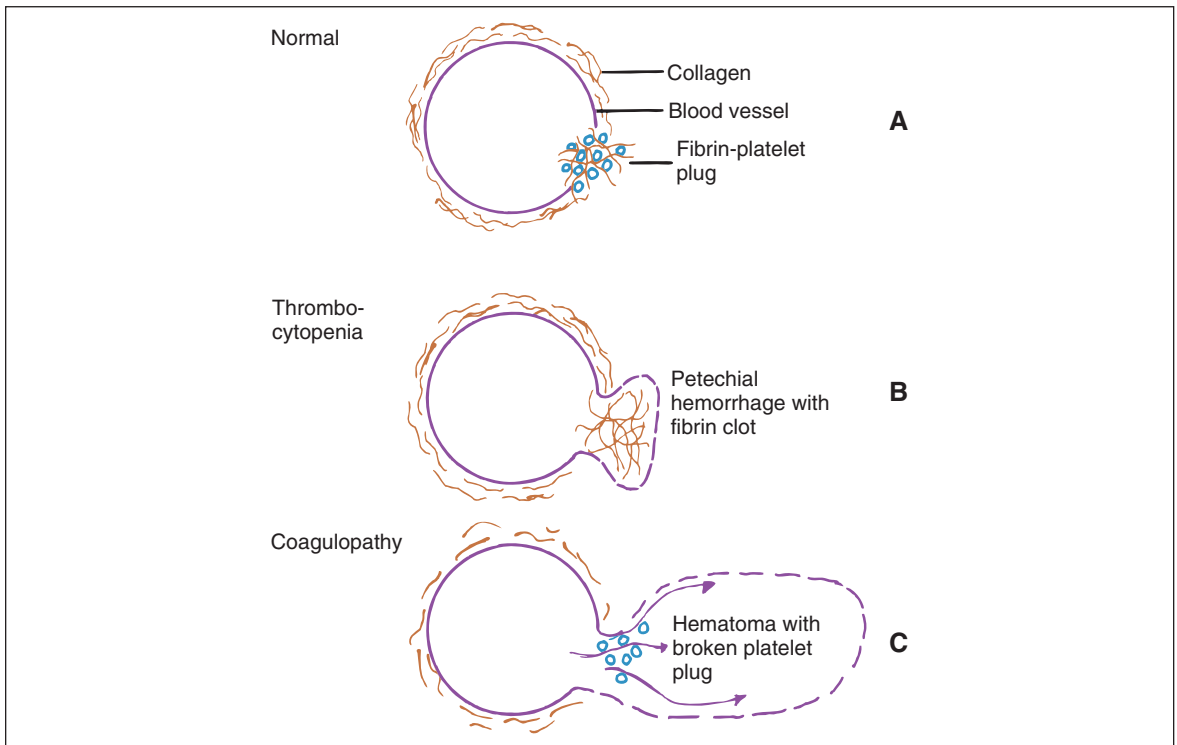


FIGURE 5-1 Physical evidence of thrombocytopenia or coagulopathy. The difference in the size of hemorrhage in thrombocytopenia versus coagulopathy is illustrated. **A**, A break in a vessel is normally repaired quickly when platelets and coagulation factors are stimulated by exposure to collagen to form a platelet plug that is rapidly stabilized by fibrin strands. **B**, A proper platelet plug is not formed in thrombocytopenia, but only a little blood leaks into the collagen before a fibrin clot is formed. **C**, A coagulation factor defect prevents rapid or firm fibrin stabilization of the platelet plug, and the platelet plug breaks, allowing abundant hemorrhage.

due to their high incidence of having vWD). Similarly, PT is the preferred single test for suspected ingestion of warfarin-type rodenticide. However, a profile of tests performed the same day is recommended to localize an unknown bleeding defect (Table 5-1; Table 5-2). Some hemostatic disorders affect multiple areas of hemostasis,

so evaluation of only one or two tests may lead to an incomplete or erroneous conclusion. Variation in production and inactivation of coagulation and anticoagulation factors and variation in production and removal of platelets and effects of treatment confuse diagnosis; therefore, performing a complete profile of tests concurrently at initial presentation permits a more likely correct diagnosis. Normal results in a profile exclude some diseases from consideration.

A reasonable profile includes a platelet count, a test of platelet function, the aPTT to assess intrinsic and common coagulation pathways, the PT to assess extrinsic and common pathways, and a test of fibrinolytic activity such as D-dimer (see Table 5-1). Alternative and additional tests are used in certain situations (see section on Laboratory Tests).

Hemostatic defects should initially be localized to a general area of the hemostatic mechanism, which has four main components: the blood vessels, the platelets, coagulation factors, and the fibrinolytic system (Table 5-2). A plug of aggregated platelets initially seals damaged vessels after circulating platelets come into contact with exposed subendothelial collagen (primary hemostasis). Platelets bind to collagen and each other with the help of vWF (Figure 5-3; see Figure 5-1). The coagulation factors stimulated by the collagen, tissue thromboplastin, and platelets form fibrin strands to stabilize the platelet plug

TABLE 5-1. HEMOSTATIC SCREENING PROFILE

PREFERRED TEST	ALTERNATIVE TEST	PARAMETER TESTED
Platelet count	Blood smear estimate	Platelet number
CBT, BMBT	PFA-100	Platelet function
aPTT	ACT	Coagulation factors (see text)
PT	Specific factor analysis	Coagulation factors (see text)
D-dimer	FDP, TEG	Fibrinolysis
vWf assay	Genetic testing	vWF, vWD

ACT, Activated coagulation time; aPTT, activated partial thromboplastin time; BMBT, buccal mucosal bleeding time; CBT, capillary bleeding time; FDP, fibrin degradation products; PFA-100, Platelet Function Analyzer; PT, prothrombin time; TEG, thromboelastography; vWD, von Willebrand disease; vWF, von Willebrand's factor.

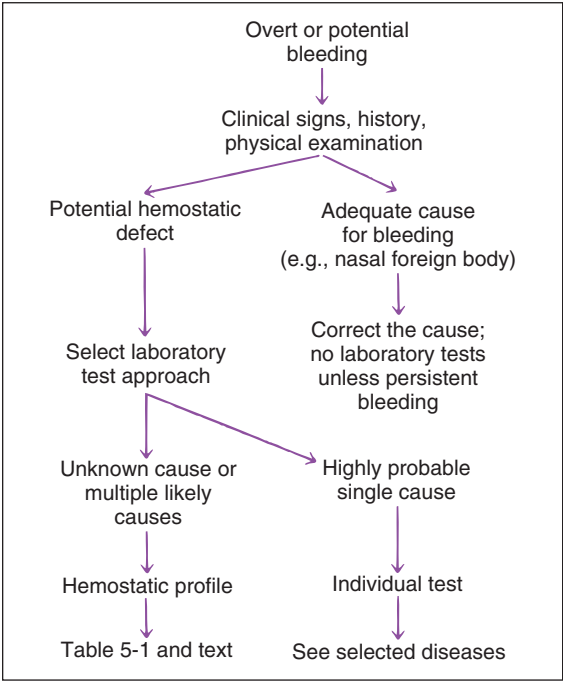


FIGURE 5-2 Selection of laboratory tests. No hemostatic tests are necessary if an adequate explanation for a bleeding process exists. If a hemostatic defect is likely and either several causes are possible or the cause is uncertain, one should screen all parts of the hemostatic mechanism for a defect with a profile of laboratory tests. When the clinical evidence suggests only one probable cause, one should select the most appropriate test to evaluate that defect.

(secondary hemostasis). The fibrinolytic system degrades the clot to reopen the vascular lumen to blood flow while the vessel heals. A platelet plug forms in coagulopathies, but easily breaks down and allows rebleeding because it is not stabilized by fibrin strands. A severe deficiency of one or more coagulation factors slows the formation of a clot, which should form rapidly after contact with the

subendothelial collagen. During this time, larger sized hemorrhages can occur before a fibrin clot is finally formed or pressure of adjacent tissues stops the bleeding (see Figure 5-1).

NOTE: It is usually more effective to use a profile of hemostatic tests during the initial diagnosis of an undefined bleeding problem than to select too few tests to localize the problem.

COMPONENTS OF NORMAL HEMOSTASIS

Vascular Endothelium and Wall

Endothelial cells secrete substances such as prostacyclin and nitric oxide that act as inhibitors of platelet adhesion. However, when the vascular endothelium is disrupted because of trauma, inflammation, neoplasia, or toxicity, coagulation is initiated by platelet adherence to exposed subendothelial collagen (primary hemostasis), which initiates the coagulation cascade and the formation of a fibrin mesh that subsequently stabilizes the clot (secondary hemostasis). Clot retraction and vasoconstriction also prevent continued blood loss.

Platelets and Primary Hemostasis

Platelets are small cytoplasmic fragments shed from megakaryocytes in the bone marrow. They circulate for approximately 5 days in dogs and about 30 hours in cats. Increased thrombopoiesis is simulated by decreased total circulating platelet mass (measured by plateletcrit) and not necessarily reduced platelet count (thrombocytopenia). For example, Cavalier King Charles spaniels with a hereditary dysplasia with fewer but larger platelets have a normal plateletcrit.^{11,54} Plateletcrit, like hematocrit, is the percentage of blood volume composed of platelets. Thus the platelet count, though universally used to judge the adequacy of platelets for hemostasis, is not as physiologic a test as the plateletcrit.

TABLE 5-2. HEMOSTATIC MECHANISMS: FUNCTIONS AND APPROPRIATE LABORATORY TESTS

	PLATELET NUMBER*	PLATELET FUNCTION*	COAGULATION FACTORS*	FIBRINOLYTIC SYSTEM*	BLOOD VESSELS*
Normal function†	Platelet plug	Platelet plug	Fibrin clot	Dissolve clot	Retain blood
Tests for evaluation‡	Platelet count	Bleeding time	aPTT and PT	D-dimer, FDP	Histopathology
Alternative tests	Platelet estimate blood smear	PFA-100	ACT or specific factor analysis	TEG	
Term for disease	<i>Thrombocytopenia</i>	<i>Thrombopathy</i>	<i>Coagulopathy</i>	<i>DIC thromboembolic disease</i>	<i>Vascular disease</i>

ACT, Activated coagulation time; aPTT, activated partial thromboplastin time; DIC, disseminated intravascular coagulation; FDP, fibrin degradation products; PFA-100, Platelet Function Analyzer; PT, prothrombin time; TEG, thromboelastography.
*Five basic portions of the hemostatic mechanism.
†Basic function of the five portions. All portions interact with the others (e.g., the fibrin clot stabilizes the platelet plug, clots stimulate fibrinolysis, platelets accelerate the clotting process).
‡The hemostatic parameter in bold type (top of columns) is evaluated by these tests. Possible abnormality that may be identified from the tests is italicized (term for disease; bottom row). Coagulation factor activity interpretation is described in the text and in Figures 5-4, 5-6, 5-7, 5-11, and 5-12.

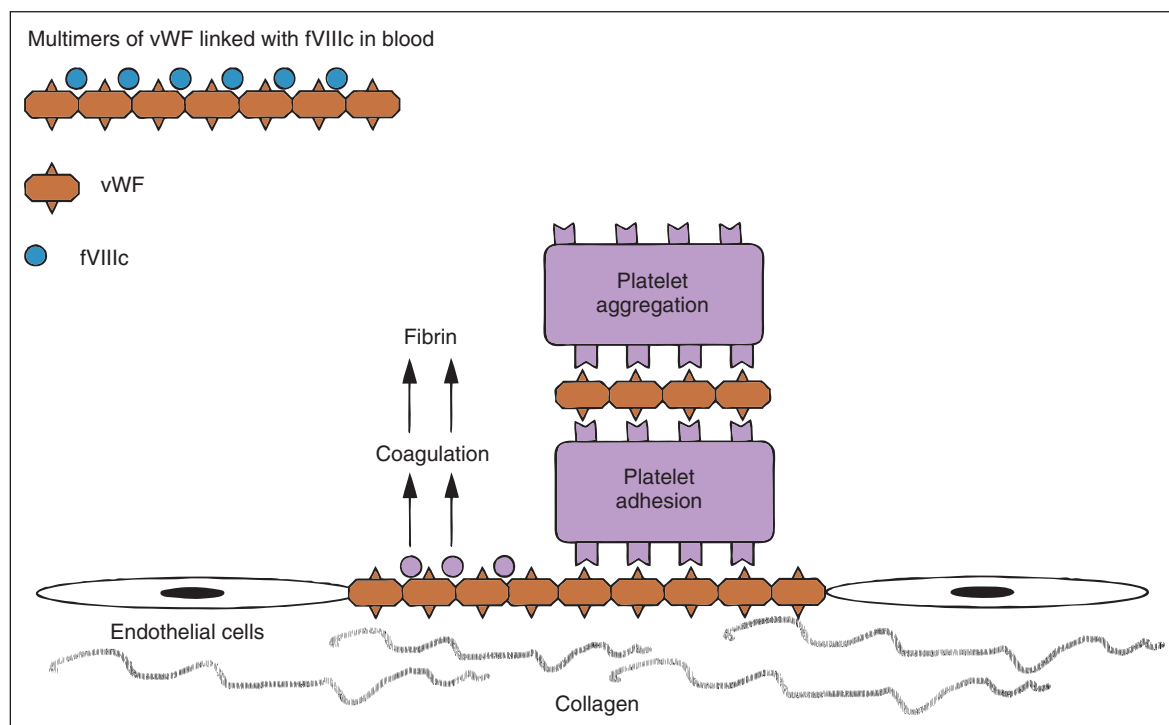


FIGURE 5-3 Depiction of the factor VIII molecular complex. von Willebrand's factor (vWF) is a long multimer of many vWF units. Factor VIII coagulant activity (fVIIIc) is a smaller molecule linked to vWF in the complex that protects fVIIIc. To facilitate platelet adhesion to sites of vascular injury, vWF binds to collagen at the sites; vWF also facilitates receptor-specific platelet aggregation. In the intrinsic system, fVIIIc acts to activate the common pathway at factor X, which ultimately results in fibrin formation to stabilize the platelet plug. (Modified from Cotran RS, Kumar V, Robbins SL: *Robbins pathologic basis of disease*, ed 5, Philadelphia, 1994, WB Saunders, p 621.)

Platelets adhere to a defect in a vascular wall and clump together as a platelet plug. Platelets then enhance and accelerate the coagulation cascade and formation of stabilizing clots. vWF is needed for primary hemostasis and can affect some tests of platelet function. The binding of platelets results in a conformational change to expose previously internalized phosphatidylserine (formerly known as PF-3, a potent receptor for factor X and prothrombin; Figure 5-4), which causes platelet activation, release of platelet granule contents, aggregation of more platelets, and initiation of secondary hemostasis by formation of a procoagulant surface of fibrinogen receptors on the primary plug. Release of adenosine diphosphate (ADP) from the dense granules causes synthesis of thromboxane A_2 , a prostaglandin that causes irreversible platelet aggregation and viscous metamorphosis and local vasoconstriction. Primary hemostasis requires adequate numbers of platelets that function adequately.

Coagulation Factors

Stabilization of the initial platelet hemostatic plug is accomplished by a mesh of fibrin formed by the coagulation cascade. The primary objective of the coagulation system is the formation of thrombin, which converts circulating fibrinogen to insoluble fibrin. These generated fibrin strands then cross-link to form a mesh, which traps

more blood cells and stabilizes the platelet plug, which is stronger than the initial plug and is resistant to proteolytic degradation by plasmin, a fibrinolytic enzyme.

Coagulation factor interactions are much more complex than this description, but may be divided into three parts—(1) intrinsic pathway, (2) extrinsic pathway, and (3) common pathway—for diagnostic purposes. These three parts are diagrammed as a letter Y (see Figure 5-4). The intrinsic pathway includes factors XII, XI, IX, and VIII and is initiated by contact activation. Factor XII is activated when it contacts a nonendothelial surface such as collagen outside a blood vessel.

The extrinsic pathway is initiated by interaction of tissue factor and activated factor VII. The extrinsic system initiates the coagulation response, whereas the intrinsic system sustains the generation of fibrin. These two pathways converge into the common pathway at the point where factor X is activated, which then leads to formation of a fibrin clot. Most coagulation tests have fibrin formation as the end point of the test. The common pathway consists of factors X, V, prothrombin, and fibrinogen.

In the case of a difficult venipuncture, tissue thromboplastin may be released from injured cells and initiate the clotting cascade *ex vivo*. Deficiencies of tissue thromboplastin and ionized calcium levels are never low enough in a patient to cause bleeding. Activated platelets are important in clot formation, because they provide a

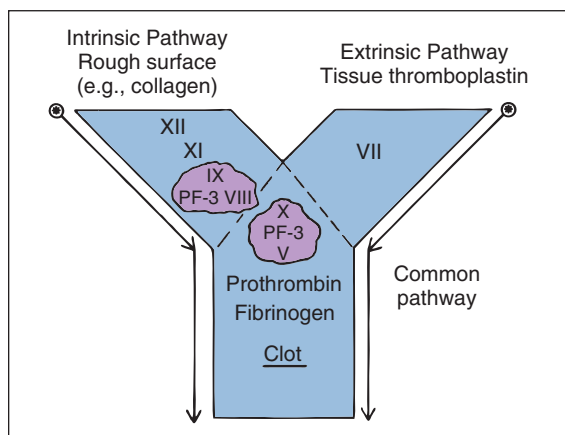


FIGURE 5-4 A simplified coagulation cascade. One may consider the cascade as a letter Y with three portions (or pathways): The intrinsic pathway includes factors XII, XI, IX, and VIII; the extrinsic pathway contains factor VII; and the common pathway includes factors X, V, prothrombin, and fibrinogen. Phosphatidylserine (i.e., platelet factor 3 [PF-3]) on the surface of activated platelets speeds the coagulation process. The intrinsic system activates the common pathway at factor X by a complex of IX, VIII, and phosphatidylserine on the activated platelet surface. The end point of the common pathway (and thus the intrinsic or extrinsic pathways) is the fibrin clot. Tissue thromboplastin from damaged cells and factor VII in the extrinsic pathway also activates the common pathway.

negatively charged phospholipid surface on which coagulation factors assemble. In severe thrombocytopenia, the coagulation process is slower (e.g., slight prolongation of the activated clotting time). The negatively charged surface of platelets reacts with factors IX and VIII as a complex in the intrinsic pathway. Activated factor IX from this complex interacts with another complex of factors X and V of the common pathway.

To keep coagulation localized to the defect, once procoagulant factors have been activated, they are inhibited by anticoagulant factors such as antithrombin III (AT III, complexed with heparin) and removed by the phagocytic system. Those that escape AT III will bind with thrombomodulin on endothelial cells, which activates protein C. Protein C is an anticoagulant and profibrinolytic factor that, with protein S as a cofactor, specifically hydrolyzes factors V and VIII and inhibits thrombin formation.³⁷ Several factors (II, VII, IX, X and protein C) are dependent on vitamin K. Vitamin K antagonists (i.e., warfarin) interfere with hemostasis by interfering with these factors. These factors have different half-lives, which affects which of them become deficient earliest during warfarin poisoning. Factor VII and protein C become deficient earliest, and lack of protein C can lead to often unexpected hypercoagulability early in warfarin poisoning or high-dose warfarin treatment.

Heparin inhibits thrombin and other procoagulant factors indirectly by activating AT III, a circulating natural anticoagulant that is the main physiologic inhibitor of thrombin. Because AT III is usually decreased in DIC,

heparin therapy may be ineffective. Heparin has been used clinically to treat hemostatic abnormalities associated with severe gastrointestinal disease, septicemia, and endotoxemia.³⁶ Decreased AT III occurs in hepatic insufficiency, owing to decreased synthesis, and in glomerular disease because of increased loss through the glomerulus. Availability of the AT III assay varies. Automated assays with chromogenic substrates are used by some veterinary laboratories and allow routine testing. However, tests of AT III function, protein C, and protein S are expensive to maintain in terms of time, quality control, and reagents, so they are available only at a few hemostasis reference laboratories. Lack of information about the activity of these anticoagulant factors impedes ability to fully understand the balance of hemostasis in a patient.

Fibrinolysis

Fibrinolysis is the process of fibrin clot dissolution once the damaged blood vessel is repaired, which reestablishes normal vascular patency. Plasmin digests fibrin clots (and even circulating fibrinogen) to produce fibrin degradation products (FDPs) or fibrinogen degradation products (FgDPs). FDPs have anticoagulant effects, which helps explain bleeding signs during DIC. Fibrinolysis may be detected by quantification of products of fibrinolysis such as D-dimer, FDPs, or alteration in TEG curves 30 to 60 minutes after clot formation.

LABORATORY TESTS

When a profile of tests is used, one should interpret each test individually and list the individual conclusions of what is normal or abnormal. Then one makes a disease diagnosis based on the total laboratory pattern and clinical evidence. Table 5-3 lists patterns expected in various diseases and may aid in diagnosis or understanding of the diseases discussed in the next section. As with all such tables, exceptions occur in the complexity of real cases.

Blood Collection for Hemostatic Testing

Proper sample collection and management avoid preanalytical errors in test results. A clean venipuncture is essential to minimize introduction of tissue factors, which could falsely activate platelets and clotting factors. One should avoid "raising" a vein by hitting it. A sharp 20-gauge (1.1-mm) or larger needle should be used. A two-syringe technique is preferred for platelet counting and coagulation testing. The first tube receiving blood with any tissue thromboplastin from the venipuncture is used for another purpose (e.g., serum clot tube for chemistry) or is discarded. Allowing blood to flow from a bare needle also flushes out tissue thromboplastins. Blood should be mixed well with the anticoagulant by inverting the tube several times without violent shaking. The collection tube may be swayed during collection to aid mixing. Additionally, turbulence, clotting, or extremes in temperature may invalidate hemostatic testing. Ethylenediaminetetraacetic acid (EDTA) is an excellent anticoagulant for canine platelet counts and routine hematology, though exceptions occur in which EDTA (and even

TABLE 5-3. EXPECTED HEMOSTATIC TEST RESULTS IN SELECTED DISEASES

DISEASE	Hemostatic Profile*				
	BLEEDING TIME	PLATELET COUNT	aPTT	PT	D-D
Thrombocytopenia (e.g., ITP)	I	D	N	N	N
Platelet dysfunction (e.g., uremia, aspirin treatment)	I	N	N	N	N
Intrinsic pathway defect (e.g., hemophilia A or B)	N [†]	N	I	N	N
Factor VII deficiency	N	N	N	I	N
Warfarin-type toxicity (multiple factor deficiency)	N [†]	N–D [‡]	I	I	N
Common pathway defect (e.g., factor X deficiency)	N [†]	N	I	I	N
Disseminated intravascular coagulation (DIC) [§]	I	D	I	I	I
von Willebrand's disease (vWD)	I	N	N	N	N

See text for details on these diseases and other diseases that may give similar patterns. Note that any of these tests may be normal with mild to variable disease changes.

aPTT, Activated partial thromboplastin time; D-D, D-dimer; ITP, immune-mediated thrombocytopenia; PT, prothrombin time.

*I, increased; D, decreased; N, normal.

[†]Initially stops in normal time period but may start bleeding again.

[‡]Thrombocytopenia occurs in some patients with warfarin toxicity.

[§]DIC can have widely variable results (see Table 5-4).

^{||}Rare cases of vWD may have prolonged aPTT.

heparin and citrate to a lesser effect) seems to promote clumping of platelets to cause pseudothrombocytopenia in certain patients. A venipuncture adequate for a routine complete blood count (CBC) is often inadequate for an accurate platelet count. (See later comments on feline platelet counting and prostaglandin E₁.)

The citrated plasma-clotting tests (i.e., aPTT, PT, fibrinogen, thrombin time [TT], FDP) require nine parts fresh whole blood and one part 3.8% trisodium citrate anticoagulant to separate plasma from cells and platelets, which must be done within 30 minutes of sample collection. Citrate or oxalate blood collection tubes must be filled completely to a mark on the tube's label to avoid errors in dilution. Plasma can be stored at 4° C for 48 hours or 20° C for 6 hours, or frozen for transport, to preserve factors VII and VIII. Furlanello has stated that samples were reasonably stable for 48 hours at room temperature and so may be sent to a laboratory.¹⁵ Certain tests require use of special tubes containing various reagents, so one should ask or read information from the laboratory before collecting the sample. Plastic tubes should be used because routine glass surfaces activate coagulation.

Blood Vessel Evaluation

Vascular disease is rarely diagnosed as the cause of bleeding in dogs and cats. Blood vessels can be evaluated histologically. Skin biopsy may document vasculitis in bleeding animals and is indicated in the presence of edema, petechiae, and a normal platelet count, or edema plus DIC. Immune-mediated vasculitis in dogs is uncommon, but antigen-antibody complexes occasionally deposit in vessels, causing an Arthus reaction and non-thrombocytopenic purpura with petechiae and edema. Widespread petechial hemorrhages seen at necropsy are a sign of septicemia-induced DIC. In rare instances, cutaneous hemorrhages are noted in dogs with

hyperadrenocorticism, in which bleeding is secondary to catabolism of supporting collagen around vessels.

Platelet Enumeration

Hematology instrument platelet counts are accurate, sensitive, and precise methods for detecting and evaluating thrombocytopenia and should be used when available. However, blood smear estimates are often adequate for initial quick diagnosis of severe thrombocytopenia. Severe thrombocytopenia is a very common cause of bleeding, and an estimate may be used during off hours or when instruments are not available. After routine preparation and staining of a blood smear, one should determine the average number of platelets in 5 to 10 microscope fields using the 100× oil objective (i.e., 1000× magnification) to estimate platelet numbers.⁵² Dogs normally have about 8 to 29 platelets/field, and cats should have 10 to 29/field. If the thrombocytopenia is severe enough to cause bleeding, only 0 to 3 platelets/field are expected. Platelets should be counted in the thin "monolayer" area. On most smears, this is where the red blood cells (RBCs) infrequently touch each other, and central pallor in canine RBCs is prominent. Anemic, bleeding animals have fewer RBCs per field in the proper area, so it is easy to look too deeply into the thicker areas of a smear. An alternative is to consider each platelet/100× oil immersion field as approximately equal to 15,000 to 20,000 platelets/μl. There is variation to the number of platelets seen per field of view depending on the microscope objective. Better objectives have a larger field of view.

One must ensure uniform platelet distribution in the sample. Blood smears should be screened for platelet aggregates to assure accuracy of any platelet count or estimate. Platelet clumps that are large enough to be seen at scanning magnification (4× or 10× objective) are usually pulled to the distal end of the smear. If many or large platelet clumps are present, all platelet methods, such as

estimated, manual, and instrument platelet counts, will be inaccurate. If an accurate platelet result is needed for the patient, then a new sample should be taken in such a manner as to avoid aggregation of platelets. Aggregation of erythrocytes or leukocytes also causes laboratory error. All tubes of anticoagulated blood should be examined for grossly visible clots and not submitted for hematologic testing.

NOTE: Severe thrombocytopenia is a common cause of bleeding, and an estimate from a blood smear is a quick, inexpensive, and usually adequate means of diagnosis.

Thrombocytopenia is a common initial clue to the presence of many diseases. Instrument platelet counts are accurate and precise and are the preferred method to detect mild to moderate thrombocytopenia. Instrument counts are preferred to monitor the course of a thrombocytopenia and response to treatment. Reference values vary with the instrument, method, and breed; thus specific reference values improve sensitivity in detection of mild thrombocytopenia.

Feline platelets have a strong tendency to clump, frequently causing inaccurate and lower platelet counts. Prostaglandin E₁ (PGE1) will prevent platelet aggregation.⁵³ If added immediately to a feline blood sample or preferably included already in the tube, PGE1 will allow accurate platelet counts that on average are 60% higher than those without PGE1. Optical counts (in instruments with laser systems) in routine feline patients can be 50% to 62% higher than impedance counts because impedance-type instruments fail to identify large platelets, which are excluded from their platelet counts.²⁹ Thus the combination of missing large platelets with impedance hematology instruments and not using PGE1 as an anticoagulant supplement will combine for substantial errors in feline platelet counts.⁵³ PGE1 is expensive and must be frozen until just before use, but need only be used in cats where thrombocytopenia is likely or other dogs with persistent platelet aggregation of blood samples. Fortunately true and severe thrombocytopenia is rare in cats. In feline myeloproliferative disorders, huge numbers of large and bizarre platelets may not be counted. Other particles (e.g., ghost cells: partially lysed RBCs) may be erroneously counted as platelets. Ghost cells are not uncommon problems in immune-mediated hemolytic anemia (IMHA) samples. An abnormal appearance of platelet dot-plots from the Advia 2120 or Sysmex XT-2000iV suggests this error (Figure 2-6).⁵¹ Small RBCs in iron deficiency anemia can be counted as platelets, especially with impedance-type instruments. This “small red” error may be detected by inspecting the platelet-RBC histogram and noting a failure of separation of the two peaks representing platelets (left peak) and RBCs (right peak) (see Figure 2-3) or by instrument error flags.

Manual counts with a glass counting chamber (hemocytometer) may be used if automated hematology cell counters are not available or, rarely, if the automated count is in question (e.g., if ghost RBCs are counted as platelets). Manual platelet counts are imprecise and labor intensive but include large platelets and are more similar

to optical instrument platelet counts than impedance feline platelet counts.²⁹ The hemocytometer must be clean and free of scratches. Because platelets are the size of dust particles, nicks and scratches in the glass may cause misidentification of dust as platelets. One very good hemocytometer should be reserved just for platelet counts. A phase-contrast microscope helps to differentiate platelets from other particles. Lowering the condenser on a regular microscope also aids in visualizing the relatively transparent platelets. A platelet stain improves platelet detection in a hemocytometer but is usually unnecessary. If the blood sample is poor (e.g., has clots or platelet aggregates), it is not appropriate to request a manual platelet count, because it too will be affected by uneven platelet distribution in the blood.

Blood must be diluted such that the number of cells in the hemocytometer is easy to count. A dilution system with plastic containers is easiest. Becton-Dickinson (BD) discontinued their Unopette System and now recommends, as an alternative, similar products of Bioanalytic GmbH (www.bioanalytic.de). Bioanalytic's Trombo-tic is advertized to lyse RBCs, disaggregate platelet clumps, and make platelets more rounded for better identification.

Delayed analysis favors platelet clumping and can lead to a lower platelet count. Various references suggest 30 minutes to 6 hours as a maximum tolerable limit. This may vary with the method, because an evaluation of 21 dogs with the Bayer H-1 hematology analyzer (precursor to the Advia 2120) suggested that clinically accurate platelet counts could be obtained with analysis of EDTA blood up to 2 days old (Figure 5-5). This suggests that EDTA blood sent to laboratories, remaining in the mail for 1 or 2 days, may still have clinically acceptable platelet count results if platelet clumping is absent or minimal.

NOTE: Do not request a manual platelet count if the blood sample has large or many platelet aggregates. Uneven distribution of platelets in the sample is a preanalytical error that will cause all types of platelet counting to be inaccurate! Take a new blood sample with special effort to avoid aggregation.

Platelet Morphology

Platelets may be characterized in different ways by various instruments, including some of those mentioned in this section. Blood smears should be evaluated routinely to ensure that the platelet count appears reasonable. Large platelets are usually seen on blood smears of dogs with thrombocytopenia. However, neither increased numbers of large platelets, increased mean platelet volume (MPV), nor abnormal platelet distribution width (PDW) have been shown in the dog to differentiate among any of the causes of thrombocytopenia or other diseases. Larger platelets and increased MPV consistently are inversely correlated to platelet numbers, which thus may assure the clinician that a thrombocytopenia in the patient is real and not a laboratory error. Large platelets are more functionally active, which may explain why some dogs with platelet counts less than 10,000/ μ l do not bleed

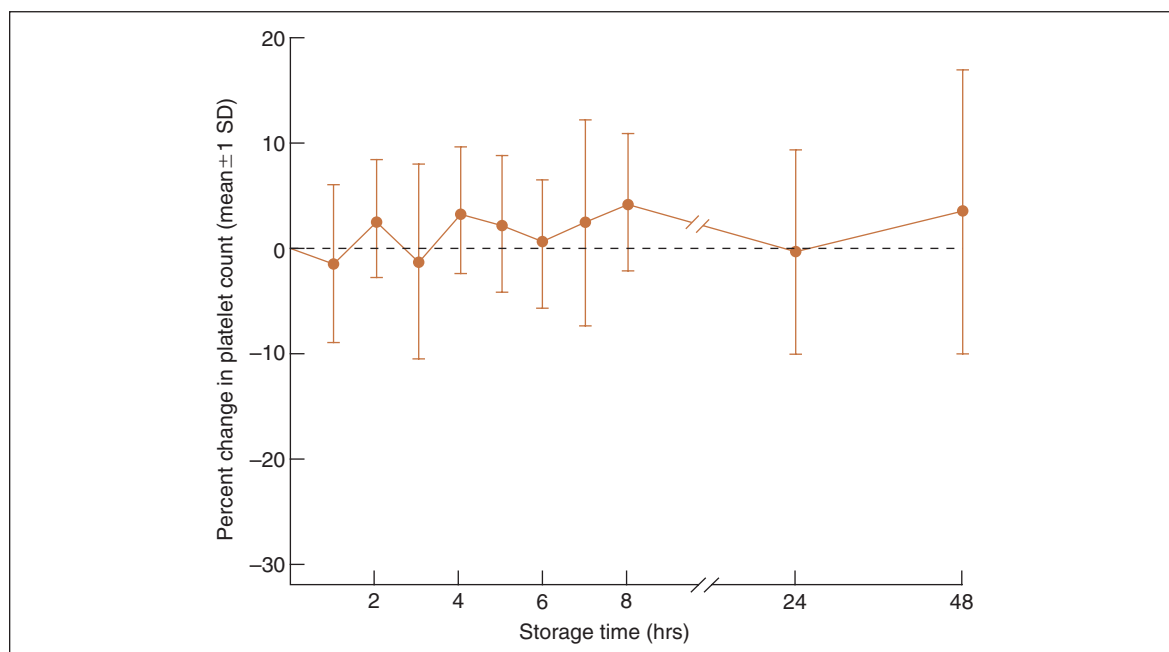


FIGURE 5-5 Stability of the platelet count with the H-1 hematology instrument (precursor to the Advia 2120). The mean percent change in the platelet count with time is compared with the initial platelet count for 21 canine ethylenediaminetetraacetic acid (EDTA) blood samples stored at room temperature for 8 hours and refrigerated for 2 days. Bars indicate the standard deviation (SD). This indicates platelet counts may be performed on blood samples sent by mail.

excessively. Cavalier King Charles spaniels often have a hereditary dysplasia with large but fewer platelets. This macrothrombocytopenia is due to a defect in beta1-tubulin.¹¹ They have no signs of bleeding, though impedance platelet counts are often less than 10,000/ μ l. The Cavalier King Charles spaniels and Norfolk terriers have a normal plateletcrit (percentage of blood volume, similar to hematocrit) despite variably low platelet counts (see Figure 2-7).⁵⁴ Plateletcrit (platelet mass) is a more physiologic measurement than platelet count, but nobody can interpret plateletcrit in % blood volume or L/L. Plateletcrit is directly measured by the IDEXX VetAutoread Hematology Analyzer (QBC) but converted to a platelet number, which most veterinarians can interpret. The QBC measures plateletcrit directly by measuring the width of the platelet layer in a centrifuged tube. The Advia 2120 performs a plateletcrit that works fairly well even in Cavalier King Charles spaniels with their very large platelets (unpublished data). Other instruments enumerate platelet numbers well, but most have problems with accurate measurement of MPV, especially when distribution of platelets by size is not typical. Plateletcrit equals platelet count multiplied by MPV multiplied by a factor.

Decreased MPV was consistently reported by the (then Technicon) H-1 Hematology analyzer in thrombocytopenic dogs with immune-mediated thrombocytopenia (ITP), but this was most likely an error in measuring MPV in very thrombocytopenic samples and not reflective of a true microthrombocytosis. Decreased MPV may occur if small particles (e.g., lipid droplets, cell fragments) are counted as platelets. This "background noise" is not noticed in samples with many platelets but has a relatively strong effect in severe thrombocytopenia.

Canine MPV may be artificially high in EDTA blood samples held more than 4 hours at 4° C because of platelet swelling.

Large, RNA-rich platelets are named the *high fluorescent platelet fraction* (HFPF) in the Sysmex XT-2000iV instrument. These HFPF platelets were shown to be equivalent to reticulated platelets.⁴¹ HFPF platelets are consistently increased during active thrombopoiesis due to thrombocytopenia or treatment with thrombopoietin. The HFPF is greater in number in thrombocytopenia due to DIC, IMHA, and ITP than in dogs with thrombocytopenia due to leukemia.^{17,41} This suggests that enumerating the absolute number of large RNA-rich platelets may be useful in differentiating causes of thrombocytopenia ("regenerative" thrombocytopenia), pending further studies. However, greater numbers of large, RNA-rich platelets (HFPF) are seen in hereditary macrothrombocytopenia in Cavalier King Charles spaniels and Norfolk terriers, which do not have increased thrombocytosis, and these large platelets are large throughout their lifespans.¹⁷

Platelets often have pseudopodia and an irregular shape on blood smears that suggests platelet activation during collection and handling of the blood. Decrease in the Advia 120 and 2120's mean platelet component (MPC) concentration is an indicator of platelet activation. MPC was more decreased in dogs with IMHA than normal dogs and sick dogs without IMHA, and more decreased in those dogs with IMHA who died.⁶¹ Primary platelet disorders such as thrombasthenic thrombopathia in otter hounds, foxhounds, and Scottish terriers are rare causes of large and morphologically bizarre platelets.^{3,5} *Ehrlichia platys* may be visible in platelets.

Platelet Function Testing

Platelet function tests are needed when a defect in primary hemostasis is suspected but platelet counts are not decreased (see also description of von Willebrand's disease). Platelet function tests include use of the Platelet Function Analyzer (PFA-100), tests of platelet aggregation (Multiplate Analyzer), and capillary bleeding time (CBT) or buccal mucosal bleeding time (BMBT). Platelet aggregation methods measure the ability of platelets to stick together and release granule contents, and thus measures platelet function *in vitro* in response to various compounds. Some in-clinic tests are described here, but to identify more specifically the mechanism of a platelet function defect, veterinarians should contact a platelet specialist such as Boudreaux.¹¹

Thrombopathy means defects in platelet function without alterations in platelet numbers. Thrombopathy is more commonly acquired than inherited and more common in dogs than cats. Thrombopathy can be caused by endocrinopathies, such as diabetes mellitus or hyperadrenocorticism, IMHA, pancreatitis, or renal failure, and may be associated with thrombosis. Inherited defects may include quantitative or qualitative defects in membrane proteins needed for platelet adhesion, cyclic adenosine monophosphate (cAMP) metabolism, prostaglandin metabolism, granule pools, vWF, and fibrinogen concentrations.

NOTE: The CBT capillary bleeding time or BMBT may be used as an in-clinic screening test for vWD, thrombocytopenia, or reduced platelet function. Either can be used as a presurgical test to exclude a problem in primary hemostasis.

Buccal Mucosal and Capillary Bleeding Time

The BMBT or CBT is typically used as a presurgical screen or to rule out defects in primary hemostasis. They are platelet function tests but also are affected by decreases in platelet number or the presence of vWD or vascular disease. The CBT has an advantage over the BMBT because severe bleeding in some vWD patients is easier to control with a bandage on the paw than is bleeding from the mouth.³⁹ The CBT is performed on the lateral, shaved surface of a front toe with the dog in lateral recumbence with a sphygmomanometer cuff on its leg (pressure 60 mm Hg). The skin is cut twice with a blood lancet, 5 mm apart near the horny part of the pad. Normally bleeding stops in about 1 to 2.5 minutes.

A spring-loaded disposable device (Suricutt) in the BMBT creates standardized cuts in the mucosal surface of the upper lip, which has been everted and tied tightly enough to cause vascular congestion by impeding venous return. The technique involves measuring the time for bleeding to cease. Blood is carefully removed by blotting near the incision with filter paper, without applying pressure to the wound. The BMBT in healthy dogs is approximately 2.6 ± 0.5 minutes and can be used to diagnose severe thrombocytopenia, vWD, and platelet dysfunction

in uremia. BMBT is affected by the type of Suricutt used and variation among operators. The BMBT is usually "normal" in pure coagulation defects such as hemophilia, because the bleeding stops initially in the expected time because of the formation of platelet plugs. However, because fibrin strands do not stabilize the platelet plug, the incision is prone to rebleed. The cuticle bleeding time test is not recommended because it is insensitive and imprecise. However, the principle may be applied when during nail trimming one clips the claws too short. The cuticle bleeding time can be determined by observing when the bleeding stops. It should stop within 5 minutes.

Platelet Function Analyzer

The Platelet Function Analyzer (PFA-100) (Dade Behring, Inc., Deerfield, IL) has been validated for dogs.³⁴ Citrate-anticoagulated blood is sucked through a capillary and membrane into various cartridges. The membrane is coated with collagen and ADP or collagen and epinephrine (two different cartridges). The collagen-epinephrine cartridge was found to be less suitable for dogs. Reference values for collagen-ADP were 53 to 98 seconds.³⁴ Decreased hematocrit also caused prolongation of closure time.

Platelet Aggregation

Aggregation of platelets after exposure to various agonists allows evaluation of platelet function. The Multiplate Analyzer (Dynabyte GmbH, Munich, Germany) is an impedance aggregometer validated for dogs.²² The platelet glass bead retention test is useful in detecting general abnormalities in hemostasis, including platelet adhesion defects in thrombopathies and vWD.⁶

Tests of Coagulation

Activated Coagulation Time

The activated coagulation time (ACT) is a simple, inexpensive, in-clinic screening test to evaluate the intrinsic and common pathways of the coagulation system. However, it is insensitive. A specific coagulation factor must be decreased to less than 5% of normal to increase the ACT. Thus only severe deficits are detected. A carrier of hemophilia, for example, has 40% to 60% of the normal concentration of factor VIII or IX and would not be detected by the ACT or even the more sensitive aPTT. The ACT may be slightly prolonged in severe thrombocytopenia ($< 10,000/\mu\text{L}$). ACT reference values are approximately 60 to 110 seconds for dogs and 50 to 75 seconds for cats. It is a whole blood procedure, obviating the need for using anticoagulants or obtaining plasma.

A special ACT Vacutainer tube that contains an activator is used for venipuncture. A heating block or water bath is used to prewarm the tubes to 37°C and maintain that temperature until the clot forms. The blood is incubated at 37°C for 60 seconds after blood enters the tube for a canine sample, or 45 seconds for a feline sample. Then, at 5-second intervals, the tube is inverted until the end point of the first visible clot is detected. The time from addition of blood to the tube until a clot first becomes visible is the ACT.

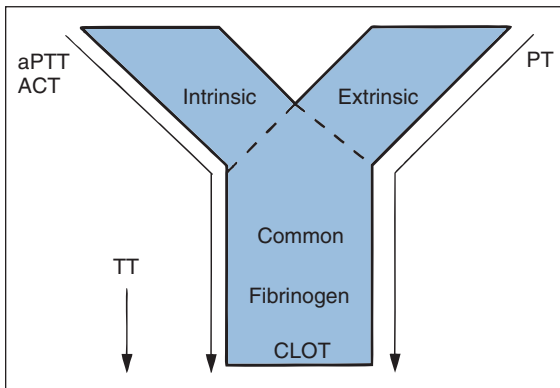


FIGURE 5-6 Hemostatic tests of the coagulation cascade. The activated partial thromboplastin time (aPTT) and activated coagulation time (ACT) test all factors of the intrinsic and common pathways. The modified thrombin time (TT) determines the fibrinogen concentration. The prothrombin time (PT) tests factor VII and the factors of the common pathway.

NOTE: The ACT is a simple in-clinic test for most coagulation defects, such as warfarin poisoning, though the aPTT is a more sensitive test for coagulation factor deficiencies in the intrinsic and common pathways.

Prothrombin Time

The PT evaluates the extrinsic and common pathways (Figure 5-6). The factors in the common pathway include X, V, II, thrombin, and fibrinogen. One of the major uses of the PT, as a single test, is detection of vitamin K antagonist poisoning (e.g., warfarin). PT is the most sensitive test of warfarin-type toxicity. Of the vitamin K-related factors (II, VII, IX, and X), factor VII has the shortest half-life. If synthesis of these factors is inhibited, factor VII deficiency develops earliest. Because factor VII is in the extrinsic pathway, the PT is prolonged earliest and has the greatest relative increase above the normal mean. The PT is used in conjunction with the aPTT (Figure 5-7) in localizing other types of coagulopathies. Factors must be reduced to less than 30% of normal to prolong a standard PT, though using a PT test optimized for dogs can detect milder deficiencies.³⁵

NOTE: PT is the most sensitive test of warfarin-type toxicity and is altered earliest and most after poisoning.

Calcium and activating agents are added during the tests, and fibrometers or photo-optical coagulometers are used to detect formation of fibrin strands or changes in the intensity of filtered light when clot formation occurs. PT measures the time required for fibrin clot formation after addition of tissue thromboplastin and calcium. PT is increased with factor deficiencies in the extrinsic or common pathways, DIC, acquired vitamin K deficiency such as in coumarin poisoning, or hepatic failure.

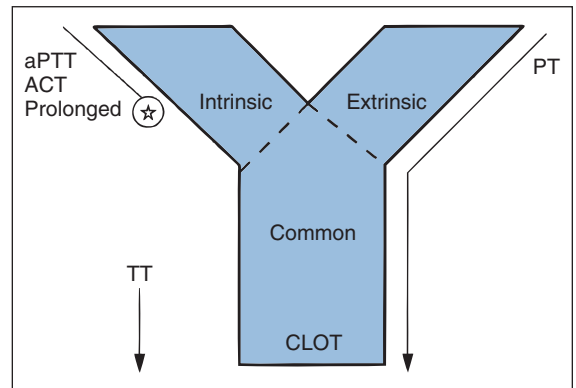


FIGURE 5-7 Test results for an intrinsic pathway defect. Only activated partial thromboplastin time (aPTT) and activated coagulation time (ACT) results should be abnormal. A star and termination of the arrow along the side of the intrinsic system depict the site of the defect. Specific factor analysis and consideration of clinical signs, history, and physical findings identify a specific factor defect from factors XII, XI, IX, and VIII. PT, Prothrombin time; TT, thrombin time.

Activated Partial Thromboplastin Time

The aPTT tests factors of the intrinsic pathway (i.e., factors XII, XI, IX, and VIII) plus the common pathway factors (i.e., all factors except VII; see Figure 5-6). It is used to detect decreased activity of one or more coagulation factors, as in hemophilia, DIC, acquired vitamin K deficiency, warfarin poisoning, or hepatic failure. The aPTT measures the time required for fibrin clot formation after addition of a contact surface activator of the intrinsic system and calcium. Factors must be less than 30% of normal to prolong the aPTT. Thus aPTT (and certainly not ACT) will not detect carriers of coagulation defects with 40% to 60% of normal factor concentrations. There is prominent variation of the sensitivity of the aPTT test while using different reagents.³³ More sensitive reagents (e.g., Hemos IL SynthAFAX; Instrumentation Laboratory) allow detection of hemophilia A (factor VIII deficiency), but some of the less sensitive reagents may give normal aPTT results even in hemophilic patients. Monitoring heparin therapy requires aPTT reagent and heparin preparation-specific calibration.

NOTE: The aPTT tests for all coagulation factors except VII, but variations in aPTT reagents cause different methods to have variable to poor sensitivity. In-clinic point-of-care instruments are available for aPTT and PT testing.

Thrombin Time

TT measures concentration of normal fibrinogen (see Figure 5-6). The TT may be used to monitor the anticoagulant activity of heparin and FDP. The modified TT has an excess of thrombin added to the reagent, making it insensitive to anticoagulants. The modified TT is the type usually used in veterinary laboratories and should not be used to monitor heparin treatment. Crude heat

precipitation methods used to measure fibrinogen concentration, for diagnosis of hyperfibrinogenemia associated with inflammatory disease, are not sufficiently sensitive to diagnose hypofibrinogenemia. Hypofibrinogenemia is primarily the result of increased consumption in DIC, but decreased production of fibrinogen may also occur in advanced liver disease.

Specific Factor Analysis

Specific factor assays are performed when a hemostatic defect has been localized to a likely factor or group of factors (e.g., early intrinsic pathway factors). Hemostasis laboratories use a modification of the aPTT to determine the percentage of specific factors (e.g., percentage of factor VIII in hemophilia A). The percentage is compared with normal pooled plasma, so about 100% is normal.

Immunologic assays (i.e., electroimmunoassay or enzyme-linked immunosorbent assay [ELISA]) of vWF are used to diagnose vWD, and several subtypes of vWD have been identified by precise characterization of the size and concentration of multimers of the molecule.⁵⁰ The incidence of vWD in some breeds (e.g., Doberman pinschers, Airedale terriers) may be 50% or more, so any bleeding tendency or need for surgery is a reasonable indication for vWF assay. One should measure vWF before any blood transfusions are administered, because transfusions add vWF, which can mask a deficiency in vWD. vWF is an acute phase protein that may increase with systemic illness, stress, strenuous exercise, azotemia, hepatic disease, parturition, and after administration of the vasopressin analogue deamino D-arginine vasopressin (DDAVP). Canine-specific vWF antigen assay has been validated in both cats and dogs to diagnose vWD. Some human vWF kits have antibodies that cross-react with canine vWF. vWF function assays (e.g., botrocetin) can also be performed.

Shipping plasma to referral laboratories requires special care. Laboratories should be asked about the required handling procedures; typically the plasma can be stored at 4° to 22° C for up to 48 hours or rapidly frozen in 1-ml aliquots and shipped on dry ice. One should use 3.8% citrate as the anticoagulant in a 1:9 ratio (e.g., 0.5 ml Na citrate + 4.5 ml blood), keep the sample refrigerated or on ice, and separate the plasma within 30 minutes of collection by high-speed centrifugation (i.e., 2500 to 3500 rpm) for 15 minutes. Plasma should be collected using plastic pipettes and containers.

D-dimer

D-dimer concentration indicates the amount of breakdown of clots in the body (fibrinolysis). D-dimer is a specific FDP from cross-linked fibrin. Cross-linked fibrin, which occurs at later stage of the formation of fibrin from fibrinogen, is produced by the action of thrombin on soluble fibrin polymers. Therefore, D-dimer is more specific for fibrinolysis than general FDP tests. Increased fibrinolysis usually indicates increased clotting in the body, which supports a diagnosis of DIC and other thromboembolic diseases. Hemorrhage (e.g., trauma) in the body creates clots that are broken down to FDP and D-dimer; therefore, an elevated D-dimer concentration is not specific for DIC. D-dimer less than 0.25 µg/ml has strong negative predictive value to more or less rule out DIC in

the patient. Various assays of D-dimer concentrations have been validated for dogs (see later discussion of DIC).

Thromboelastography

Thromboelastography (TEG) measures the viscoelastic properties of whole blood during clotting under low shear conditions and indicates changes in the strength of the clot over time (30 to 120 minutes). TEG tests for a net effect of the interaction of cellular elements (mainly platelets) and soluble elements (coagulation factors [mainly fibrinogen] and anticoagulation factors) in clot formation. Interpretations are that the net hemostatic effect is either hypercoagulation, normocoagulation, or hypocoagulation, and the test indicates how severely abnormal the results are (Figure 5-8). Fibrinolysis less often may be indicated by lysis values (%) at 30 minutes (LY30) or 60 minutes (LY60) or by interpretation of the curve. Figure 5-9 shows a decrease in amplitude (width) of curve over time suggesting fibrinolysis. Fibrinolysis should be concluded with caution because similar changes in the TEG curve may be seen in normal dogs (perhaps due to platelet contraction), giving a false impression of fibrinolysis.

Different instruments and reagents are used for TEG and may vary from the following description. The basic principle is that calcium chloride and an activator of coagulation are added to anticoagulated blood. Development of a clot over time is measured in a plastic chamber with a rotating piston or pin. The time from adding the activator to initial formation of the clot (*R*) is similar to the ACT. The rate of initial clotting is described by the time (*K*) for the clot to reach an amplitude of 20 mm, and *K* is inversely related to the angle (in degrees) of the curve during this time period (see Figure 5-8). The maximum amplitude (*MA*) reflects the maximal width or strength of the clot and is strongly affected by amount of fibrin and number of platelets. *G* is a "global" or summation value calculated from other results. Certain defects such as hemophilia or warfarin toxicity may prevent formation of a clot. Decreasing the hematocrit of a sample will cause increase *MA* and *G*, suggesting a hypercoagulable TEG result. Trust in the usefulness of TEG testing is generally stronger in Europe than the United States.

DISORDERS OF HEMOSTASIS

In this section, a few selected diseases are discussed that are either common or illustrate a defect in a part of the hemostasis system not previously discussed.

Disseminated Intravascular Coagulation • DIC is a very common diagnosis. "Disseminated intravascular coagulation" is the usual term used for a patient with excessive coagulation, thrombus formation, infarction, fibrinolysis and consumption of platelets and plasma factors, and bleeding. However, there is disagreement on what criteria are needed to diagnose DIC compared to "thromboembolic disease" or hypercoagulability of variable intensity and duration. Many veterinarians restrict the term *DIC* to a very severe form with signs of bleeding, thrombosis, and death, while DIC to others also includes milder, subclinical, and chronic disorders. Therefore,

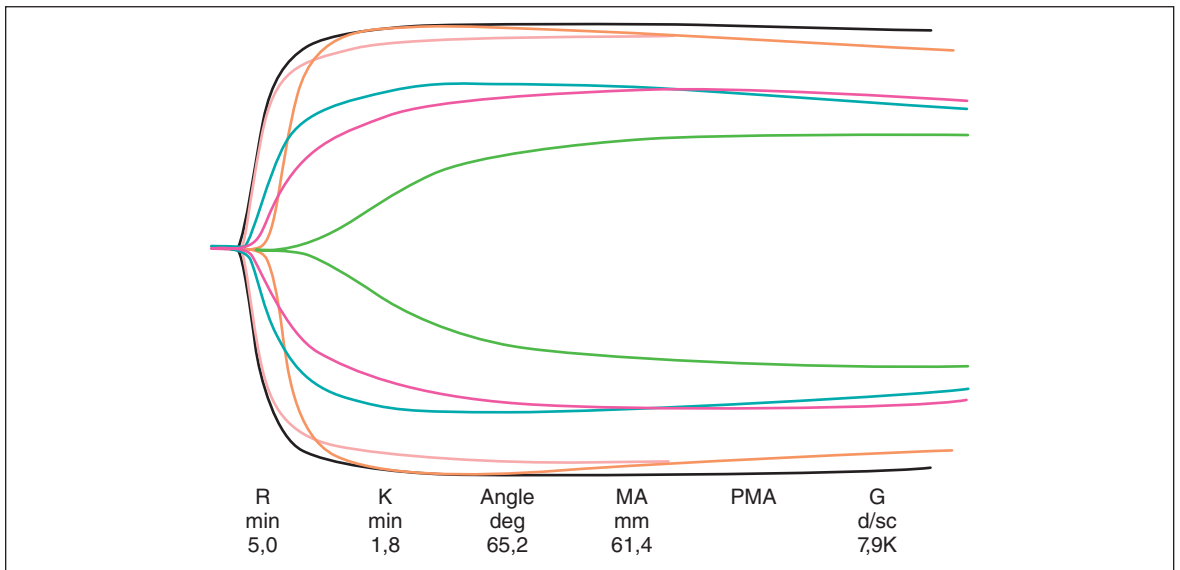


FIGURE 5-8 Examples of thromboelastography responses. The blue-green and red-violet lines were from two normal dogs. The inner light green line shows a hypocoagulable response. The outer three lines (black, orange, and light pink) show hypercoagulable responses. The values given at the bottom were from one normal dog (blue-green line). The width of the curves is described by maximum amplitude (MA), which is the maximal width of the developing clot in millimeters (mm). The three hypercoagulable responses had a distinctly wider curve and larger MA. K and angle describe the initial stages of clotting. The rate of initial clotting is described by the time (K) for the clot to reach an amplitude of 20 mm, and K is inversely related to the angle (in degrees) of the curve during this time period. G is a "global" or summation value calculated from other results. The three hypercoagulable lines have a steeper angle than in normal dogs and thus shorter time (K) during initial clotting. The hypocoagulable line in green had a less acute angle during initial coagulation and thus longer time during this change (K). R is the time from adding calcium to start of coagulation. The hypocoagulable line in green had a longer R and its curve starts to deviate from the zero point later than the other examples. Ignore PMA which is not used in diagnosis.

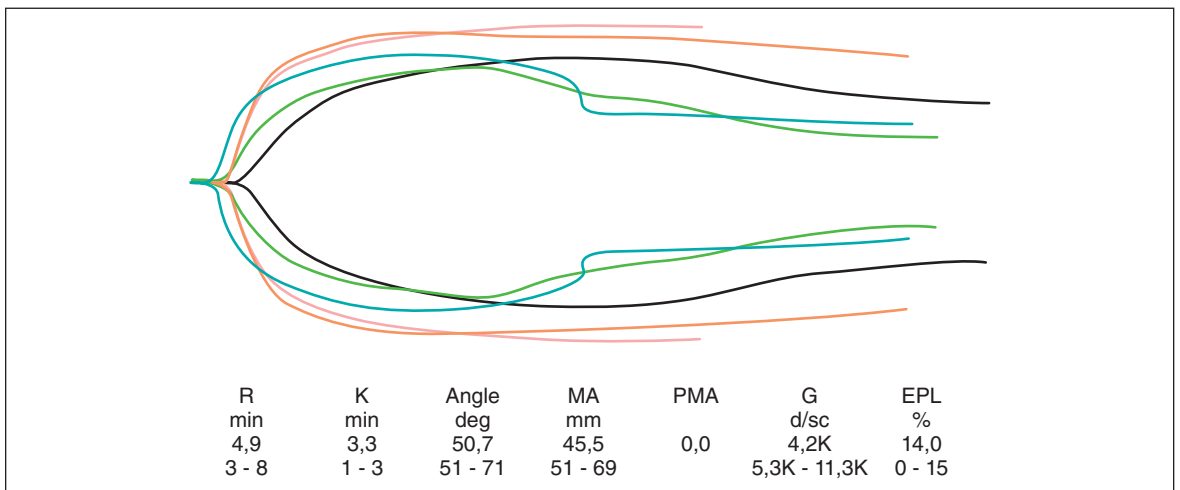


FIGURE 5-9 Thromboelastography responses including a fibrinolysis pattern. If fibrinolysis occurs during the TEG test, there is a decrease in the width of the curve (amplitude) with time. This figure shows a likely true case of fibrinolysis (black and light green lines), an example of an artifact that looked like fibrinolysis (blue line), and the curves of two normal dogs (outer pink and orange lines). The canine patient with fibrinolysis had pyometra, an adrenal tumor, and DIC. Note that this dog's two curves decrease in width with time (time is along the x axis). This was mild the first day (black line; LY30 was 4.6% while normal dogs have none, 0%), but was worse the next day (green line; LY30 was 14%). LY30 is the amount of lysis (decrease from maximal amplitude) at 30 minutes. The black line was from a normal blood donor dog without fibrinolysis but with an LY30 of 17.3%. Clot retraction may explain an artifact of increased LY30. Ignore PMA and EPL which are not used in diagnosis. Other abbreviations as in Figure 5-8.

TABLE 5-4. FREQUENCY OF LABORATORY ABNORMALITIES IN DISSEMINATED INTRAVASCULAR COAGULATION (DIC)

ABNORMALITY	FELDMAN ¹⁴	KOCIBA ²⁵	MARUYAMA ³¹
Increased FDP	61%	94%	50%
Prolonged aPTT	87%	95%	70%
Prolonged PT	80%	72%	45%
Decreased PLT	80%	48%	39%

aPTT, Activated partial thromboplastin time; FDP, fibrin degradation products; PLT, platelet count; PT, prothrombin time.

*No test is 100% sensitive or specific for DIC.

description of laboratory findings in DIC varies greatly with the authors, the methods they used, and their definition of DIC (Table 5-4). For example, Maruyama evaluated dogs with malignant neoplasia and reported thrombocytopenia more commonly (61%) in dogs that were classified as non-DIC patients than those with DIC (39%).³¹ But the mean platelet count in DIC dogs (121,000/ μ l) was clearly more severely decreased than that in non-DIC dogs with neoplasia (369,000/ μ l). Maruyama required four or more abnormal results to diagnose DIC, thus dogs with malignant neoplasia and one to three abnormal hemostasis test results were not classified as having DIC. Dogs with malignant neoplasia and thrombocytopenia and/or coagulation defects had some hemostatic disorder, but many were classified as non-DIC. Tvedten would have included many more of Maruyama's cases as having DIC. DIC is used in this chapter to include a wide variety of hemostatic disorders. Wiinberg and others (2010) described a system for diagnosis of canine DIC similar to a human scoring system proposed by the International Society on Thrombosis and Haemostasis.⁶⁰ Rather than argue on the requirements for diagnosis of DIC, one should identify a hemostatic abnormality is present and to characterize it as best one can with the tests available.

DIC (thromboembolic disease, consumptive coagulopathy, hypercoagulation, etc.) is a common, variably severe disorder of hemostasis that affects primary and secondary hemostasis and fibrinolysis and even is often associated with vasculitis. Thus any and all components of hemostasis may be affected. DIC occurs secondary to a wide variety of diseases, including neoplasia, inflammation, infection, trauma, snake venom, necrotic tissue, rough surfaces, and vasculitis. The extrinsic pathway of the coagulation system is initiated by tissue thromboplastin from damaged cells; the intrinsic pathway is activated by abnormal surfaces such as exposed collagen beneath damaged endothelial cells. Inflammatory diseases create areas of necrosis and exposed collagen, which stimulate clotting. Many infections, such as canine infectious hepatitis or feline infectious peritonitis (FIP),^{57,58} induce a DIC-type episode.

Neoplasms (e.g., hemangiosarcoma) often have necrotic, inflamed areas, and chemotherapy of neoplasms may create additional necrosis and increase the likelihood of DIC. IMHA produces abundant RBC debris

during hemolysis. Vasculitis may induce DIC, because vessels have damaged endothelial cells. Endothelial cells provide a complete barrier between plasma and subendothelial collagen and generate factors that inhibit platelet aggregation. DIC, as considered in this chapter, can be localized or chronic and not always disseminated and peracute. Death in many diseases is secondary to infarction, thrombosis, or bleeding because of DIC.

Platelets and coagulation factors are consumed during excessive clotting. Breakdown of these clots increases FDP and D-dimer, which act as anticoagulants and interfere with platelet function and clotting by competing with fibrinogen for platelet membrane receptors. Thus a textbook pattern (see Table 5-3) would have all tests in a hemostasis profile abnormal. However, variably increased production of coagulation factors and platelets compensate for variably severe consumption, and no single result is predictable (see Table 5-4). Confidence in a DIC diagnosis is increased with the number of hemostatic tests that are abnormal and how abnormal they become. Any hemostatic abnormality in a patient with a disease that commonly causes DIC should suggest DIC is present. When both thrombocytopenia and coagulopathy occur concurrently, DIC is likely. Thrombocytopenia and coagulopathy occur in warfarin toxicity, but this is likely due to a hypercoagulable state that occurs the first few days after poisoning. Warfarin-type agents cause a more rapid decrease in vitamin K-related anticoagulation factors (protein C) prior to sufficient decrease in procoagulant factors (II, IX, and X) to cause hypocoagulation and bleeding. Platelets are likely consumed during the hypercoagulable phase and are not decreased because of blood loss. Decreased AT III is a sensitive indicator of DIC. Hepatic insufficiency and protein-losing states may also decrease AT III. AT III testing is not commonly available.

Thrombocytopenia • Thrombocytopenia is the most common cause of bleeding in dogs. Thrombocytopenia occurs in about 15% of canine patients (see Chapter 2). Most of these cases, however, have mild thrombocytopenia and no signs of bleeding. Mild to moderate thrombocytopenia is often detected during initial laboratory testing of patients and may be an early indicator of thromboembolic problems or infectious diseases (see Chapter 15). True thrombocytopenia is fortunately uncommon in cats, but pseudothrombocytopenia due to platelet aggregation and platelets too large to detect with impedance-type hematology instruments is unfortunately very common with feline blood samples. Platelet counts are often inaccurate in cats.

Causes of thrombocytopenia include decreased platelet production because of infectious, toxic, drug-induced, or neoplastic bone marrow disease or accelerated platelet destruction or use because of infectious, inflammatory, or immune-mediated disease. The most common causes of canine thrombocytopenia are immune-mediated, infectious disease, and neoplasia, whereas thrombocytopenia in cats is most often the result of infectious disease, neoplasia, or thromboembolism.¹⁹ Platelet sequestration in the spleen can be accentuated with splenomegaly, hypothermia, splenic congestion, or splenic hyperplasia. Tvedten dislikes the diagnosis of "hypersplenism," which

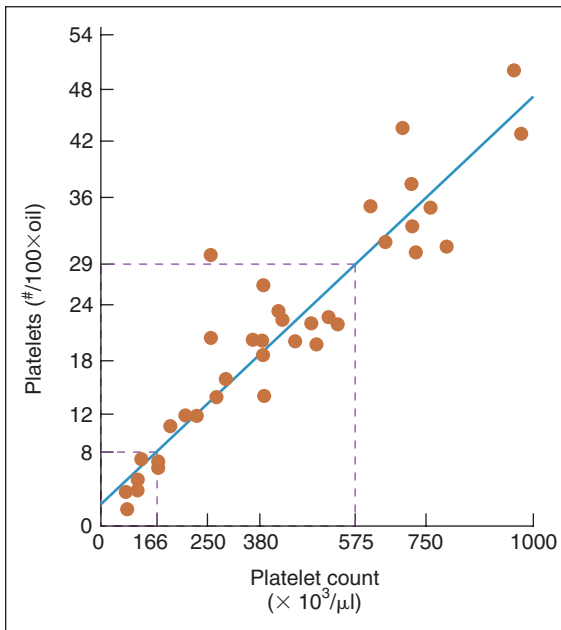


FIGURE 5-10 Estimation of platelet numbers for canine blood smears. The number of platelets in an average 100 $\times$ oil immersion field may be used to estimate the platelet count. Between 8 and 29 platelets/100 $\times$ oil immersion field represents a normal canine platelet count. A regression line indicates the relationship of the two factors.

only gives a surgeon a reason to remove a large spleen doing what it is supposed to do.

NOTE: Thrombocytopenia is the most common cause of clinical signs of bleeding in dogs. Thus platelet numbers should be evaluated first in unexplained bleeding disorders. A platelet estimate from a blood smear is simple, quick, inexpensive, and sufficient to detect severe thrombocytopenia.

Thrombocytopenia is usually defined as a platelet count less than the appropriate reference range (Figure 5-10). Platelet mass (plateletcrit) is a more physiologically useful measurement but is not commonly available, and few clinicians are comfortable interpreting the units (%). Diagnosis of thrombocytopenia first requires confirming that thrombocytopenia is not an error of collection, the result of sample-handling delay, or laboratory error. The severity of the thrombocytopenia aids in interpretation and in treatment decisions. Persistent mild thrombocytopenia (100,000 to 175,000 platelets/ μl) is not specific for a particular disease. Very severe thrombocytopenia (<20,000/ μl platelets) is highly associated with immune-mediated thrombocytopenia (ITP), at least in areas where infectious causes of thrombocytopenia are uncommon. Access to tests of antiplatelet antibodies is very limited; thus ITP is often diagnosed by exclusion of other common causes and response to immunosuppressive therapy. Clinical signs (e.g., epistaxis, hematochezia, melena, hematuria, hematemesis, cutaneous petechial or ecchymotic

hemorrhage) become likely with severe thrombocytopenia (<20,000 platelets/ μl). Some animals may not bleed with 10,000 platelets/ μl , whereas others may bleed at 30,000 to 50,000 platelets/ μl . Platelet size, platelet function, blood vessel and endothelial support, concentration of anticoagulant and procoagulant factors, and severity of the challenge to the hemostatic mechanism all contribute to the presence or absence of bleeding. Linking the severity of change in laboratory data to clinical signs is important. For example, if the platelet count is greater than 50,000/ μl and the patient is bleeding, additional factors (e.g., defective platelet function associated with DIC) are likely. In contrast, disorders of coagulation tend to have large areas of hemorrhage, hematomas, body cavity hemorrhages, and hemarthroses rather than petechial and ecchymotic hemorrhages.

The three common causes of thrombocytopenia are (1) destruction (e.g., ITP), (2) bone marrow production defects (e.g., estrogen toxicity, immune-mediated destruction of megakaryocytes, leukemia), and (3) consumption of platelets (e.g., DIC). Infections such as *Ehrlichia*, *Anaplasma*, or Rocky Mountain spotted fever, and other bacterial and viral infections, commonly cause thrombocytopenia. These infections should be investigated in areas where they are common. Bone marrow aspirate, biopsy, or both usually document megakaryocytic numbers and morphology (see Chapter 2). Bleeding at the aspiration or biopsy site is usually easily controlled, so bone marrow collection should not be feared. DIC is diagnosed with a profile of hemostatic tests. ITP diagnosis lacks commonly available direct testing.

von Willebrand Disease • vWD is the most common inherited hemostatic defect that has been reported in many breeds of dogs, including Doberman pinschers, Scottish terriers, Shetland sheepdogs, and Chesapeake Bay retrievers.^{7,55} vWF is required for adhesion of platelets to subendothelium at sites of vascular damage and to other platelets (see Figure 5-3). Therefore, vWD appears similar to a defect of primary hemostasis.

NOTE: vWD is the most common inherited hemostatic defect. vWD may be detected by prolonged bleeding time, vWF assays, or genetic testing.

The clinical signs of vWD are often mild and variable, which can obscure diagnosis. Often owners and surgeons are unaware of any bleeding tendency in affected dogs until a surgical procedure is performed. A bleeding time test may be done in-clinic as a presurgical screen in breeds with a high frequency of vWD. A CBT is recommended over the BMBT (see earlier description) because excessive bleeding in some vWD patients is easier to control with a bandage on the paw than pressure on the gums. vWF assay or genetic testing allows specific diagnosis. Most of the common hemostatic tests (i.e., aPTT, ACT, TEG) fail to identify vWD despite very low levels of vWF. Predisposition to bleeding tends to be noted with vWF levels of 30% or less, and bleeding tends to be greater with worsening deficiency of vWF. Type I vWD is characterized by decreased vWF antigen with proportional decreases in all

TABLE 5-5. DIFFERENTIATION OF VON WILLEBRAND'S DISEASE AND HEMOPHILIA A

TEST	VON WILLEBRAND'S DISEASE (vWD)	HEMOPHILIA A
aPTT	Usually normal	Increased
BMBT	Prolonged	Normal
vWF	Decreased	Normal to increased

aPTT, Activated partial thromboplastin time; BMBT, buccal mucosal bleeding time; vWF, von Willebrand's factor.

multimers, and it has been reported in many canine breeds. Type II vWD has decreased vWF antigen, but only the largest multimers have diminished concentrations; it has been reported in German shorthaired and wirehaired pointers. Type III vWD has essentially no vWF in the homozygotes and has the most severe clinical signs; it has been reported in Scottish terriers, Shetland sheepdogs, and Chesapeake Bay retrievers.

One function of vWF is to bind with factor VIII to stabilize the molecule and prevent rapid clearance from the circulation. Therefore, diagnosis of vWD was confused earlier with hemophilia A.⁸ vWD is clearly differentiated from hemophilia A by vWF concentration (Table 5-5); vWF is normal to increased in hemophilia A. The aPTT should be increased in hemophilia A (assuming the reagents are optimized for dogs), whereas in vWD the aPTT is usually normal despite the deficiency of vWF. Treatment recommendations include administration of fresh frozen plasma (or cryoprecipitate) for the treatment or prophylaxis of hemorrhagic episodes in dogs with vWD or hemophilia.⁴⁸

Disorders of Vascular Endothelium

Inherited Vascular Wall Disease

Ehlers-Danlos Syndrome • Several, rare inherited disorders of the vasculature result in aberrant hemostasis. Ehlers-Danlos–like syndrome is one disorder that occurs in dogs and cats.⁴⁶ Cutaneous asthenia, or dermatosparaxis, is an inherited disease of abnormal collagen synthesis that results in increased vascular fragility, joint laxity, and hyperextensibility of the skin. Skin tears are accompanied by hemorrhage and defects in platelet function that have been managed successfully with administration of desmopressin (i.e., DDAVP).

Familial Vasculopathy • A second inherited vascular wall disease is familial vasculopathy, which is a rare idiopathic inherited vasculopathy that results in vasculitis and collagenolysis. This disorder has been reported in several canine breeds, including the German shepherd, beagle, Scottish terrier, and greyhound.⁵⁶

Acquired Vascular Wall Disease

Commonly reported acquired vascular wall diseases include Rocky Mountain spotted fever, heartworm disease, infectious canine hepatitis, leishmaniasis, neoplasia, purpura hemorrhagica, and various endocrinopathies.

Rocky Mountain Spotted Fever • Rocky Mountain spotted fever (i.e., *Rickettsia rickettsii* infection) results in vascular disease approximately 2 weeks after infection. The organism replicates in and alters endothelial cells, which induces platelet and fibrinolytic activation, causing thrombocytopenia and DIC. Hematologic abnormalities include altered vascular permeability, petechiae, ecchymosis, edema, splenomegaly, hyperfibrinogenemia, activation of the fibrinolytic system, thrombosis, and DIC.¹⁰ Decreased plasma factor VIII and increased factor V activity indicate a poor patient prognosis.⁴³ Many infectious diseases kill animals by DIC-type episodes secondary to vascular damage, tissue necrosis, or both. A common gross lesion seen at necropsy with septicemia is widespread petechial hemorrhages as the result of DIC.

Heartworm Disease • Adult heartworms reside in the right ventricle and pulmonary artery and can cause partial blood flow obstruction and interference with tricuspid valve closure, with resultant caval syndrome because of retrograde migration of adult heartworms. Adult worms cause direct endothelial damage, leading to exposure of subendothelial collagen, and hypercoagulability, thrombocytopenia, and possible pulmonary thromboembolism or DIC. Administration of thiactarsemide adulticide may contribute to the incidence of thromboembolism via platelet aggregation.⁴

Leishmaniasis • Hemostatic abnormalities caused by leishmaniasis include epistaxis and thrombocytopenia. DIC may be triggered by endothelial damage. Thrombocytopenia, thrombopathia, and altered plasma fibrin and FDP concentrations have been reported. Epistaxis may be caused by local nasal inflammation in the absence of detectable hemostatic defects.

Acquired Hemostatic Disease • Acquired bleeding disorders are better investigated in people, but these mechanisms may be considered in confusing cases. A relatively rare acquired form of vWD is associated with human hematoproliferative disorders. Autoantibodies directed against vWF may lead to its more rapid clearance from the circulation or interference with its function. Other diseases causing acquired hemostatic disorders include neoplasia, which causes altered angiogenesis, alterations in tumor-associated vascular endothelium, hypercoagulability, and venous thromboembolism, partially the result of increased synthesis of thrombin, tissue factor, clotting factors, and vascular endothelial growth factor by tumor cells or tumor-associated cells. Diabetes mellitus and hypoadrenocorticism may alter coagulation, including impaired fibrinolysis, platelet aggregation, and increased plasminogen activator inhibitor. Acquired vascular lesions occur in FIP-associated vasculitis, immune-mediated disease such as hemorrhagic vasculitis,³⁸ drug reactions, trauma, gastrointestinal ulceration, and vitamin C deficiency.

Thrombocytosis • Thrombocytosis is not uncommon and was seen in 5% of canine and 3% of feline hematology samples. Causes include iron deficiency anemia, neoplasia, endocrine disease, splenic contraction, splenectomy, and immunosuppressive therapy (e.g., prednisolone,

vincristine). Nonspecific bone marrow stimulation occurs during leukocytosis or anemia because of cross-stimulation from the cytokines interleukin-3, interleukin-6, granulocyte-macrophage colony-stimulating factor, and erythropoietin. It also occurs with infectious and inflammatory disease, tumors, blood loss, or endocrinopathies. The spleen sequesters approximately one fourth to one third of the total circulating platelets, so splenic contraction from an excitement (i.e., epinephrine) response may result in a pseudothrombocytosis. Thrombocytosis is typically seen in canine iron deficiency anemia. Thrombocytosis in cats is often associated with myeloproliferative disorders or leukemia. Primary thrombocytosis, otherwise known as essential thrombocythemia, may cause thrombosis in some human patients. But it is less clear how much risk of thrombosis exists in dogs and cats with thrombocytosis and whether they should be treated preventatively.

Inherited Disorders of Primary Hemostasis

Greyhounds have asymptomatic lower circulating platelet counts.⁴⁹ Macrothrombocytopenia in Cavalier King Charles spaniels is an autosomal recessive trait, with a predominance of large platelets in circulation.⁴² It is due to a beta1-tubulin defect¹¹ (see earlier discussion on plateletcrit). Norfolk terriers also have a macrothrombocytopenia.¹⁷

Defective platelet function may be attributed to certain drugs or vWD (Table 5-6). Platelet dysfunction (i.e., thrombopathia) is documented by a platelet function test in animals with adequate platelet numbers. Rare disorders of membrane glycoproteins, platelet adhesion, dense body and alpha granule secretion, ATP production, and generation of procoagulant activity include basset hound thrombopathia, Spitz thrombopathia, thrombasthenic thrombopathia of otter hounds, Bernard-Soulier syndrome, Hermansky-Pudlak syndrome, Wiskott-Aldrich syndrome, Glanzmann's thrombasthenia of Great Pyrenees, and dense granular storage pool disease of American cocker spaniels.^{3,5,9,40}

Acquired Disorders of Primary Hemostasis

Acquired disorders of platelet numbers and function may arise after infectious or neoplastic disease or secondary to hyperthermia or drug therapy. For example, aspirin and nonsteroidal anti-inflammatory agents, which irreversibly

or reversibly acetylate platelet cyclooxygenase, prevent generation of thromboxane A₂ needed for secretion and aggregation. Toxins include estrogen (including testicular tumors), phenylbutazone, chemotherapeutic agents, and dapsone. Often myelofibrosis or a fatty marrow remains without evidence of the causative agent. A bone marrow aspirate is indicated when a patient exhibits bicytopenia, pancytopenia, or abnormal peripheral blood cell morphology (see Chapter 2).

Immune-Mediated Thrombocytopenia • ITP is a common disorder in which binding of antibodies results in platelet removal.⁵⁹ ITP is often diagnosed by ruling out a marrow production problem, DIC, and infectious diseases. Response to immunosuppressive therapy⁴⁴ further supports the diagnosis. Detection of antiplatelet antibodies on platelets in blood or in serum has been validated in dogs but not in cats. Tests are not widely available but include highly sensitive flow cytometric tests for detection of serum platelet-bindable immunoglobulin G (IgG) and platelet-bound IgG.^{26,27} Antimegakaryocytic antibodies may be identified on bone marrow smears. A platelet count less than 20,000/ μ l is highly suggestive for ITP and therefore are also clinical signs of bleeding because of so low platelet count. ITP may be secondary to drugs, infections, multiple transfusions, or lupus, or it may be idiopathic.

Ehrlichiosis • Thrombocytopenia, anemia, bicytopenia, or pancytopenia in a dog warrant considering *Ehrlichia*. Different species of *Ehrlichia* infect dogs; *Ehrlichia canis* is discussed here. Thrombocytopenia is typically found in ehrlichiosis. The platelet count is often greater than 20,000/ μ l and yet may still be associated with bleeding (e.g., epistaxis, hyphema, petechia). Platelet dysfunction in canine ehrlichiosis may be caused by antiplatelet antibodies.¹⁸ If the animal does not have bone marrow destruction as the result of *Ehrlichia*, the platelet count is usually 75,000 to 175,000/ μ l. Other hematologic findings associated with ehrlichiosis include mild to severe nonregenerative anemia (approximately 90% of cases), as well as leukopenia and neutropenia (approximately 50% of cases). Neutropenia may be severe enough to allow overwhelming sepsis. In some dogs, the bone marrow has ineffective hematopoiesis with a paradoxical pattern of normal to increased cellularity, despite pancytopenia. Anemia is not common in acute cases. Severe anemia and severe leukopenia are generally only seen when chronic disease and loss of bone marrow cellularity are found. The acute phase of the disease resembles other infections, with fever, anorexia, weight loss, and lymphadenopathy.

The chronic form is more variable, although hyperproteinemia and hypergammaglobulinemia are frequently present; the latter is occasionally severe enough to induce serum hyperviscosity. Polyclonal gammopathy is expected, but occasional serum protein electrophoretograms with oligoclonal peaks may mimic the monoclonal peaks of a lymphoid neoplasm. Numerous plasma cells in the bone marrow may also mislead one into diagnosing a plasma cell myeloma. The exuberant immune response may lead to an immune-complex glomerular disease and proteinuria.

TABLE 5-6. SELECTED CAUSES OF ABNORMAL PLATELET FUNCTION

CAUSE	
Drugs	Aspirin, ibuprofen, phenylbutazone, indomethacin
Acquired	Lymphoproliferate disorders, disseminated intravascular coagulation (DIC), uremia
Hereditary	von Willebrand's disease (vWD) and rare disorders in basset hounds, otter hounds, foxhounds, Spitz, Great Pyrenees, Scottish terriers, American cocker spaniels

Anaplasma phagocytophilum • *Anaplasma phagocytophilum* is a common tick-borne infection of dogs and occasional cats in Sweden.²⁸ Thrombocytopenia is moderate (occasionally $<60,000/\mu\text{L}$) and transient and is worse at peak parasitemia. Blood smear evaluation is diagnostic during the acute phase (about a week). Polymerase chain reaction (PCR) and antibody titer tests are available.

Hemolytic Uremic Syndrome • Hemolytic uremic syndrome has been reported in dogs and cats. It is associated with acute renal failure and thrombocytopenia after infectious and inflammatory disease, and has been observed after renal transplantation in cats and gastroenteritis in dogs.^{2,20} Platelet hyperaggregability and platelet thrombi, tissue ischemia, thrombocytopenia, and microangiopathic hemolytic anemia characterize this syndrome. Alteration of the thrombogenic properties of the endothelium with increased expression of tissue factor and enhanced platelet deposition can occur after 24 to 72 hours of exposure to uremic serum.

Retroviral Disease • Cats with feline leukemia virus (FeLV), feline immunodeficiency virus (FIV), or both often have moderate nonregenerative anemia with or without concurrent granulocytopenia and thrombocytopenia.⁴⁵ One should always test for FeLV, FIV, FIP, and rickettsial disease in thrombocytopenic cats. Platelets from FeLV-infected cats or those with DIC may have decreased granularity or vacuolation.

Hyperthermia • Hyperthermia has been used with some success in the treatment of certain types of cancer and viral infections. However, hyperthermia may cause thrombocytopenia, increased plasma FDPs, prolonged clotting times, and evidence of spontaneous bleeding, caused (in part) by hyperthermia-induced liver injury.¹²

Drug-Associated Platelet Dysfunction • Drugs that may affect platelet function include corticosteroids, sulfonpyrazone, methylxanthines, furosemide, carbenicillin and other synthetic penicillins, cephalosporins, nitrofurantoin, chloroquine, levamisole, phenothiazine, sympathetic blocking and stimulatory agents, prostacyclin, estrogen, ethanol, caffeine, heparin, antihistamine, dextran, vitamin E, and dipyridamole. A return of normal platelet function 4 to 5 days after discontinuing use of a drug suggests a cause-and-effect relationship. More rigorous evidence is needed to prove the effect.

Disorders of Secondary Hemostasis

Abnormal coagulation test results indicate a coagulopathy (i.e., impaired ability to form a clot). Shortened PT or aPTT results are usually ignored, because they do not correlate with clinical hypercoagulability. Abnormal test results (reported in seconds) usually are prolonged (the PT may be reported as a percent of normal from some human and European veterinary laboratories). A 3-second increase in the PT or a 5-second increase in the aPTT over reference values is significant. Lesser increases are possibly abnormal, but this conclusion must be strengthened with other information or repeated testing.

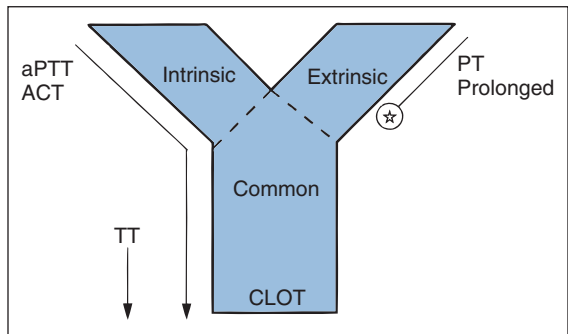


FIGURE 5-11. Test results of a factor VII deficiency. Only the prothrombin time (PT) would be abnormal, as depicted by a star and termination of the arrow at the site of factor VII in the extrinsic system. ACT, Activated coagulation time; aPTT, activated partial thromboplastin time; TT, thrombin time.

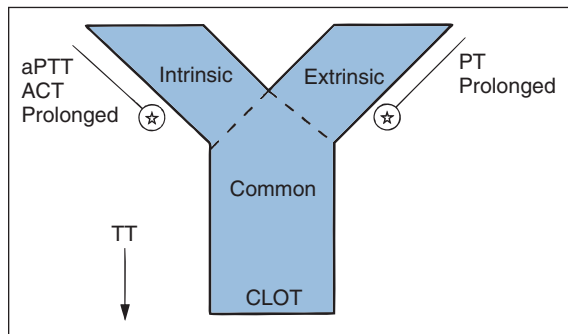


FIGURE 5-12. Test results for vitamin K antagonist toxicity. Inhibition of the vitamin K-related factors (II, VII, IX, and X) in warfarin poisoning creates defects in all three pathways. The thrombin time (TT) should be normal, because fibrinogen is not primarily affected. A factor X deficiency would have the same laboratory test pattern, because a defect in the common pathway slows clot formation despite normal intrinsic and extrinsic pathways. Hepatic failure may have a similar pattern unless fibrinogen deficiency is present. ACT, Activated coagulation time; aPTT, activated partial thromboplastin time; PT, prothrombin time.

A defect in the intrinsic pathway (i.e., factors XII, XI, IX, VIII) would be identified by the combination of a normal PT and prolonged aPTT (see Figure 5-7). A defect in factor VII is identified by the combination of a normal aPTT and prolonged PT (Figure 5-11).

A defect in the common pathway (i.e., factors X, V, prothrombin, fibrinogen) or multiple defects involving the intrinsic, common, and extrinsic pathways would prolong both the PT and aPTT. All tests except the TT would have prolonged results in vitamin K antagonist toxicity, because the vitamin K factors (i.e., II, VII, IX, X) would be deficient, creating defects in the intrinsic, extrinsic, and common pathways; however, the concentration of fibrinogen should be adequate (Figure 5-12). Because the liver synthesizes most procoagulant and anticoagulant factors, and removes activated factors from blood, severe hepatic failure causes multiple defects in the

coagulation cascade, including decreased synthesis of clotting and inhibitor factors, decreased clearance of activated factors, quantitative and qualitative platelet defects, hyperfibrinolysis, and accelerated intravascular coagulation.¹ DIC is common in animals with hepatic disease. A defect in the common pathway prolongs both the PT and aPTT, because the normal intrinsic and extrinsic pathways must pass through the common pathway to form a clot (see Table 5-3).

Clinical signs, breed, sex, and history provide important clues to the diagnosis before specific factor analysis. For example, if only some of the males in a litter of young dogs are affected with severe clinical signs of bleeding (including hematomas) and the dogs have a normal PT but prolonged aPTT, the problem is with either factor VIII or factor IX. The combination of prolonged aPTT and normal PT has localized the defect to the intrinsic pathway. The historical evidence of a sex-linked hereditary problem has implicated factors VIII and IX, the only two sex-linked defects. The severity of the bleeding rules out factors XII and XI in the intrinsic pathway, because bleeding is absent with factor XII or mild with factor XI deficiency. Factor IX and VIII defects (i.e., hemophilia B and A) have similar clinical bleeding problems and genetic implications. The cost:benefit ratio of more specific testing compared with the economic impact of the diagnosis determines whether additional tests for a more definitive diagnosis are appropriate.

Inherited Disorders of Secondary Hemostasis

Hemophilia A (factor VIII deficiency) is identified by prolongation of the aPTT. It is the most commonly reported inherited canine coagulopathy and has been reported cats with multiple factor deficiencies, leading to defects in intrinsic coagulation. The causative mutation has been determined to be an intron 22 inversion found in approximately 45% of severely affected hemophilia A patients.²¹ Several commercial tests have been validated to diagnose factor VIII:C and factor IX deficiencies from the plasma of hemophiliac dogs.³³

Factor IX deficiency (i.e., hemophilia B or Christmas disease) is sex-linked like hemophilia A and is detected by prolonged aPTT. Factor IX deficiency is much less commonly reported and has more consistently severe clinical signs. It affects German shepherds and British shorthair, Siamese, and American domestic shorthaired cats.

Factor X deficiency occurs in cocker spaniels and Jack Russell terriers.¹³ Factor XI deficiency in Kerry blue terriers, springer spaniels, and Pyrenees mountain dogs is a severe bleeding disorder with postoperative hemorrhage and prolonged aPTT.²⁴ These dogs tend to bleed after trauma or surgery but do not have spontaneous hemorrhage in joints and soft tissue characteristic of hemophiliacs.¹⁶

Devon rex cats may have vitamin K-dependent coagulopathies. They are treated successfully with oral vitamin K₁ or plasma transfusion, which normalizes clotting factor II, VII, IX, and X concentrations.³⁰ Prothrombin deficiency occurs in cocker spaniels, boxers, and otter hounds and, as a common pathway defect, will have prolonged aPTT and PT.

Autosomal dominant factor VII deficiencies have been reported in Alaskan malamutes, Labrador retrievers,

and beagles.^{32,47} Deficiency of factor XII, or Hageman factor, has been determined to have an autosomal recessive pattern in cats, similar to that which is reported in humans (i.e., Hageman trait).²³ A Chinese shar-pei was found to have a combined factor XII deficiency and impaired prekallikrein activity that resulted in episodes of intestinal hemorrhage and diarrhea. This is uncommonly reported in the dog, and is not typically associated with hemorrhagic tendencies.

Acquired Disorders of Secondary Hemostasis

Vitamin K Antagonism • Warfarin-type anticoagulant rodenticides are common causes of acquired bleeding disorders. Second-generation rodenticides have a long functional half-life (i.e., 15 to 20 days for diphacene, compared with 40 hours for warfarin). These rodenticides act by causing a functional vitamin K deficiency and reduced hepatic synthesis of functional forms of factors II, VII, IX, and X. Sulfaquinoxaline treatment (i.e., coccidiostat) of dogs may cause bleeding by antagonism of vitamin K. Post-hepatic biliary obstruction or severe enteric disease rarely causes vitamin K deficiency. The PT is preferred to monitor the coagulation defect. When factor synthesis is inhibited, the factor with the shortest half-life becomes deficient earliest. The half-life of factor VII is 2 to 4 hours, compared with 14 to 16 hours for factors IX and X and 41 hours for prothrombin (factor II). Because factor VII is in the extrinsic system, the earliest and greatest relative increase is noted in the PT. The thrombotest or PIVKA (proteins induced by vitamin K absence or antagonism) is sensitive for the precursor coagulation proteins from the liver that accumulate and spill into the circulation when vitamin K antagonism exists. After effective vitamin K₁ therapy, the liver can reach maximum prothrombin synthesis in 9 to 11 hours and the PT should be approaching normal within 24 hours. Warfarin also blocks protein C production, which, due to a short half-life like factor VII, is deficient earlier than factors II, IX, and X. This may cause a hypercoagulable situation early before hypocoagulation occurs. This hypercoagulable state may cause thrombocytopenia.

Other acquired coagulopathies arise secondarily from maldigestive and malabsorptive gastrointestinal disorders, exocrine pancreatic deficiency, infiltrative enteritis, bile duct obstruction, or hepatic disease, or because of increased circulating heparin from mast cells.

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6

Electrolyte and Acid-Base Disorders

Stephen P. DiBartola

Electrolyte and acid-base disorders may result from many different diseases. Timely correction of fluid, electrolyte, and acid-base disturbances is often of more immediate benefit to patients than a specific diagnosis, although both are important.

SERUM POTASSIUM CONCENTRATION

Commonly Indicated • Common indications to measure serum potassium concentration include prolonged anorexia, vomiting, diarrhea, muscle weakness, bradycardia, supraventricular arrhythmias, oliguria, anuria, and polyuria. Serum potassium concentrations should be measured if hypoadrenocorticism, acute or chronic renal failure, diabetic ketoacidosis, prolonged vomiting, urethral obstruction, uroabdomen, or postobstructive diuresis are suspected, or if prolonged use of diuretics (e.g., furosemide, thiazides, spironolactone) or angiotensin-converting enzyme inhibitors (e.g., enalapril) has occurred.

Analysis • Serum potassium concentrations are measured in serum, plasma, or urine by dry reagent methods, ion-specific potentiometry, and flame photometry (rarely used now). Different methods provide comparable results. Measured potassium concentrations obtained with the new “point-of-care” instruments do not always correlate well with results determined by traditional analyzers. Point-of-care units that measure potassium in whole blood typically give results approximately 0.5 mEq/L less than those obtained with other instruments.

Normal Values • Dogs and cats, 3.5 to 5.5 mEq/L (mEq/L are the same as mmol/L for univalent ions).

Danger Values • Concentrations less than 2.5 mEq/L (muscle weakness) or greater than 7.5 mEq/L (cardiac conduction disturbances) are considered dangerous. Severely hyponatremic animals seem less able to compensate for hyperkalemia.

Artifacts • Serum potassium concentrations exceed plasma concentrations because potassium is released from platelets during clotting. This difference is most pronounced when thrombocytosis occurs. Hemolysis causes hyperkalemia if red blood cells (RBCs) have a high potassium content. Most dog and cat RBCs contain little potassium; however, RBCs in some breeds (e.g., neonates, Akitas, English springer spaniels) have a higher potassium content (i.e., ≥ 20 mEq/L), and hemolysis may cause hyperkalemia. In animals with white blood cell (WBC) counts greater than 100,000/ μ l, enough WBCs may lyse and release potassium during clotting that serum potassium is artifactually increased. These are causes of pseudohyperkalemia, because they only occur *in vitro*. Using lithium heparin tubes for collection plus prompt separation of plasma from cells prevents these problems. Samples contaminated by drawing them through improperly cleared intravenous (IV) catheters may yield falsely increased or decreased potassium concentrations, depending on the fluid being administered. When obtaining blood from an IV catheter, one should remove and discard enough blood to clear the catheter before collecting the sample. Using ethylenediaminetetraacetic acid (EDTA) or potassium oxalate as an anticoagulant may markedly alter measured values. Large amounts of bilirubin may slightly increase potassium concentrations measured with ion-selective electrodes.

Drugs That May Alter Serum Potassium Concentration • Hypokalemia may be caused by administration of furosemide, thiazides, acetazolamide, laxatives, mineralocorticoids (e.g., fludrocortisone, desoxycorticosterone pivalate), insulin, sodium bicarbonate, amphotericin B, large doses of sodium penicillin G given IV, chronic administration of ammonium chloride, potassium-free fluids, and glucose-containing crystalloid solutions. Peritoneal dialysis can be responsible if potassium-free dialysate is used long term.

Hyperkalemia may be caused by excessive potassium chloride (either IV or oral), heparin solutions containing chlorbutol, massive digitalis overdose, and potassium penicillin G given IV. It may also be caused by trimethoprim, angiotensin-converting enzyme inhibitors (e.g., enalapril), blood transfusions (if from a dog with high

intracellular potassium), potassium-sparing diuretics (e.g., spironolactone, amiloride), mannitol infusions causing acute hypertonicity, nonspecific beta blockers, and nonsteroidal anti-inflammatory drugs (if they cause renal failure).

Causes of Hypokalemia • The three possible mechanisms for hypokalemia are (1) decreased intake, (2) translocation of potassium from extracellular to intracellular fluid, and (3) loss via the kidneys or gastrointestinal tract (Box 6-1 and Figure 6-1). Dilution of serum potassium concentration by giving potassium-free fluids, especially those containing glucose, may contribute to hypokalemia. Decreased intake may aggravate hypokalemia caused by increased loss or translocation, but it is unlikely to cause hypokalemia by itself. Hypokalemia often results from a combination of decreased intake plus urinary or gastrointestinal losses (e.g., administering potassium-free fluids to anorexic animals).

Translocation of potassium from extracellular to intracellular fluid may occur with bicarbonate administration or insulin-mediated glucose uptake by cells. Both situations typically are iatrogenic (e.g., aggressive treatment for diabetic ketoacidosis). Total parenteral nutrition may do likewise if sufficient potassium is not present in the solution. Hypothermia may cause potassium to enter cells (this effect is reversed when hypothermia is corrected). Hypokalemic periodic paralysis in young Burmese cats causes potassium to move intracellularly and is characterized by recurrent episodes of limb muscle weakness and neck ventroflexion, increased creatine kinase activity, and hypokalemia.

Excessive gastrointestinal (e.g., vomiting, diarrhea) and urinary (e.g., polyuria) losses commonly cause hypokalemia. Vomiting gastric contents causes loss of potassium and chloride. The resulting hypochloremia and metabolic alkalosis causes additional urinary loss of potassium and hydrogen ions. Aldosterone secretion due to dehydration from any cause results in sodium retention but further potassium excretion. Loop diuretics (e.g., furosemide) cause renal potassium wasting. Hypokalemia occurs in approximately 20% to 30% of cats and 10% of dogs with chronic renal failure.

Hypokalemic nephropathy characterized by tubulointerstitial nephritis may develop in cats fed high-protein diets with inadequate potassium, especially with diets that also contain urinary acidifiers.

Hypokalemia commonly occurs during the postobstructive diuresis after relief of feline urethral obstruction. Hypokalemia may occur in canine hyperadrenocorticism because of mineralocorticoid effects of endogenous steroids and is more common with adrenal tumors than in pituitary-dependent disease.

The most common causes of moderate to severe hypokalemia (i.e., <2.5 to 3.0 mEq/L) are vomiting of gastric contents, urinary losses (e.g., postobstructive diuresis, polyuric chronic renal failure), use of loop diuretics (especially in anorexic animals), aggressive insulin and sodium bicarbonate therapy (e.g., treatment of diabetic ketoacidosis), and inappropriate fluid therapy in anorexic animals. Causes of hypokalemia can usually be ascertained from history and physical

BOX 6-1. CAUSES OF HYPOKALEMIA

Pseudohypokalemia (infrequent and rarely causing significant change)

Increased Loss (most common and important category)

Gastrointestinal ($FE_K < 6\%$)

Vomiting of gastric contents (common and important)

Diarrhea (common and important)

Urinary ($FE_K > 20\%$)

Chronic renal failure in cats (common and important)

Diet-induced hypokalemic nephropathy in cats (important)

Postobstructive diuresis (common and important)

Inappropriate fluid therapy (especially with inadequate potassium supplementation) (common and important)

Diuresis caused by diabetes mellitus/ketoacidosis (common and important)

Dialysis (uncommon)

Drugs

Loop diuretics (e.g., furosemide) (common and important)

Thiazide diuretics (e.g., chlorothiazide, hydrochlorothiazide)

Amphotericin B

Penicillins (rare)

Albuterol overdose (rare)

Distal (type I) RTA (rare)

Proximal (type II) RTA after NaHCO_3 treatment (rare)

Mineralocorticoid excess (rare)

Hyperadrenocorticism (mild changes)

Primary hyperaldosteronism (i.e., adenoma, hyperplasia)

Translocation (Extracellular Fluid → Intracellular Fluid)

Glucose-containing fluids $\pm$ insulin (common and important)

Total parenteral nutrition solutions (uncommon, but important)

Alkalemia (uncommon)

Catecholamines (rare)

Hypokalemic periodic paralysis (Burmese cats) (rare)

Hypothermia (questionable)

Decreased Intake (Unlikely to cause hypokalemia by itself unless diet is severely deficient)

Administration of potassium-free fluids (e.g., 0.9% NaCl, 5% dextrose in water)

FE_K , Fractional excretion of potassium; RTA, renal tubular acidosis. Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 93.

examination. Additional laboratory tests are rarely needed.

Causes of Hyperkalemia • The three mechanisms for hyperkalemia are (1) increased potassium intake, (2) translocation of potassium from intracellular to extracellular fluid, and (3) decreased urinary potassium excretion

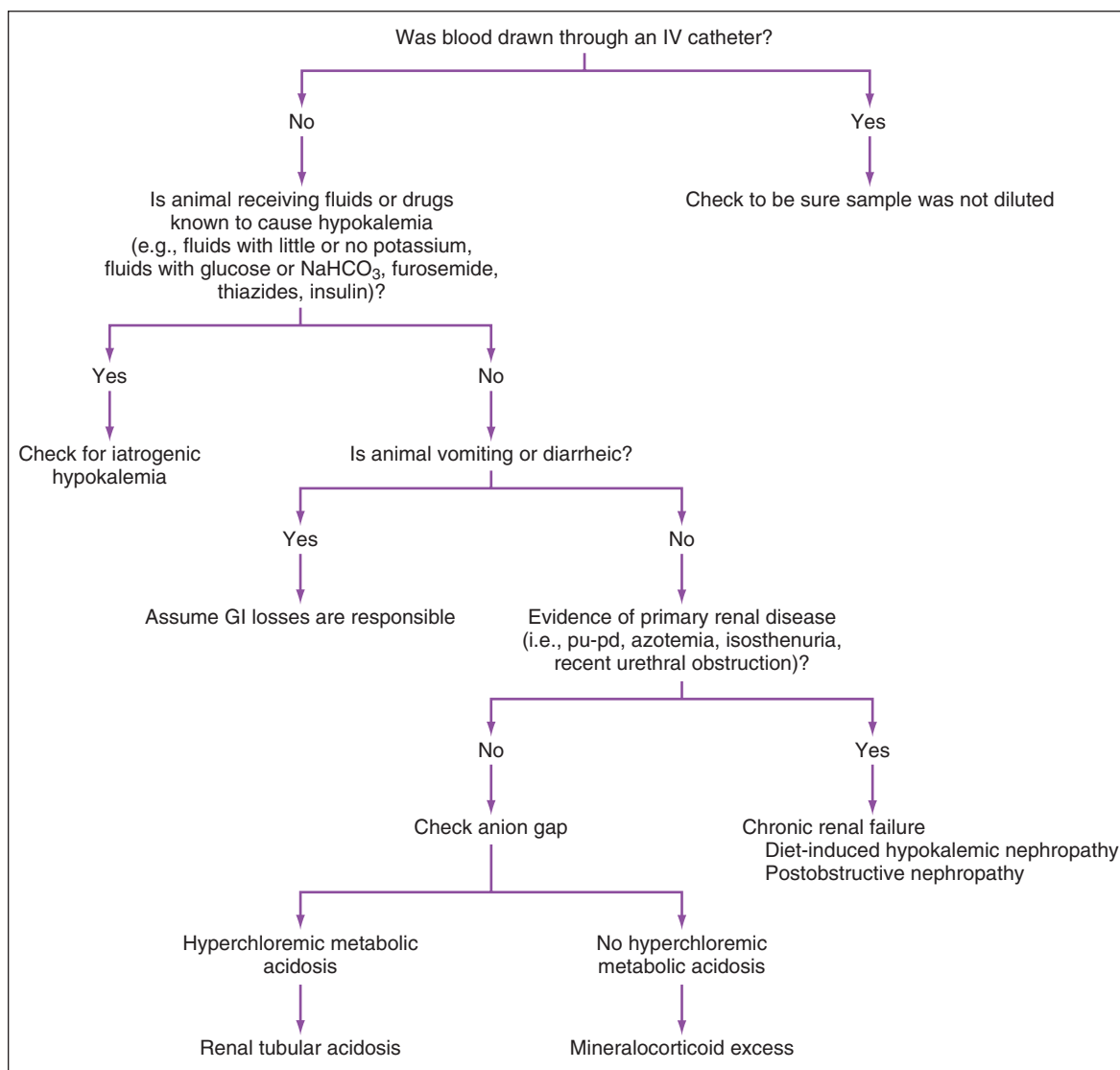


FIGURE 6-1 Algorithm for clinical approach to hypokalemia. *GI*, Gastrointestinal; *IV*, intravenous; *pu-pd*, polyuria-polydipsia. (Modified from DiBartola SP: *Fluid therapy in small animal practice*, Philadelphia, 1992, WB Saunders, p 98.)

(most common) (Box 6-2 and Figure 6-2). Increased intake is seldom the cause, unless potassium administration is greatly excessive or concurrent renal or adrenal impairment exists.

Translocation of potassium from cells to extracellular fluid may occur with acute inorganic acidosis, massive tissue damage (e.g., acute tumor lysis) or potassium retention (caused by acute renal failure), insulin deficiency, and acute hypertonicity. Acute acidosis due to inorganic acids (e.g., NH_4Cl , HCl) but not organic acids (e.g., lactic acid, keto acids) may cause potassium to shift out of cells (uncommon). The effect of inorganic metabolic acidosis on serum potassium concentration varies, usually raising potassium 0.17 to 1.67 (mean, 0.75) mEq/L per 0.1-unit decrement in pH; however, this rule

of thumb is not reliable. Respiratory acidosis has minimal effect on potassium. Acute tumor lysis syndrome rarely occurs after radiation or chemotherapy for lymphoma. Other causes of massive tissue damage include reperfusion injury and crush injury (rare). Insulin deficiency and hyperosmolality may cause hyperkalemia in diabetic ketoacidosis. Acute hypertonicity (e.g., mannitol infusion, hyperglycemia) may cause water and potassium to exit cells and enter the extracellular space, causing hyperkalemia (uncommon).

Decreased excretion is the most important mechanism; hyperkalemia seldom occurs if renal function is normal. The most common causes of decreased urinary potassium excretion are urethral obstruction, ruptured bladder (or ureter), anuric or oliguric renal failure, and

BOX 6-2. CAUSES OF HYPERKALEMIA**Pseudohyperkalemia**

Thrombocytosis (usually mild, but can produce marked changes)

WBCs > 100,000/ μ l (rare cause, but can cause significant changes)

Hemolysis in breeds or individuals with high RBC potassium concentration (e.g., Akitas, English springer spaniels, neonates, occasional other dogs)

Decreased Urinary Excretion (most common)

Urethral obstruction (common and important)

Ruptured bladder/ureter (uncommon but important)

Anuric or oliguric renal failure (common and important)

Hypoadrenocorticism (uncommon but important)

Selected gastrointestinal diseases (e.g., trichuriasis, salmonellosis, perforated duodenal ulcer)

Chylothorax with repeated pleural fluid drainage (rare)

Hyporeninemic hypoaldosteronism (with diabetes mellitus or renal failure) (rare)

Drugs (angiotensin-converting enzyme inhibitors [e.g., enalapril],* potassium-sparing diuretics [e.g., spironolactone, amiloride, triamterene],* prostaglandin inhibitors,* heparin*)

Increased Intake

Unlikely with normal renal/adrenal function, unless administration is greatly excessive (e.g., IV administration of fluids with high KCl concentrations, administration of large doses of potassium penicillin G)

Translocation (Intracellular Fluid $\rightarrow$ Extracellular Fluid)

Insulin deficiency (e.g., diabetic ketoacidosis) (uncommon and transient)

Acute inorganic acidosis (e.g., HCl, NH_4Cl) (rare)

Massive tissue damage (e.g., acute tumor lysis syndrome [rare], reperfusion of extremities after aortic thromboembolism in cats with cardiomyopathy [rare], crush injuries [rare])

Hyperkalemic periodic paralysis (rare)

Drugs (nonspecific beta blockers [e.g., propranolol*])

IV, Intravenous; RBC, red blood cell; WBC, white blood cell.

*Only likely to cause hyperkalemia in conjunction with other contributing factors (e.g., decreased renal function, concurrent administration of potassium supplements).

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 100.

hypoadrenocorticism. Hyperkalemia may occur within 48 hours of feline urethral obstruction, but it does not usually occur for at least 48 hours after urinary bladder rupture. Hyperkalemia seldom occurs in chronic renal failure and then usually only in oliguric patients. Hyperkalemia, hyponatremia, and Na/K ratios less than 27:1 are often (but not always) found in animals with hypoadrenocorticism or renal failure. An adrenocorticotrophic hormone (ACTH) stimulation test (see Chapter 8) is necessary to diagnose hypoadrenocorticism, because identical electrolyte abnormalities can occur because of oliguric renal failure, whipworms, salmonellosis, and pleural or peritoneal effusions.

Very rarely, hyporeninemic hypoaldosteronism impairs urinary potassium excretion, causing hyperkalemia in patients with diabetes or renal failure. This disease is diagnosed by measuring aldosterone (not cortisol) concentrations before and after ACTH administration. Hyperkalemic periodic paralysis is another rare cause of hyperkalemia that has been reported in only one dog.

The most important causes of serious hyperkalemia (i.e., > 6.0 mEq/L) are oliguric and anuric acute renal failure (e.g., ethylene glycol ingestion), urethral obstruction in male cats, and hypoadrenocorticism. Pseudohyperkalemia should be eliminated first. If serum potassium concentration is greater than 7.0 mEq/L and the patient is asymptomatic (e.g., normal electrocardiogram and physical examination), serum potassium concentration should be rechecked using lithium heparin plasma. After artifact has been eliminated, history should be examined for iatrogenic causes. If hyperkalemia might be iatrogenic, the drug in question should be discontinued and serum potassium rechecked in 1 to 2 days. Diagnostic evaluation should continue in case another disease is present, however. Hyperkalemia is usually an indication for evaluation of some or all of the following: serum creatinine, blood urea nitrogen (BUN), urinalysis, and a resting serum cortisol concentration (see Chapter 8).

URINARY FRACTIONAL EXCRETION OF POTASSIUM

Seldom Indicated • Fractional excretion of potassium (FE_K) helps distinguish renal from nonrenal potassium loss. FE_K is calculated as follows:

$$[(U_K / S_K) / (U_{Cr} / S_{Cr})] \times 100$$

where

U_K = urine concentration of potassium (mEq/L)

S_K = serum concentration of potassium (mEq/L)

U_{Cr} = urine concentration of creatinine (mg/dl)

S_{Cr} = serum concentration of creatinine (mg/dl)

Normal Values • Dogs and cats, 6% to 20%.

Abnormalities • FE_K should be less than or equal to 6% if the animal has nonrenal sources of potassium loss (e.g., gastrointestinal loss). Values greater than 20% in hypokalemic patients with normal renal function indicate excessive renal potassium losses.

SERUM SODIUM CONCENTRATION

Commonly Indicated • Serum sodium determination is useful in systemic diseases characterized by vomiting, diarrhea, polydipsia and polyuria, muscle weakness, abnormal behavior, abnormal mentation, seizures, edema, pleural or peritoneal effusion, or dehydration. Serum sodium should be determined whenever adrenal, renal, hepatic, or cardiac failure has been diagnosed; in cases of prolonged fluid or diuretic therapy; or in patients

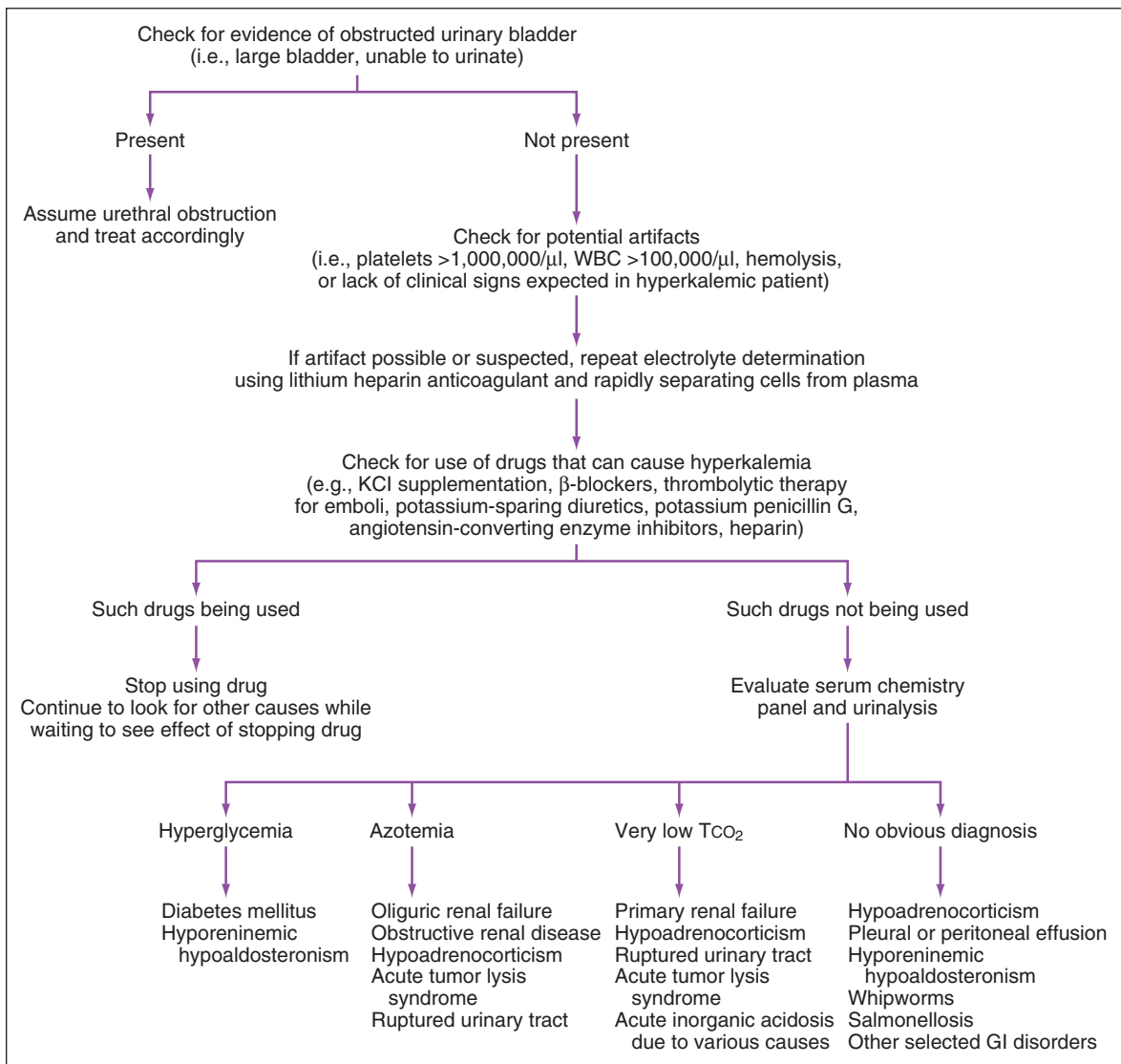


FIGURE 6-2 Algorithm for clinical approach to hyperkalemia. *GI*, Gastrointestinal; TCO_2 , total carbon dioxide; *WBC*, white blood cell. (Modified from DiBartola SP: *Fluid therapy in small animal practice*, Philadelphia, 1992, WB Saunders, p 107.)

that are not drinking water. Results obtained by using point-of-care instruments usually correlate well with results obtained by traditional instruments.

Analysis • Serum sodium is measured in serum, plasma, or urine by ion-specific potentiometry and dry reagent methods.

Normal Values • Dogs, 140 to 150 mEq/L; cats, 150 to 160 mEq/L (mEq/L are the same as mmol/L).

Danger Values • Clinical signs of hyponatremia and hypernatremia are more related to rapidity of onset than to magnitude of change and associated plasma hypo-osmolality or hyperosmolality. Neurologic signs (e.g., disorientation, ataxia, seizures, coma) may occur at serum

sodium concentrations less than 120 or greater than 170 mEq/L in dogs.

Artifacts • Historically, when flame photometry or indirect potentiometry was used and hyponatremia plus normal plasma osmolality was found, this was called pseudohyponatremia and was caused by hyperlipidemia or severe hyperproteinemia. Excessive lipid and protein in serum caused the machine to inaccurately determine the concentration. Pseudohyponatremia rarely occurs when ion-specific electrodes are used. Hyperviscosity caused by hyperproteinemia can lead to “short samples” (and artifactual hyponatremia) with certain aspiration techniques. Samples drawn through improperly cleared IV catheters may yield falsely increased or decreased sodium concentrations, depending on the fluid being

administered. When obtaining blood from an IV catheter, one should remove and discard enough blood to clear the catheter before collecting the sample. Sodium salts of various anticoagulants (e.g., oxalate, fluoride, citrate) increase measured values.

Drugs That May Alter Serum Sodium Concentration • Hyponatremia may develop because of thiazides, furosemide, spironolactone, or trimethoprim combined with a diuretic. Drug-induced syndrome of inappropriate antidiuretic hormone secretion (SIADH) is reported in people (e.g., with vincristine), but not dogs or cats. Hypernatremia may develop because of desoxycorticosterone acetate or pivalate, fludrocortisone, sodium bicarbonate, lactulose, inappropriate therapy with physiologic or hypertonic saline solutions, or sodium phosphate enemas.

Abnormal Serum Sodium Concentrations • Serum sodium concentration is the amount of sodium relative to the volume of water in the blood; it does not reflect total body sodium content. Hyponatremic and hypernatremic patients may have decreased, normal, or increased total body sodium. Hypernatremia almost always causes hyperosmolality, whereas hyponatremia usually implies hypo-osmolality.

Causes of Hyponatremia • Accurate evaluation of hyponatremia requires measuring plasma osmolality. Most hyponatremic patients are hypoosmolar, but hyperglycemia (i.e., diabetes mellitus) or mannitol administration (Box 6-3) may cause hyponatremia with hyperosmolality. The next step in evaluating hyponatremia is to estimate hydration status. History may indicate fluid loss. Physical examination allows some evaluation of a patient's hydration status (e.g., skin turgor, moistness of mucous membranes, capillary refill time, pulse rate and character, appearance of jugular veins, presence or absence of ascites).

Dehydrated hyponatremic patients have lost water and sodium, but more sodium than water. Nonrenal or renal routes may result in loss of sodium-rich fluid. Nonrenal losses may be gastrointestinal (e.g., vomiting, diarrhea), third space (e.g., pancreatitis, peritonitis, uroabdomen, pleural effusion), or cutaneous (e.g., burns). Gastrointestinal fluid losses may lead to hyponatremia if the loss of sodium is greater than the loss of water or if subsequent replacement of the lost fluids by drinking water dilutes the remaining sodium. Hypoadrenocorticism, diuretics, diabetes mellitus, or renal disease may cause renal fluid and salt loss. Once again, drinking water replaces water but not sodium, causing hyponatremia. Hyponatremia also has been associated with chronic hemorrhage and hemoabdomen in dogs.

Overhydrated hyponatremic patients (e.g., ascites, edema) may have increased total body sodium. Impaired water excretion causes fluid retention, which dilutes serum sodium. Clinical signs of hypervolemia may not be visible, because the retained water may be intracellular or interstitial. Hypervolemic hyponatremia primarily occurs in congestive heart failure, severe hepatic disease, nephrotic syndrome, and advanced renal failure.

BOX 6-3. CAUSES OF HYPONATREMIA

With Normal Plasma Osmolality (Pseudohyponatremia) (rare with current instruments)

Hyperlipidemia

Marked hyperproteinemia (rare)

With High Plasma Osmolality

Hyperglycemia (common)

Mannitol infusion

With Low Plasma Osmolality

Overhydration (i.e., hypervolemia)

Severe hepatic disease causing ascites (common)

Congestive heart failure causing effusion (common)

Nephrotic syndrome causing effusion (common)

Advanced renal failure (primarily oliguric or anuric)

Dehydration (i.e., Hypovolemia)

Gastrointestinal loss (common) (i.e., vomiting or diarrhea)

Third-space loss (i.e., pancreatitis, peritonitis, uroabdomen [common], chylothorax with repeated pleural fluid drainage)

Cutaneous loss (i.e., burns)

Hypoadrenocorticism (uncommon but important)

Diuretic administration (including osmotic diuretics)

Normal Hydration (i.e., Normovolemia)

Inappropriate fluid therapy with 5% dextrose, 0.45% saline solution, or hypotonic fluids (important)

Psychogenic polydipsia

Syndrome of inappropriate antidiuretic hormone secretion (SIADH) (rare)

Antidiuretic drugs (e.g., heparin solutions containing chlorbutol, vincristine, cyclophosphamide, nonsteroidal anti-inflammatory drugs)

Myxedema coma of hypothyroidism (rare)

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 60.

Normovolemic hyponatremia may be caused by primary (i.e., psychogenic) polydipsia, fluid therapy (e.g., 5% dextrose or 0.45% saline), SIADH (rare), drugs with antidiuretic effects, and myxedema coma from hypothyroidism (rare). Primary polydipsia (see Chapter 7) usually occurs in large breeds of dogs. These dogs have severe polydipsia, polyuria, severe hyposthenuria, mild hyponatremia, and mild plasma hypo-osmolality. SIADH refers to excessive antidiuretic hormone (ADH) release despite lack of normal stimuli; it can be caused by malignancy, pulmonary disease, or central nervous system (CNS) disorders. Diagnosis of SIADH requires eliminating adrenal, renal, cardiac, and hepatic disease and finding inappropriately high urine osmolality (> 100 mOsm/kg) despite serum hypo-osmolality. Drugs that stimulate ADH release or potentiate its renal effects may lead to hyponatremia with normovolemia.

The most common causes of moderate to marked hyponatremia (i.e., Na <135 mEq/L) in dogs and cats include vomiting, hypoadrenocorticism, and advanced

BOX 6-4. CAUSES OF HYPERNATREMIA**Loss of Free Water without Adequate Replacement (important)**

Normal insensible water loss without normal replacement

Water unavailable or patient unable to drink

Abnormal thirst mechanism

Primary hypodipsia (e.g., miniature schnauzers) (*rare*)

Central nervous system (CNS) neoplasia

Increased insensible water loss without replacement

High environmental temperature, fever, tachypnea/panting

Urinary loss of free water

Diabetes insipidus (either central or nephrogenic)

Loss of Hypotonic Fluids without Adequate Replacement of Water (important)

Extrarenal

Gastrointestinal (i.e., vomiting, diarrhea, small intestinal obstruction)

Third-space loss (i.e., peritonitis, pancreatitis)

Cutaneous (e.g., burns)

Renal

Diuresis (osmotic [e.g., diabetes mellitus, mannitol], chemical [e.g., drugs])

Renal failure, postobstructive diuresis

Increased Intake of Sodium

Hypertonic fluid administration (e.g., hypertonic saline, sodium bicarbonate, total parenteral nutrition solutions, sodium phosphate enema)

Inappropriate maintenance fluid therapy with sodium-containing fluids (*important*)

Salt poisoning

Hyperaldosteronism (*rare*)

Hyperadrenocorticism (*mild changes*)

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 53.

congestive heart failure (with or without concomitant diuretic therapy). History or physical examination usually reveals the cause, but a resting serum cortisol should be measured (see Chapter 8) if the clinician suspects hypo-adrenocorticism. If the cause is still unknown, plasma osmolality measurements are recommended.

Causes of Hypernatremia • Hypernatremia is caused by loss of water, gain of sodium, or both (Box 6-4). It is rare for animals with normal thirst mechanisms and adequate access to water to become hypernatremic unless they are unable to ingest water.

Free-water loss (i.e., loss of water without appreciable amounts of electrolytes) occurs in diabetes insipidus and insensible losses. Central diabetes insipidus (see Chapter 7) is due to lack of ADH production and release. Affected animals have severe polydipsia and polyuria, and hypernatremia is common. Nephrogenic diabetes insipidus is a category that includes many disorders characterized by renal urine concentration abnormalities (see Chapter 7). Insensible losses (i.e., normal respiratory tract losses)

occur in all animals; if the patient cannot or will not drink (e.g., hypodipsia caused by an abnormal CNS thirst mechanism in young female miniature schnauzers), hypernatremia results. Clinical signs include anorexia, lethargy, weakness, disorientation, ataxia, and seizures.

Hypotonic water loss is loss of both water and electrolytes, but more water than sodium. Such losses may be renal or extrarenal (e.g., gastrointestinal, third space, cutaneous). Vomiting, diarrhea, and small intestinal obstruction may cause hypotonic gastrointestinal losses. Third-space losses include pancreatitis and peritonitis. Cutaneous losses are rarely important in dogs and cats. Renal losses may result from lack of ADH, osmotic or drug-induced diuresis, or renal disease that affects concentrating ability.

NOTE: If a patient with hypotonic fluid losses replaces fluids by drinking water, it may become *hyponatremic* instead of *hypernatremic*, because it is diluting remaining sodium with water it drinks.

Administration of excessive sodium (e.g., hypertonic saline, sodium bicarbonate, inappropriate fluid therapy) causes hypernatremia if the patient does not ingest adequate water. Hyperadrenocorticism, sodium phosphate enemas, and primary hyperaldosteronism (*rare*) may cause hypernatremia.

Clinically significant hypernatremia (i.e., $\text{Na} > 160 \text{ mEq/L}$ in dogs and 170 mEq/L in cats) usually is due to a pure water deficit (e.g., unable or unwilling to drink), loss of hypotonic fluid (e.g., gastrointestinal or renal losses), or fluid therapy. History is usually adequate to determine the cause of hypernatremia.

URINARY FRACTIONAL EXCRETION OF SODIUM

Seldom Indicated • Determining fractional sodium excretion (FE_{Na}) may help differentiate prerenal from primary renal azotemia (seldom needed for this purpose), and renal from extrarenal sodium loss in dehydrated patients with hypernatremia or hyponatremia. FE_{Na} is calculated by using the equation:

$$[(U_{\text{Na}}/S_{\text{Na}})/(U_{\text{Cr}}/S_{\text{Cr}})] \times 100$$

where

U_{Na} = urine concentration of sodium (mEq/L)

S_{Na} = serum concentration of sodium (mEq/L)

U_{Cr} = urine concentration of creatinine (mg/dl)

S_{Cr} = serum concentration of creatinine (mg/dl)

Normal Values • FE_{Na} should be less than 1% in normal dogs and cats.

Abnormalities • FE_{Na} should be less than 1% in animals with prerenal azotemia; greater than 1% suggests primary renal azotemia. Prerenal azotemia with FE_{Na} greater than 1% may occur despite normal renal function if the animal is receiving diuretics (e.g., furosemide). In dehydrated patients, FE_{Na} values less than 1% suggest nonrenal losses

(e.g., gastrointestinal, third space); values greater than 1% suggest renal losses (e.g., hypoadrenocorticism, diuretic administration, renal disease).

SERUM CHLORIDE CONCENTRATION

Commonly Indicated • Serum chloride concentration commonly is measured in systemic diseases characterized by vomiting, diarrhea, dehydration, polyuria, and polydipsia or in patients likely to have metabolic acid-base abnormalities.

Analysis • Serum chloride is measured in serum, plasma, or urine by dry reagent systems, colorimetric titration, spectrophotometry (i.e., autoanalyzers), ion-specific potentiometry, and coulometric and amperometric titration. Results obtained by using point-of-care instruments do not always correlate well with results obtained by traditional methods.

Normal Values • Changes in water balance change chloride and sodium concentrations proportionately. Chloride concentration can also change primarily; therefore, evaluation of chloride concentration must be done in conjunction with evaluation of sodium concentration. Using this approach, chloride disorders can be divided into artifactual (sodium and chloride change proportionately) and corrected (changes in chloride are proportionately greater than changes in sodium) categories (Boxes 6-5 and 6-6). Changes in free water are responsible for the chloride changes in artifactual disorders. In corrected chloride disorders, chloride ion itself changes. Corrected chloride can be estimated as:

$$[\text{Cl}^-] \text{ corrected} =$$

$$[\text{Cl}^-] \text{ measured} \times 146 / [\text{Na}^+] \text{ measured (for dogs)}$$

$$[\text{Cl}^-] \text{ corrected} =$$

$$[\text{Cl}^-] \text{ measured} \times 156 / [\text{Na}^+] \text{ measured (for cats)}$$

BOX 6-5. CAUSES OF HYPOCHLOREMIA

Artifactual (Dilutional)

Corrected Hypochloremia

Pseudohypochloremia (lipemic samples using titrimetric methods)

Excessive loss of chloride relative to sodium

Vomiting of stomach contents (*common and important*)

Therapy with thiazide or loop diuretics (*common and important*)

Chronic respiratory acidosis

Hyperadrenocorticism

Exercise

Therapy with solutions containing high sodium concentration relative to chloride

Sodium bicarbonate

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 78.

where $[\text{Cl}^-]$ measured and $[\text{Na}^+]$ measured are the patient's serum chloride and sodium concentrations, respectively. The values 146 and 156 reflect the mean value for serum sodium concentrations in dogs and cats. Normal $[\text{Cl}^-]$ corrected is approximately 107 to 113 mEq/L in dogs and 117 to 123 mEq/L in cats. These values may vary among laboratories and analyzers.

Danger Values • Unknown. Metabolic alkalosis and decreased ionized calcium concentration probably cause muscle twitching or seizures in hypochloremic animals, whereas clinical signs associated with hyperchloremia are probably caused by hyperosmolality.

Artifacts • Pseudohypochloremia results when chloride is measured in lipemic or markedly hyperproteinemic samples via techniques that are not ion-selective. Hyperviscosity may cause problems in machines that dilute samples before analysis. In lipemic samples, chloride concentration is underestimated by some titrimetric methods and overestimated by colorimetric methods. Halides (e.g., bromide, iodide) are measured as chloride, falsely increasing reported values (especially important in animals receiving potassium bromide as an anticonvulsant).

Drugs That May Alter Serum Chloride Concentration • Administration of NH_4Cl , KCl, physiologic saline solution (with or without KCl), hypertonic saline solution, or total parenteral nutrition solutions containing arginine HCl and lysine HCl may add excessive Cl to the body. Acetazolamide may cause renal chloride retention. Hypochloremia may be caused by excessive renal loss of chloride relative to sodium (e.g., furosemide, thiazides)

BOX 6-6. CAUSES OF HYPERCHLOREMIA

Artifactual (Concentration)

Corrected Hyperchloremia

Pseudohyperchloremia

Lipemic samples using colorimetric methods

Potassium bromide therapy (*common and important*)

Excessive loss of sodium relative to chloride

Small bowel diarrhea (*common and important*)

Excessive gain of chloride relative to sodium

Therapy with chloride salts (e.g., NH_4Cl , KCl)

Total parenteral nutrition

Fluid therapy (e.g., 0.9% NaCl, hypertonic saline, KCl-supplemented fluids)

Salt poisoning

Renal chloride retention

Renal failure

Renal tubular acidosis

Hypoadrenocorticism

Diabetes mellitus

Chronic respiratory alkalosis

Drugs (e.g., spironolactone, acetazolamide)

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 79.

or excessive intake of sodium without chloride (e.g., NaHCO_3).

Causes of Hypochloremia • Many causes of hyponatremia also produce hypochloremia. If changes in sodium are proportional to changes in chloride (hypochloremia with normal corrected chloride or artifactual hypochloremia), it is usually easier to search for the cause of the hyponatremia. Corrected hypochloremia results from excessive loss of chloride relative to sodium or administration of fluids containing high sodium concentration relative to chloride (see Box 6-5). The most common causes of corrected hypochloremia are chronic vomiting of gastric contents and aggressive furosemide or thiazide therapy. Administration of sodium without chloride (e.g., NaHCO_3) also may cause corrected hypochloremia. Hypochloremia caused by increased renal chloride excretion is a normal adaptation to chronic respiratory acidosis. Persistent hypochloremia is an indication to determine serum sodium, potassium, and total carbon dioxide (TCO_2) concentrations (preferably by blood gas analysis).

Causes of Hyperchloremia • Most causes of hypernatremia produce concurrent hyperchloremia. If changes in sodium are proportional to changes in chloride (hyperchloremia with normal corrected chloride concentration or “artifactual” hyperchloremia), it is usually easier to search for the cause of the hypernatremia. Corrected hyperchloremia results from excessive sodium loss relative to chloride, excessive chloride gain relative to sodium, or renal chloride retention (see Box 6-6). Small bowel diarrhea can cause hyperchloremic metabolic acidosis because of loss of bicarbonate-rich, chloride-poor fluid (i.e., excessive sodium loss). Salt poisoning or therapy with NH_4Cl , KCl , cationic amino acids, hypertonic saline, or 0.9% NaCl with or without added KCl represent excessive chloride gain (e.g., physiologic saline solution has 154 mEq chloride/L but contains 174 mEq chloride/L if supplemented with 20 mEq KCl /L).

The most common cause of hyperchloremia is hypotonic fluid loss leading to hyperchloremic (normal anion gap) metabolic acidosis. Persistent hyperchloremia is an indication for determining serum sodium, potassium, and TCO_2 concentrations and blood gas analysis.

OSMOLALITY AND OSMOLAL GAP

Osmolality refers to the number of osmotically active particles in a solution. *Tonicity* describes the osmolality of a solution relative to plasma. A solution with the same osmolality as plasma is said to be *isotonic*, whereas one with greater osmolality than plasma is *hypertonic*. A solution with osmolality lower than that of plasma is *hypotonic*. Tonicity depends on the ability of these particles to exert oncotic pressure and whether or not the particles can rapidly cross a semipermeable membrane (e.g., a cell membrane). For example, urea does not cause hypertonicity (i.e., exert oncotic pressure), because it rapidly diffuses across cell membranes and equilibrates throughout the body. Sodium and glucose cannot rapidly cross membranes; therefore, they tend to stay on one side and cause

hypertonicity (i.e., exert oncotic pressure), attracting fluids. Everything that affects tonicity (e.g., sodium) also affects osmolality, but not everything that affects osmolality also affects tonicity (e.g., urea).

Occasionally Indicated • Serum or plasma osmolality helps differentiate causes of hyponatremia, aids in early diagnosis of ethylene glycol intoxication, evaluates hydration status and renal concentrating ability during water deprivation testing, and sometimes helps evaluate patients with diabetic ketoacidosis and those being treated with mannitol for cerebral edema.

Disadvantage • Special equipment (e.g., freezing point depression or vapor pressure osmometer) is required.

Analysis • Osmolality is measured in serum, plasma, or urine by freezing point or vapor pressure osmometry (citrate anticoagulants cause artifactual increases). It is estimated (i.e., calculated) by various formulas. In the absence of excessive unmeasured osmoles (e.g., ethylene glycol), the following formula closely estimates osmolality:

Osmolality (mOsm/kg) =

$$1.86([\text{Na}^+] + [\text{K}^+]) + (\text{glucose}/18) + (\text{BUN}/2.8) + 9$$

where serum sodium and potassium are expressed in mEq/L and BUN and glucose in mg/dl. However, $2 \times [\text{Na}]$ may be used as a quick estimate of osmolality. Tonicity may be estimated by the following formula:

$$\text{Tonicity} = \text{Plasma osmolality} - (\text{BUN}/2.8)$$

where tonicity and osmolality are expressed in mOsm/kg and BUN is expressed in mg/dl.

Normal Values • Serum or plasma osmolality: dogs, 290 to 310 mOsm/kg; cats, 308 to 335 mOsm/kg. Urine osmolality values vary widely. Typical ranges are 50 to 2800 mOsm/kg (dogs) and 50 to 3000 mOsm/kg (cats).

Danger Values • Signs caused by hypo-osmolality or hyperosmolality are related more to rapidity of change than magnitude of change. Neurologic signs (e.g., disorientation, ataxia, seizures, coma) may occur when serum or plasma osmolality is less than 250 mOsm/kg or tonicity is greater than 360 mOsm/kg.

Osmolal gap is defined as measured serum osmolality – calculated serum osmolality. An increased gap is due to unmeasured osmoles (e.g., ethylene glycol metabolites), pseudohyponatremia (i.e., normal osmolality plus hyponatremia), or laboratory error. Vapor pressure osmometry does not detect volatile solutes (e.g., methanol). If measured osmolality is less than calculated osmolality, a laboratory error is probably responsible.

Normal Values (Osmolal gap) • Dogs, 10 to 15 mOsm/kg; cats, unknown.

Danger Values (Osmolal gap) • An osmolal gap greater than 25 mOsm/kg indicates the presence of an unmeasured osmole, usually as a result of intoxication (e.g., ethylene glycol, methanol, ethanol).

Causes of Serum or Plasma Hypo-osmolality • See Causes of Hyponatremia.

Causes of Serum or Plasma Hyperosmolality • Hyperosmolality is caused by hypernatremia, hyperglycemia, severe azotemia, glycerin, and intoxications (e.g., ethylene glycol, ethanol, methanol). The most common causes of serum osmolality greater than 360 mOsm/kg are diabetic ketoacidosis, azotemia, and hypernatremia. Citrate anticoagulants may cause increased readings. Hyperosmolality is an indication to measure serum sodium, potassium, urea nitrogen, and glucose concentrations plus calculate anion and osmolal gaps.

Causes of Increased Osmolal Gap • Pseudohyponatremia, glycerin, ethylene glycol, methanol, ethanol, and possibly other intoxications can increase the osmolal gap. Mannitol or lactic acid might also be responsible. If pseudohyponatremia is ruled out, an increased osmolal gap mandates a search for recent exposure to these toxins. The increase in osmolal gap in dogs with ethylene glycol intoxication peaks at 6 hours, persists for at least 12 hours, but may be normal 24 hours after ingestion. If ethylene glycol intoxication is likely, urinalysis looking for calcium oxalate crystals, blood gas analysis, anion gap, and appropriate toxicologic analyses (see Chapter 17) are indicated.

BLOOD GAS ANALYSIS

Occasionally Indicated • Acid-base evaluation is useful in severely ill animals (e.g., severe dehydration, vomiting, diarrhea, oliguria and anuria, hyperkalemia, tachypnea). Blood gas analysis is also necessary to evaluate gas exchange and TCO_2 alterations in patients with respiratory disorders (see Chapter 11). Urine pH does not necessarily reflect systemic pH and cannot substitute for blood gas analysis.

Advantages • Blood gas analysis allows precise identification of the different acid-base disturbances and aids in evaluation of pulmonary function.

Disadvantages • Equipment is expensive, and careful technique is required in obtaining and handling blood specimens to prevent artifacts. The need for rapid analysis may prohibit use of remote laboratories; however, point-of-care units (e.g., immediate response mobile analysis [IRMA]) allow immediate determinations and are often affordable for busy practices.

Analysis • Blood gas analyzers are equipped with specific electrodes to measure pH, carbon dioxide partial pressure or tension (PCO_2), and oxygen partial pressure or tension (PO_2). The bicarbonate (HCO_3^-) is calculated. Arterial blood is required to evaluate PO_2 for pulmonary function, but free-flowing jugular blood is acceptable for acid-base analysis. Pulmonary artery, jugular vein, and cephalic vein samples usually have similar values in normal dogs, whereas arterial blood has a slightly lower HCO_3^- (21 mEq/L versus 22 to 23 mEq/L for venous blood) and much lower PCO_2 (37 mm Hg versus 42 to

TABLE 6-1. NORMAL BLOOD GAS VALUES

	pH	PCO_2 (mm Hg)	HCO_3^- (mEq/L)	PO_2 (mm Hg)
Dog venous	7.32-7.40	33-50	18-26	
Dog arterial	7.36-7.44	36-44	18-26	≈100
Cat venous	7.28-7.41	33-45	18-23	
Cat arterial	7.36-7.44	28-32	17-22	≈100

43 mm Hg for venous blood). Abnormal cardiovascular function may change this relationship.

For routine blood gas analyzers, a 3-ml syringe with a 25-gauge needle is used to collect 0.5 to 1.5 ml of blood. Heparin (1000 U/ml) is drawn into the syringe (coating the interior) and it and all air are expelled, leaving the needle hub filled with heparin (approximately 0.1 to 0.2 ml). For point-of-care units, as little as 0.125 ml blood is required. After the blood is collected, air bubbles must be dislodged and expelled. Inserting the needle into a rubber stopper or placing a tightly fitting cap over the syringe hub prevents exposure of the sample to room air. The syringe is rolled between the palms of the hands to mix the sample, and then submitted. Analysis should occur within 15 to 30 minutes of collection (if stored at 25° C) or within 2 hours if the sample is immersed in an ice-water bath. Handheld devices (e.g., IRMA) developed for use at the bedside (or cage) have been marketed as point-of-care units. These units can provide rapid blood gas data (as well as electrolytes and selected other determinations) on critically ill patients that can aid in decision making while waiting for routine laboratory results. There appears to be good correlation between results obtained with these small units and those coming from larger laboratory units.

Normal Values • Normal blood gas values are shown in Table 6-1.

Danger Values • pH less than 7.10 indicates life-threatening acidosis, which may impair myocardial contractility; pH greater than 7.60 denotes severe alkalosis.

Artifacts • PCO_2 decreases, whereas pH and PO_2 increase, if the sample is exposed to air. Air bubbles in the sample may produce the same artifacts, especially if they occupy greater than or equal to 10% of sample volume. PCO_2 increases and pH decreases if analysis is delayed. Aerobic metabolism by WBCs may decrease PO_2 . Cooling the sample from 25° to 4° C slows these changes. Prolonged venous stasis during venipuncture increases PCO_2 and decreases pH. Excessive heparin (>10% of the sample volume) decreases pH, PCO_2 , and HCO_3^- , whereas citrate, oxalate, or EDTA may decrease pH. Blood gas analyzers calculate HCO_3^- from pH and PCO_2 ; TCO_2 is measured on serum chemistry autoanalyzers but calculated on many blood gas analyzers. TCO_2 usually is 1 to 2 mEq/L higher than HCO_3^- .

Drugs That May Alter Blood Gas Results • Acetazolamide, NH_4Cl , and CaCl_2 may cause acidosis. Antacids, sodium bicarbonate, potassium citrate or gluconate, and

TABLE 6-2. RENAL AND RESPIRATORY COMPENSATIONS FOR PRIMARY ACID-BASE DISORDERS IN DOGS

DISORDER	PRIMARY CHANGE	COMPENSATORY RESPONSE
Metabolic acidosis	↓ [HCO ₃ ⁻]	0.7 mm Hg decrement in PCO ₂ for each 1-mEq/L decrement in [HCO ₃ ⁻]
Metabolic alkalosis	↑ [HCO ₃ ⁻]	0.7 mm Hg increment in PCO ₂ for each 1-mEq/L increment in [HCO ₃ ⁻]
Acute respiratory acidosis	↑ PCO ₂	1.5-mEq/L increment in [HCO ₃ ⁻] for each 10 mm Hg increment in PCO ₂
Chronic respiratory acidosis	↑ PCO ₂	3.5-mEq/L increment in [HCO ₃ ⁻] for each 10 mm Hg increment in PCO ₂
Acute respiratory alkalosis	↓ PCO ₂	2.5-mEq/L decrement in [HCO ₃ ⁻] for each 10 mm Hg decrement in PCO ₂
Chronic respiratory alkalosis	↓ PCO ₂	5.5-mEq/L decrement in [HCO ₃ ⁻] for each 10 mm Hg decrement in PCO ₂

From DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 196.

loop diuretics may cause alkalosis. Salicylates may cause metabolic acidosis, respiratory alkalosis, or both.

Analysis of Blood Gas Results • The clinician should begin by evaluating the pH. If it is abnormal, an acid-base disturbance exists. If the pH is within the normal range, the clinician should check the PCO₂ and HCO₃⁻. If they are abnormal, a mixed acid-base disturbance is probably present. If the pH is low and the HCO₃⁻ is decreased, metabolic acidosis is present. If the pH is low and the PCO₂ is increased, respiratory acidosis is present. If the pH is high and the HCO₃⁻ is increased, metabolic alkalosis is present. If the pH is high and the PCO₂ is decreased, respiratory alkalosis is present.

Next, the clinician should calculate the expected compensatory response (e.g., respiratory alkalosis is compensation for metabolic acidosis; metabolic alkalosis is compensation for respiratory acidosis) using the guidelines in Table 6-2. These guidelines are for dogs only. If a patient's compensatory response is within the expected range (i.e., within 2 mm Hg or 2 mEq/L of the calculated values), the acid-base disturbance is simple. If the compensatory response falls outside of the expected range, more than one acid-base disorder (i.e., a mixed disorder) is probably present.

After classifying the type of disturbance and whether it is simple or mixed, the clinician should determine whether the acid-base disturbance is compatible with the patient's history and clinical findings. If the acid-base disturbance does not fit with the patient's history, clinical

BOX 6-7. CAUSES OF METABOLIC ACIDOSIS**Increased Anion Gap (Normochloremic)**

Ethylene glycol intoxication (*important*)
 Diabetic ketoacidosis* (*common and important*)
 Uremic acidosis† (*common and important*)
 Lactic acidosis (*common and important*)
 Salicylate intoxication
 Other rare intoxications (e.g., paraldehyde, methanol)

Normal Anion Gap (Hyperchloremic)

Hypoadrenocorticism‡ (*uncommon but important*)
 Diarrhea
 Carbonic anhydrase inhibitors (e.g., acetazolamide)
 Dilutional acidosis (e.g., rapid administration of 0.9% saline)
 Ammonium chloride (*infrequent*)
 Cationic amino acids (e.g., lysine, arginine, histidine) (*rare*)
 Post-hypocapnic metabolic acidosis (*rare*)
 Renal tubular acidosis (RTA) (*rare*)

*Patients with diabetic ketoacidosis may have some component of hyperchloremic metabolic acidosis in conjunction with increased anion gap acidosis.

†The metabolic acidosis early in renal failure may be hyperchloremic and later convert to increased anion gap acidosis.

‡Patients with hypoadrenocorticism typically have hypochloremia caused by impaired water excretion (dilutional effect) and absence of aldosterone.

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 213.

findings, and other laboratory data, the blood gas analysis should be questioned.

Metabolic Acidosis • Metabolic acidosis (i.e., decreased pH and HCO₃⁻, with a compensatory decrease in PCO₂) is caused by addition of acid, failure to excrete acid, loss of HCO₃⁻, or a combination thereof (Box 6-7). Addition of acid may be iatrogenic (e.g., ethylene glycol, salicylates, NH₄Cl, cationic amino acids) or spontaneous (i.e., lactic acidosis, ketoacidosis). Decreased acid excretion is due to renal dysfunction (e.g., renal failure, hypoadrenocorticism, type I renal tubular acidosis [RTA]). Loss of HCO₃⁻ is usually caused by small bowel diarrhea (i.e., diarrheic fluid has more HCO₃⁻ than plasma); renal losses of HCO₃⁻ (e.g., carbonic anhydrase inhibitors, type II RTA) are rare. Metabolic acidosis is usually caused by renal failure, diabetic ketoacidosis, lactic acidosis from poor perfusion, hypoadrenocorticism, and perhaps small bowel diarrhea. The anion gap sometimes helps differentiate these causes and is discussed later. Measurement of blood lactate concentrations may help determine the cause of the acidosis and may also be prognostic (i.e., increased blood lactate is associated with a poorer prognosis). See Chapter 14 for a brief discussion of blood lactate measurement.

Respiratory Acidosis • Respiratory acidosis (i.e., decreased pH, increased PCO₂, with a compensatory increase in HCO₃⁻) is due to hypoventilation (which increases PCO₂) and is synonymous with "primary

BOX 6-8. CAUSES OF RESPIRATORY ACIDOSIS**Airway Obstruction**

Aspiration (e.g., foreign body, vomitus)

Respiratory Center Depression

Neurologic disease (e.g., brainstem, high cervical spinal cord lesion)

Drugs (e.g., narcotics, sedatives, barbiturates, inhalation anesthetics)

Toxemia

Cardiopulmonary Arrest (common)**Neuromuscular Defects**

Myasthenia gravis, tetanus, botulism, polyradiculoneuritis, polymyositis, tick paralysis, hypokalemic periodic paralysis in Burmese cats, hypokalemic myopathy in cats

Drug-induced (i.e., succinylcholine, pancuronium, aminoglycosides administered with anesthetics, organophosphates)

Restrictive Diseases

Diaphragmatic hernia, pneumothorax, pleural effusion, hemothorax, pyothorax, chest wall trauma, pulmonary fibrosis

Pulmonary Diseases (less common)

Respiratory distress syndrome, pneumonia, severe pulmonary edema, diffuse metastatic disease, smoke inhalation, pulmonary thromboembolism, chronic obstructive pulmonary disease, pulmonary mechanical ventilation fibrosis

Inadequate Ventilation

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 246.

hypercapnia" (Box 6-8). Hypoventilation may be caused by airway obstruction, cardiopulmonary arrest, restrictive respiratory diseases (e.g., diaphragmatic hernia, pneumothorax, pleural effusion, hemothorax, chest wall trauma, pulmonary fibrosis, pyothorax), severe pulmonary diseases, and inadequate mechanical ventilation. Hypoventilation may also result from respiratory paralysis from neuromuscular disease (e.g., myasthenia gravis, tetanus, botulism, polyradiculoneuritis, tick paralysis), as well as by neuromuscular blocking drugs (e.g., succinylcholine, pancuronium, aminoglycosides combined with anesthetics). Airway obstruction, cardiac arrest, and respiratory paralysis usually cause respiratory acidosis. These patients are invariably hypoxemic if breathing room air. Breathing oxygen-enriched air (i.e., anesthesia) sometimes causes normal or increased PO_2 .

Metabolic Alkalosis • Metabolic alkalosis (i.e., increased pH and HCO_3^- , with a compensatory increase in PCO_2) is caused by loss of acid from or addition of alkali to the body (Box 6-9). Loss of acid usually is due to vomiting gastric fluid, but loss of Cl^- via the kidneys (i.e., caused by furosemide) may be responsible. Metabolic alkalosis is usually due to vomiting of gastric contents (especially but not exclusively because of gastric outflow

BOX 6-9. CAUSES OF METABOLIC ALKALOSIS

Vomiting of gastric contents (common and important)

Diuretic therapy (e.g., loop diuretics, thiazides) (important)

Oral administration of sodium bicarbonate or other organic anions (e.g., lactate, citrate, gluconate, acetate)

Hyperadrenocorticism (infrequent)

Post-hypercapnia (rare)

Primary hyperaldosteronism (rare)

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 231.

obstruction) or administration of furosemide. Adding alkali may occur by administering $NaHCO_3$, lactated Ringer's solution, or potassium citrate. Normal kidneys eliminate administered alkali, however, and iatrogenic alkalosis seldom results unless renal dysfunction is present.

Respiratory Alkalosis • Respiratory alkalosis (i.e., increased pH, decreased PCO_2 , with a compensatory decrease in HCO_3^-) is due to tachypnea (which decreases PCO_2) and is synonymous with "primary hypocapnia" (Box 6-10). It may be the result of pulmonary disease, pulmonary thromboembolism, hypoxemia, direct stimulation of the medullary respiratory center (e.g., Gram-negative sepsis, hepatic disease, salicylates, xanthines, CNS disease, heat stroke), and excessive mechanical ventilation. Unexplained respiratory alkalosis may suggest Gram-negative sepsis or pain. Pulmonary edema may cause respiratory alkalosis, metabolic acidosis, or respiratory acidosis. Respiratory alkalosis may occur during recovery from metabolic acidosis because hyperventilation (the compensation for metabolic acidosis) persists for 24 to 48 hours after correction of the acidosis. These patients are sometimes hypoxemic. Respiratory disease

BOX 6-10. CAUSES OF TACHYPNEA RESULTING IN RESPIRATORY ALKALOSIS**Hypoxemia from Almost Any Cause**

Right-to-left shunting, decreased PIO_2 (e.g., residence at high altitude), congestive heart failure, severe anemia, pulmonary disease

Central Nervous System (CNS) (direct stimulation of medullary respiratory center)

Central neurologic disease, hepatic disease, Gram-negative sepsis, drugs (i.e., salicylate intoxication, xanthines such as aminophylline), heat stroke, fear, pain, fever, hyperthyroidism

Mechanical Ventilation

PIO_2 , Partial pressure of inspired oxygen.

Modified from DiBartola SP: *Fluid therapy in small animal practice*, ed 2, Philadelphia, 2000, WB Saunders, p 247.

sometimes initially causes tachypnea and consequently hypocapnia, which can change to hypercapnia if the disease worsens.

TOTAL CARBON DIOXIDE FOR ACID-BASE EVALUATION

TCO_2 is synonymous with HCO_3^- in samples handled aerobically.

Frequently Indicated • In any severe systemic disease process, TCO_2 helps determine if blood gas analysis is needed. Diseases in which TCO_2 determinations and blood gas analysis are indicated include ethylene glycol or salicylate intoxication, severe diabetic ketoacidosis, and severe uremia.

Advantage • Abnormal TCO_2 may be an indication to obtain blood gas analysis.

Disadvantages • One cannot accurately define metabolic and respiratory acid-base disorders using just TCO_2 . High TCO_2 could be the result of either metabolic alkalosis or compensated respiratory acidosis. Low TCO_2 could be the result of either metabolic acidosis or compensated respiratory alkalosis.

Analysis • TCO_2 is measured in serum or plasma by enzymatic and dry reagent methods. Serum or plasma analyzed within 15 to 20 minutes of collection is preferred. Samples may be stored in a capped syringe on ice at 4° C for up to 2 hours before analysis.

Normal Values • Dogs and cats, 17 to 23 mEq/L.

Danger Values • Less than 12 mEq/L (implies but does not confirm diagnosis of severe metabolic acidosis).

Artifacts • TCO_2 determined by dry reagent methods is not affected by hyperlipidemia. Falsely decreased TCO_2 occurs if processing of the blood sample is delayed for several hours, if the blood collection tube is underfilled, and if heparin anticoagulant occupies greater than 10% of the sample volume.

Drugs That May Alter TCO_2 • Acetazolamide and NH_4Cl cause metabolic acidosis, reducing TCO_2 . Furosemide, thiazides, and sodium bicarbonate cause metabolic alkalosis, increasing TCO_2 .

Causes of Decreased TCO_2 • TCO_2 concentrations are decreased in metabolic acidosis (most common cause) and compensated respiratory alkalosis. A hyperventilating animal with decreased TCO_2 usually has metabolic acidosis but could have chronic respiratory alkalosis. Blood gas analysis may be necessary to determine which is present. Severely decreased TCO_2 in a patient with a recognized cause of metabolic acidosis (e.g., diabetic ketoacidosis) is usually assumed to represent metabolic acidosis. Blood gas analysis is necessary to confirm the presence of metabolic acidosis and assess the severity of the change in pH. TCO_2 less than or equal to 12 mEq/L in a patient with

undiagnosed systemic disease is an indication for blood gas analysis. If blood gas analysis is unavailable, the clinician must correlate TCO_2 with the clinical setting and decide if NaHCO_3 therapy is indicated. This approach, however, can be dangerous because the actual pH is not known. Measuring serum electrolyte concentrations allows optimal fluid therapy (i.e., disturbances that affect acid-base balance often also cause electrolyte abnormalities).

Causes of Increased TCO_2 • TCO_2 concentrations are increased in metabolic alkalosis (most common) and compensated respiratory acidosis (rare). Serum sodium, potassium, and chloride concentrations should be measured, because hypochloremia and hypokalemia are common in metabolic alkalosis. If these changes occur, they should be corrected (i.e., administration of 0.9% NaCl + KCl) and the underlying cause (e.g., pyloric obstruction) diagnosed.

ANION GAP

Infrequently Indicated • The anion gap sometimes helps differentiate causes of metabolic acidosis and may help clarify mixed acid-base disorders. Metabolic acidosis with a high anion gap usually comes from acids that do not contain chloride (e.g., lactic acid, keto acids, salicylic acid, metabolites of ethylene glycol, phosphates, sulfates). Metabolic acidosis characterized by a normal anion gap has an increased plasma chloride concentration and is called hyperchloremic acidosis.

Advantage • Only a simple calculation from values already measured is required.

Disadvantage • The anion gap is affected by several factors and can be difficult to interpret.

Analysis • The anion gap is calculated as $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)$ or $\text{Na}^+ - (\text{Cl}^- + \text{HCO}_3^-)$, depending on the clinician or laboratory's preference. The anion gap and its component values are expressed in mEq/L. If the patient is severely hypoalbuminemic, the anion gap may not reflect expected findings. For each 1 g/dl decrease in serum albumin, the anion gap decreases approximately 2.4 mEq/L.

Normal Values • The normal anion gap calculated by $(\text{Na}^+ + \text{K}^+) - (\text{Cl}^- + \text{HCO}_3^-)$ is approximately 12 to 24 mEq/L in dogs and 13 to 27 mEq/L in cats.

Danger Values • Greatly increased values may be the result of acute ethylene glycol intoxication and warrant a careful review of the patient's history. There may be a correlation between increasing anion gap and mortality in seriously ill animals.

Causes of Decreased Anion Gap • Hypoalbuminemia is probably the most common cause of a decreased anion gap; immunoglobulin G (IgG) multiple myeloma may also be responsible. The magnitude of increase in unmeasured cations (e.g., calcium, magnesium) necessary to

lower the anion gap would probably be fatal. Laboratory errors resulting in overestimation of TCO_2 or Cl^- or in underestimation of sodium may artifactually decrease the anion gap. A decreased anion gap is seldom clinically significant.

Causes of Normochloremic (Increased Anion Gap)

Acidosis • The most common causes of an increased anion gap in acidotic patients are lactic acidosis, diabetic ketoacidosis, uremic acidosis, ethylene glycol intoxication, and laboratory error.

Causes of Hyperchloremic (Normal Anion Gap) Acidosis

• Severe, acute small bowel diarrhea causes HCO_3^- loss and produces hyperchloremic (normal anion gap) acidosis. Carbonic anhydrase inhibitors (e.g., acetazolamide) inhibit proximal renal tubular reabsorption of HCO_3^- and produce self-limiting hyperchloremic metabolic acidosis. Acidosis resulting from administration of NH_4Cl decreases HCO_3^- , but serum Cl^- increases and the anion gap is unchanged. Infusion of cationic amino acids (e.g., lysine HCl, arginine HCl) during total parenteral nutrition may cause hyperchloremic metabolic acidosis, because H^+ ions are released when urea is generated. Renal acid excretion decreases during chronic respiratory alkalosis, with consequent reduction in plasma HCO_3^- and increase in Cl^- . When the stimulus for hyperventilation is removed and Pco_2 increases, pH decreases because it requires 1 to 3 days for the kidneys to increase acid excretion and increase plasma HCO_3^- . This transient phenomenon is called post-hypocapnic metabolic acidosis and is associated with hyperchloremia.

Dilutional acidosis occurs when extracellular volume is expanded via an alkali-free chloride-containing solution (e.g., 0.9% NaCl). The high Cl^- of physiologic saline solution (i.e., 154 mEq/L) and the highly resorbable nature of Cl^- in renal tubules contribute to decreased plasma HCO_3^- and hyperchloremia. RTA is a rare disorder characterized by hyperchloremic metabolic acidosis because of either decreased HCO_3^- reabsorption (type II RTA) or defective acid excretion (type I RTA).

Other Causes of Increased Anion Gap • Severe dehydration may increase both serum albumin concentration and the anion gap. Alkalemia may increase the anion gap slightly. Excessive standing of serum, especially in uncapped containers, also may increase the anion gap (a common error in samples not analyzed until the next day).

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7

Urinary Disorders

Jeanne A. Barsanti

DIFFERENTIATION OF POLYURIA-POLYDIPSIA, DYSURIA, AND INCONTINENCE

These three abnormalities must be differentiated early in the diagnostic workup by history or direct observation of urination. *Polyuria-polydipsia* (pu-pd) means urine production and water consumption in excess of normal. *Dysuria* means difficult urination and implies unduly frequent urination with small or minimal amounts voided each time. Clients may assume that increased frequency means increased volume. The diagnostician must ascertain whether the amount of urine voided with each micturition is large, small, or unknown. Animals with pu-pd or dysuria are conscious of voiding and do not wake up in puddles of urine, unless they are too weak or in too much pain to get up. Dogs and cats with urinary *incontinence* may wake up soaked in urine, leave a spot of urine where they slept, or dribble urine as they move.

Polyuria-Polydipsia

A precise history and consistently low urine specific gravities (<1.030) suggest pu-pd. Measurement of total water consumption (including water in food) over at least a 24-hour period is necessary to definitively verify polydipsia (normal water intake is 20 to 70 ml/kg/day). This measurement is best performed at the client's home because some polyuric animals will not readily drink water at a clinic. Quantifying urine production is difficult unless a metabolism cage is available (normal 20 to 45 ml/kg/day).

Pu-pd has many possible causes (Table 7-1). History and physical examination are crucial in evaluating patients with pu-pd (Figure 7-1). Iatrogenic causes must be sought from the history (e.g., diuretics, glucocorticoids, anticonvulsants, high-salt or very-low-protein diets, excessive thyroid supplementation). Aminoglycosides tend to produce polyuric, acute renal failure. Glucocorticoids can cause pu-pd, even when administered rectally or topically. Pyometra is usually suggested by history (2 months post-estrus) or physical examination findings (enlarged uterus, vaginal discharge). If the

clinician is unsure whether pyometra is likely, a complete blood count (CBC) and abdominal imaging are usually definitive (neutrophilic leukocytosis with left shift and enlarged uterus). Weight loss plus pu-pd in a cat suggests hyperthyroidism, renal failure, or diabetes mellitus (hyperthyroidism is rare in dogs). Enlarged thyroid glands often may be palpated in the neck in hyperthyroid patients. Feline kidneys are usually palpable; size and contour should be assessed. Postoliguric diuresis is usually diagnosed from the history (e.g., a male cat that has undergone removal of a urethral obstruction). Pu-pd is the most common presenting complaint of owners of hyperadrenal dogs; most affected dogs have a pendulous abdomen, hepatomegaly, alopecia, or a combination thereof on physical examination. Such dogs should undergo adrenal function testing (see Chapter 8). Recent onset of cataracts suggests diabetes mellitus. Peripheral lymphadenopathy suggests lymphoma causing hypercalcemia. Other neoplasms (e.g., anal sac apocrine gland adenocarcinomas) and certain infectious diseases (e.g., systemic mycoses) may also cause hypercalcemia.

If the cause is not obvious, a urinalysis, CBC, and biochemical profile are the next steps (see Figure 7-1). Urinary tract infections (UTIs) may be secondary to hyperadrenocorticism or diabetes mellitus, but renal infection (i.e., bacterial pyelonephritis) can cause pu-pd. Neutrophilic leukocytosis, azotemia, white blood cell (WBC) casts, renal pain, or hyposthenuria may occur with pyelonephritis. Pyelonephritis can be difficult to diagnose; excretory urography is the diagnostic method of choice (although abnormalities may also be found with ultrasonography if the renal pelvis can be imaged). Sometimes a presumptive diagnosis can only be made by the necessity for long-term antibiotic therapy after failure of short-term therapy. Clinicopathologic screening often reveals changes indicative of the cause of pu-pd (e.g., renal failure, hyperadrenocorticism, hepatic insufficiency, hypercalcemia, diabetes mellitus) (see Figure 7-1). Serum thyroxine determinations are always indicated in older cats (i.e., >10 years old).

Many dogs with hyperadrenocorticism are obviously cushingoid on physical examination (i.e., cutaneous abnormalities, potbellied appearance, and hepatomegaly). Common CBC and serum chemistry profile changes

TABLE 7-1. CAUSES OF POLYURIA-POLYDIPSIA (PU-PD) IN DOGS AND CATS

CAUSES	REMARKS
Iatrogenic; drugs (e.g., diuretics, corticosteroids, thyroxine, anticonvulsants, aminoglycosides, or amphotericin B); salty or very-low-protein diets	History is informative BUN should be low with low-protein diets
Renal disease	Urine specific gravity should be persistently 1.008–1.029 (dogs) May be azotemic or nonazotemic For nonazotemic renal failure, measurement of GFR is useful for diagnosis of renal dysfunction as the cause
Upper urinary tract infection	May be hyposthenuric Excretory urography is diagnostic test of choice, but ultrasound may be helpful
Fanconi's syndrome	Usually nonazotemic, hyperchloremic acidosis, glucosuric, and aminoaciduric
Diabetes mellitus	Hyperglycemic Note: Cats are prone to stress-induced hyperglycemia; urine glucose measured at the same time is usually (but not always) negative
Central diabetes insipidus	Hyposthenuric when euhydrated (see Figure 7-8)
Nephrogenic diabetes insipidus	Hyposthenuric when euhydrated May be primary (congenital), idiopathic, or secondary (pyelonephritis, hyperadrenocorticism, hypercalcemia, hypokalemia, pyometra, prostatic abscessation, <i>E. coli</i> septicemia, hypoadrenocorticism)
Hyperadrenocorticism	Isosthenuric, hyposthenuric, or concentrated urine Common cause of pu-pd in old dogs, rare in cats
Hypoadrenocorticism	Pu-pd occurs in approximately 20% of patients; may closely resemble renal failure but differentiated by absence of a stress leukogram and presence of hyperkalemia despite polyuria; ACTH response test is necessary for confirmation
Hypercalcemia	Isosthenuric or hyposthenuric, azotemic or nonazotemic
Hepatic insufficiency	Isosthenuric, hyposthenuric, or concentrated; may resemble hyperadrenocorticism (hepatic enzymes may be increased), but can also have normal ALT and SAP
Hyperthyroidism	Primarily in older cats but may be iatrogenic because of supplementation
Hyponatremia	Loss of sodium from any cause May cause isosthenuria whenever <120 mEq/L
Post-urethral obstruction	Occurs occasionally after removal of a urethral obstruction that has resulted in uremia
Hypokalemia	Must be persistent and severe to cause polyuria
Polycythemia vera	Rare
Apparent psychogenic polydipsia	Concentrate urine in response to water deprivation (see Figure 7-8)
Acromegaly	Rare

ACTH, Adrenocorticotrophic hormone; ALT, alanine aminotransferase; BUN, blood urea nitrogen; GFR, glomerular filtration rate; SAP, serum alkaline phosphatase.

include lymphopenia, eosinopenia, and increased serum alkaline phosphatase (SAP), alanine aminotransferase (ALT), and serum cholesterol. Approximately 40% to 50% of hyperadrenal dogs have UTI. Bacteriuria is often the only abnormality on urinalysis (i.e., no hematuria or pyuria). Adrenal function tests (see Chapter 8) or hepatic biopsy may be necessary to distinguish hyperadrenocorticism from primary hepatic disease.

Renal failure, hypercalcemic nephropathy, and hypoadrenocorticism can resemble each other. The first two usually produce pu-pd, whereas the last causes it in 15% to 25% of affected dogs. Each may have azotemia, decreased renal concentrating ability (e.g., specific gravity 1.012 to 1.029), and hypercalcemia (10% to 15% of renal failure patients and 30% of hypoadrenal dogs). Most hypoadrenal patients have a serum Na:K of less than or

equal to 27:1 with hyponatremia, hyperkalemia, or both. Classically, no stress leukogram exists despite the animal being ill. An adrenocorticotrophic hormone (ACTH) stimulation test (see Chapter 8) is needed to confirm the diagnosis, because other disorders may cause similar changes. Most azotemic renal failure patients are hyperphosphatemic and only mildly hypercalcemic (i.e., <13 mg/dl), whereas most animals with hypercalcemia of nonrenal origin are normophosphatemic or mildly hypophosphatemic and may be markedly hypercalcemic. Nevertheless, distinguishing whether hypercalcemia is the cause or consequence of renal failure can be difficult if persistent hypercalcemia has produced renal damage plus hyperphosphatemia (especially when hypercalcemia is mild [11.5 to 14 mg/dl]). Such patients need a thorough search for neoplasia and require measurement of ionized

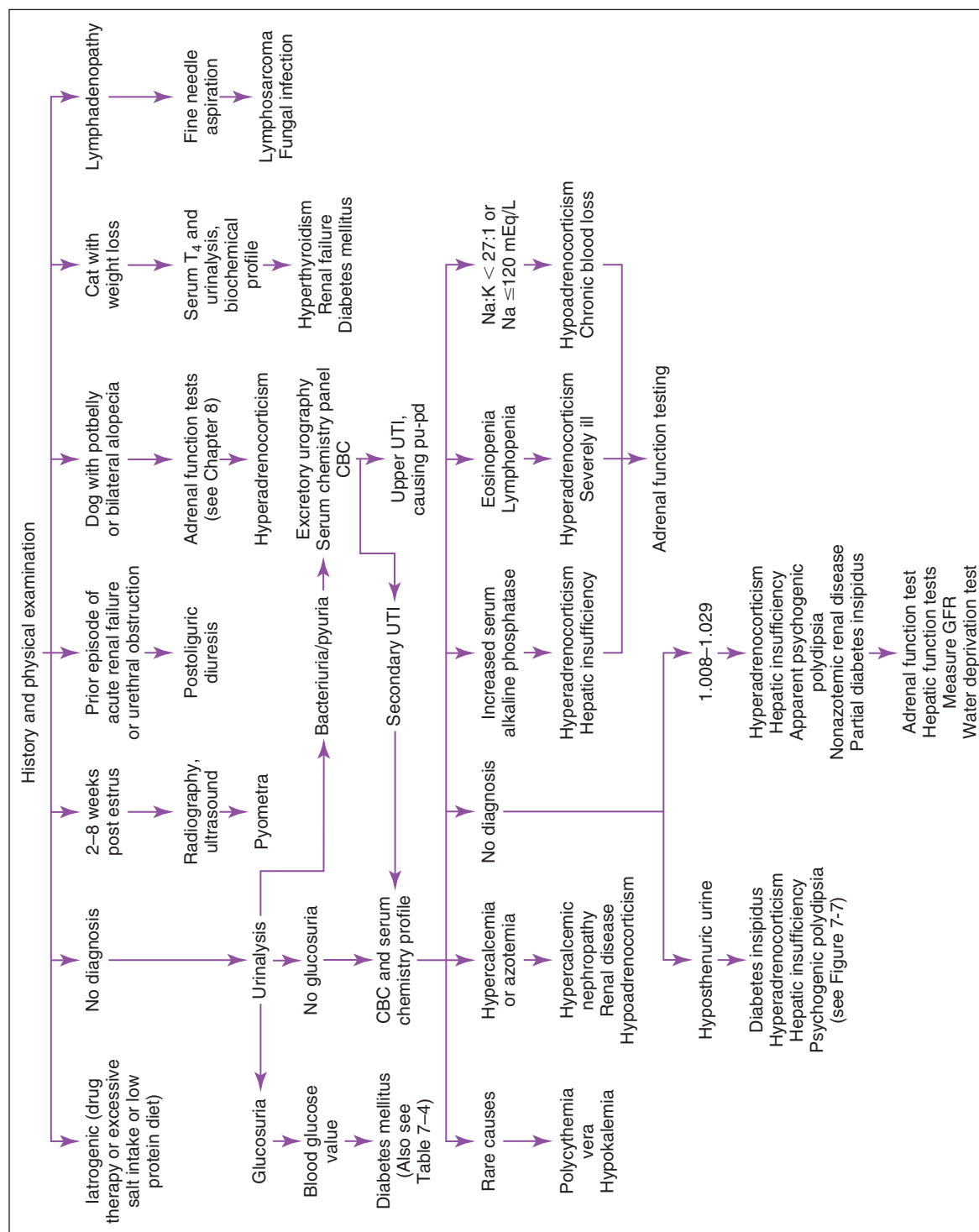


FIGURE 7-1 Diagnostic approach in a dog or cat with a history of polyuria-polydipsia (pu-pd); persistently hyposthenuric, isosthenuric, or inadequately concentrated urine; or a water balance study documenting water consumption greater than 70 ml/kg/day. CBC, Complete blood count; GFR, glomerular filtration rate; T_{ur} , thyroxine; UTI, urinary tract infection.

calcium and parathyroid hormone (PTH) concentrations (see Chapter 8). Most dogs with hypoadrenocorticism and primary renal failure have normal to decreased ionized calcium, whereas animals with primary hypercalcemic disorders (i.e., hyperparathyroidism, hypercalcemia of malignancy) have increased ionized calcium concentrations.

More extensive testing is necessary if the diagnosis is still uncertain (see Figure 7-1). Some dogs with renal failure are polyuric because of loss of functional nephron number (may occur with 67% reduction in renal function) but maintain a sufficient glomerular filtration rate (GFR) to avoid azotemia (which requires a 75% reduction in GFR). A creatinine or iothexol clearance test is a noninvasive way to identify these patients. Water deprivation and antidiuretic hormone (ADH) response testing can be useful if the patient is not azotemic. Elimination of other causes also allows a reasonable tentative diagnosis.

NOTE: Renal failure typically produces urine specific gravities between 1.008 and 1.020, but rare dogs in renal failure may be hyposthenuric (1.006 to 1.007), and some dogs with 67% reduction in renal function can concentrate urine to 1.027. Some cats with renal failure have a urine specific gravity greater than or equal to 1.035.

Persistent hyposthenuria suggests diabetes insipidus, although hyperadrenocorticism, hepatic insufficiency, and psychogenic polydipsia are also possible. Adrenal and hepatic function testing, water deprivation and ADH response testing, or both may be indicated. Water deprivation testing should not be performed unless a CBC, urinalysis, and biochemical profile have been evaluated. Animals with metabolic causes of pu-pd can be harmed by iatrogenic dehydration.

Dysuria

Alterations in behavior associated with urination (other than polyuria) generally suggest disorders affecting the urinary bladder, urethra, or both (Figure 7-2). Irritative or inflammatory (septic or nonseptic) lesions that do not impede urine flow typically cause animals to urinate small volumes and to urinate more often, perhaps with apparent discomfort (i.e., dysuria). Urethral obstruction causes animals to make repeated voiding efforts that are either unproductive (i.e., complete obstruction) or somewhat productive but the bladder cannot be emptied (i.e., partial obstruction). Some animals with urethral obstruction have urinary incontinence. Such paradoxical incontinence occurs when accumulated urine is forced past an obstruction by markedly increased intravesicular pressure.

Urinary obstruction must be promptly identified because uremia, hyperkalemia, and death occur within 48 to 72 hours of complete urethral obstruction, and severe structural damage occurs with chronic, partial urethral or ureteral obstruction. Urethral or trigonal obstruction typically causes an enlarged, turgid, and inexpressible bladder. Partial obstruction is more difficult to identify; however,

observation of voiding plus assessment of residual urine volume in the bladder after micturition (i.e., palpation, ultrasonography, or catheterization) is usually diagnostic. Palpation per rectum of the urethra in male and female dogs and the prostate gland in male dogs is important. Mechanical obstructions, which may be intraluminal (e.g., uroliths, urethral plugs, neoplasms) or extraluminal (e.g., caused by displacement as the result of bladder entrapment in a perineal hernia, strictures, inflammation or edema of the urethra, severe prostatic diseases) are the most common. In these cases, passing a urinary catheter can be both diagnostic and therapeutic.

Even if a urinary catheter passes easily, a urethrocytogram with bladder distention should be performed when the animal cannot empty its bladder in order to eliminate anatomic obstruction (a urinary catheter can pass by an obstruction, depending on the size of the catheter, the size of the urethra, and the nature of the obstruction). Detrusor muscle dysfunction and functional urethral obstruction (i.e., detrusor-sphincter dyssynergia) are neuromuscular causes of inability to urinate. Detrusor muscle dysfunction is usually secondary to prolonged bladder overdistention secondary to urethral obstruction or neurologic diseases. Detrusor-sphincter dyssynergia is diagnosed only when anatomic causes of obstruction have been excluded. With functional obstruction, neurologic examination usually detects deficits; however, these deficits may be subtle.

Most dysuric dogs that are not experiencing obstruction have lower urinary tract inflammation, UTI being a common cause. Finding bacteriuria, often with hematuria, proteinuria, and pyuria, identifies UTI. Urine culture is the definitive test, as discussed under Bacteriuria later in this chapter. If no evidence of UTI exists or if UTI does not respond as expected to therapy, calculi, neoplasia, cyclophosphamide therapy, and rarely parasitism of the bladder should be considered.

Cats with dysuria and inappropriate urination may have UTI, especially if they are over 10 years of age. Younger dysuric cats (i.e., 2 to 6 years of age) usually have idiopathic sterile cystitis. Cats with sterile cystitis usually have hematuria with little or no pyuria. The second most common cause of these signs is urolithiasis. Idiopathic sterile cystitis typically resolves spontaneously within 7 days. If the problem does not resolve, radiography or ultrasonography of the urinary bladder or both may be needed.

If surgery is performed because of a lower urinary tract problem, the bladder and, in intact male dogs, the prostate gland should undergo biopsy regardless of how normal or neoplastic the organs appear. Bladder abnormalities may occur without gross lesions, whereas polypoid cystitis may mimic malignancy. If a urine culture is negative, cultures of bladder mucosal biopsies are also indicated.

Incontinence

Urinary incontinence is defined as the lack of voluntary control of micturition. The diagnostic approach to incontinence starts with a thorough history. The age of onset of the problem, reproductive status of the animal, relationship between the onset of incontinence and neutering,

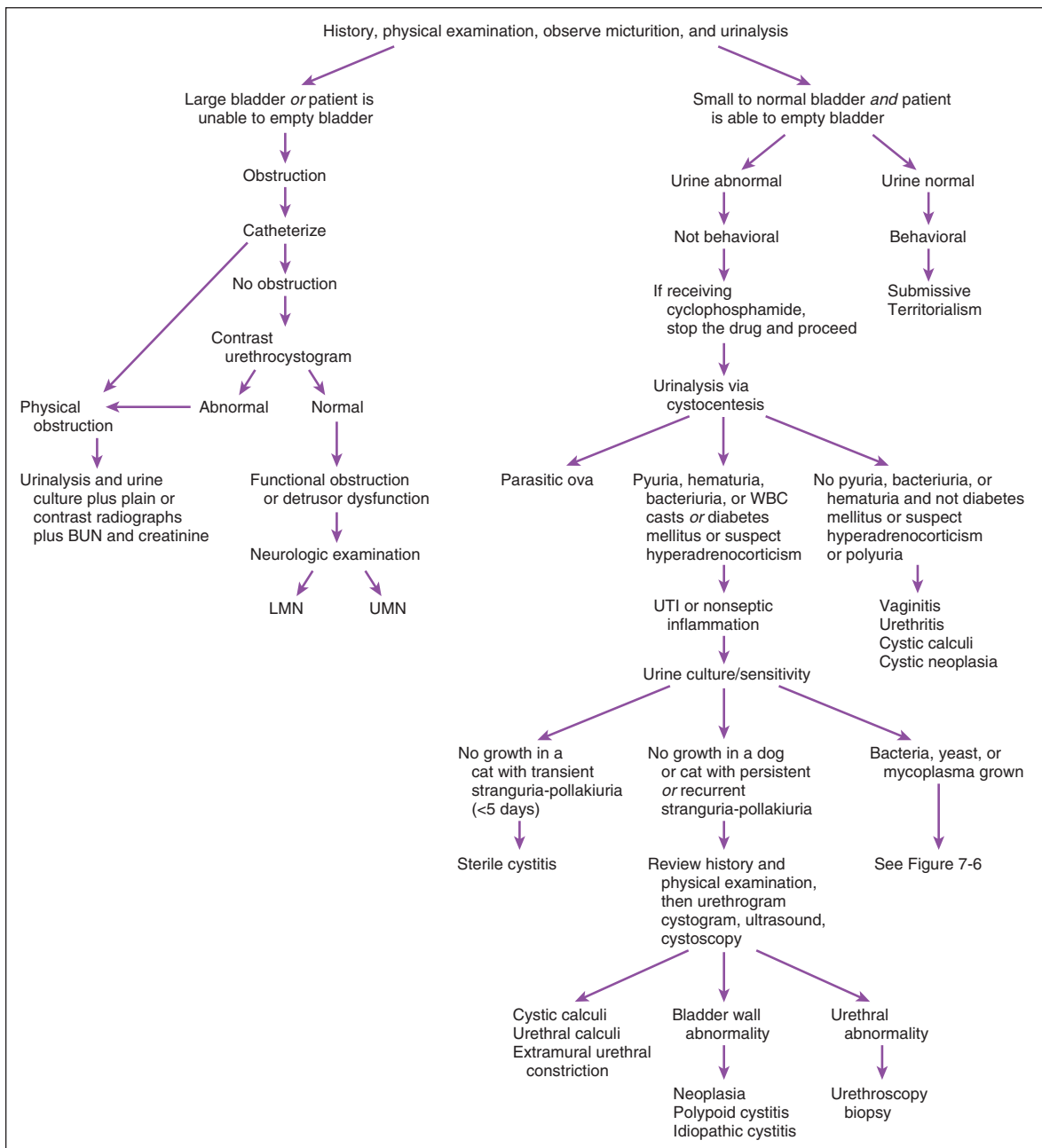


FIGURE 7-2 Diagnostic approach to dysuria in dogs and cats. *BUN*, Blood urea nitrogen; *LMN*, lower motor neuron; *UMN*, upper motor neuron; *UTI*, urinary tract infection; *WBC*, white blood cell.

chronologic course of the incontinence, associated urinary tract problems, history of other neurologic abnormalities, whether normal micturition occurs at all, and when incontinence occurs in relation to micturition are all important, as well as any drug usage or dietary changes. Any drug or dietary change or development of a disease that stimulates a polyuria could precipitate a latent predisposition to incontinence by increasing the amount of urine the bladder must store.

A complete physical examination follows. Special attention should be paid to the bladder. Is it large and distended, small and contracted, or normal? In a male dog the prostate gland should be palpated. Anal tone and the integrity of the perineal reflex should be evaluated. Any abnormal neurologic signs should be pursued by a complete neurologic examination. Micturition should be observed and residual volume determined if there is any question about the ability of the bladder to empty.

Residual volume has not been measured in large numbers of dogs and cats, but most have only a small residual volume, less than 20 ml. One must remember that some male dogs may want to repeatedly mark territory before the bladder is completely emptied. If complete micturition occurs, the empty bladder should be palpated for calculi, soft tissue masses, and wall thickness. If the bladder is not emptied, the urethra should be carefully palpated externally and per rectum in males and females. Any yellow fluid found dripping from the urethra in intact male dogs should be compared to urine since fluid from a prostatic cyst communicating with the urethra may have the same color as urine.

After the history and physical examination, the incontinence should be classified as neurogenic (associated with other neurologic problems) or not (unassociated with other neurologic signs). When the neurologic examination is abnormal, the lesion should be localized and further diagnostic tests pursued to identify the cause. Even when the neurologic examination is normal, a local neuromuscular cause (such as detrusor dysfunction) may be the cause of incontinence. Incontinence in animals with a normal neurologic examination is best approached by subdividing it into two categories on the basis of the physical examination: a distended bladder with inability to completely empty or a normal bladder with ability to void (Table 7-2).

URINALYSIS

Commonly Indicated • Urinalysis is part of a thorough evaluation of patient health. It is essential in patients with a urinary tract problem.

Advantages • Urinalysis is easy and inexpensive to perform and provides important information in patients with systemic and urinary diseases.

Analysis • Urine is preferably obtained by cystocentesis. Exceptions are patients with coagulopathy, those with mild hematuria in which a voided sample is preferred to avoid mild iatrogenic hemorrhage associated with cystocentesis, and those which may have pyometra or other intra-abdominal abscess in the vicinity of the bladder. If cystocentesis cannot be performed, a mid-stream voided or catheterized sample can be used. The method of collection should be recorded so abnormalities can be correctly interpreted.

For greatest accuracy (especially regarding casts, cellular components, and crystalluria), urine sediment should be examined while the specimen is as fresh as possible. If the urinalysis cannot be performed within 30 minutes, the urine should be held at 4° C and brought back to room temperature just before analysis.

Analysis consists of four important steps: (1) determining color and turbidity, (2) chemical analysis using multitest dipsticks, (3) measurement of specific gravity, and (4) microscopic analysis of the sediment. One should thoroughly mix the sample and determine the color and turbidity first; then immerse the reagent strip into the urine and rapidly remove it, tapping the edge of the strip on the edge of the container to remove excess urine. The dipstick should be held level to avoid runoff between pads and prevent mixing reagents from different test pads. One should compare the color of the pads with the scale provided by the manufacturer at the proper time interval. Automated dipstick analyzers have been shown to have results similar to those of human dipstick

TABLE 7-2. CAUSES OF INCONTINENCE AND DIAGNOSTIC TESTS

CATEGORY	RULE OUTS	POSSIBLE DIAGNOSTIC TESTS
ASSOCIATED WITH OTHER NEUROLOGIC SIGNS	Cerebral lesions	MRI, CT, CSF
	Brainstem lesions	MRI, CT, CSF
UNASSOCIATED WITH OTHER NEUROLOGIC SIGNS; Inability to Empty the Bladder	Spinal cord lesions (cervical, thoracic, lumbar)	MRI, CT, spinal radiographs, myelography, CSF, EMG
	Lesions of the sacral spinal cord, roots, pelvic or pudendal nerves	MRI, CT, epidurogram, CSF, EMG
UNASSOCIATED WITH OTHER NEUROLOGIC SIGNS; Ability to Empty the Bladder	Urethral obstruction	Palpation per rectum, radiography, urethrography, urethrosocopy
	Mass in the bladder neck area	Palpation, bladder ultrasound, cystourethrography, cystoscopy, aspiration, biopsy
UNASSOCIATED WITH OTHER NEUROLOGIC SIGNS; Inability to Empty the Bladder	Detrusor-urethra dyssynergia	Diagnosis by exclusion
	Detrusor dysfunction	History, CMG
UNASSOCIATED WITH OTHER NEUROLOGIC SIGNS; Ability to Empty the Bladder	Urethral incompetence	History, urinalysis, urine culture, urethral pressure profile
	Ectopic ureter(s)	History, vaginourethrogram, excretory urography, urethrocystoscopy
UNASSOCIATED WITH OTHER NEUROLOGIC SIGNS; Ability to Empty the Bladder	Mass in the bladder neck area	Palpation, bladder ultrasound, cystourethrography, cystoscopy, aspiration, biopsy
	Patent urachus	Physical examination
UNASSOCIATED WITH OTHER NEUROLOGIC SIGNS; Ability to Empty the Bladder	Reduced bladder capacity	Bladder ultrasound, cystourethrography, CMG, cystoscopy, aspiration, biopsy

CMG, Cystometrogram; CSF, cerebrospinal fluid; CT, computed tomography; EMG, electromyography; MRI, magnetic resonance imaging.

readers. Urine specific gravity should be determined using a refractometer. The accuracy of the refractometer should be checked periodically to be sure that a reading of 1.000 is obtained with distilled water.

Next, 3 to 5 ml of urine should be centrifuged at approximately 2000 rpm for 5 minutes. Standardization of volume, speed, and duration of centrifugation is important for comparing results from different samples. If the uncentrifuged urine sample was visibly hemorrhagic or very turbid, the clinician or technician should repeat the dipstick analysis and the specific gravity on the supernatant. Next, most of the supernatant should be decanted, leaving approximately 0.5 ml in the tube, and the sediment in the remaining supernatant should be resuspended. The clinician or technician should transfer a drop of the reconstituted sediment to a microscope slide and place a coverslip over it. The intensity of the microscope light should be dimmed, and the clinician or technician should examine under low power (10×) for casts, crystals, and cells. The number of casts per low-power field (lpf) should be counted, and then the specimen should be examined under high power (40×) to identify cells and bacteria. The clinician or technician should count the number of WBCs and red blood cells (RBCs) per high-power field (hpf) and estimate the number of bacteria (i.e., trace, moderate, many). Sediment stains can be used. Visualization of bacteria is enhanced by the use of either Gram² or modified Wright staining.⁴⁹ Finally, the results should be recorded, with a notation of how urine was collected and how many milliliters of urine were spun if it was less than 3 to 5 ml.

Color and Turbidity

Analysis • Color and turbidity are analyzed by visual inspection. Normal urine is clear to slightly turbid and light yellow to amber. Dilute urine tends to be colorless, and concentrated urine is dark yellow. Different colors and their significance are listed in Table 7-3. Significant disease may exist even if the urine is normal in color and turbidity. If urine discoloration is noted, one should review the patient's history for drug administration and carefully examine the urine sediment. Hematuria, hemoglobinuria, and bilirubinuria are the most common causes of discolored urine. Pyuria, hematuria, crystaluria, and lipiduria are common causes of increased turbidity.

Specific Gravity

Analysis • Specific gravity is determined with a refractometer. One should periodically check the refractometer's calibration by verifying that distilled water gives a reading of 1.000. Storage of urine in capped containers at room or refrigeration temperature for at least 24 hours does not affect measurement of specific gravity.¹ Some dipsticks have a test pad that indicates specific gravity; however, these results are inaccurate.⁶ Use of dipstick test pads to evaluate urine specific gravity is not recommended.

Normal Values • Specific gravity varies in normal dogs and cats, and any random specific gravity may be normal

TABLE 7-3. POTENTIAL CAUSES OF DISCOLORED URINE IN DOGS AND CATS

URINE COLOR	CAUSES
Dark yellow	Concentrated urine
Pale yellow	Normal urochromes, urobilin
Yellow-orange	Bilirubin Fluorescein Concentrated urine Phenazopyridine
Green-blue	Methylene blue Dithiazanine Biliverdin
Brown-black	Bile pigments Myoglobin Methemoglobin
Yellow-brown	Bile pigments
Red	Hemoglobin RBCs Myoglobin Dyes Phenazopyridine Phenolsulfonphthalein
Milky	Pyuria Lipiduria Phosphate crystals
Colorless	Dilute

RBCs, Red blood cells.

in euhydrated animals. Specific gravities less than 1.020 may be associated with polyuria evident to the owner. Specific gravity is important to assess renal function in dehydrated or azotemic animals. The urine of a dog with evident dehydration and normal renal function should have a specific gravity greater than 1.030, and a cat's should be greater than 1.035. Puppies do not concentrate urine until they reach 4 weeks of age.¹⁷

Danger Values • None.

Therapy and Diets That May Alter Results • Low urine specific gravity may be caused by fluid therapy, glucocorticoids, diuretics, anticonvulsants, and excessive thyroid hormone supplementation. Persistent use of low-protein diets can result in inability to maximally concentrate urine (usually blood urea nitrogen [BUN] concentrations are below normal in this situation). Increased specific gravity may be caused by radiographic contrast media if the preadministration urine specific gravity was less than 1.040 (if preadministration urine specific gravity was >1.040, urine specific gravity may decrease because of osmotic diuresis).

NOTE: It is important to obtain urine specific gravity before treatment, especially fluid or diuretic therapy.

Causes of Altered Urine Specific Gravity • Urine that is less than or equal to 1.007 is hyposthenuric. Hyposthenuria indicates renal function capable of diluting

glomerular filtrate and suggests that renal failure is absent; however, some dogs with renal failure excrete mildly hyposthenuric urine. Persistent hyposthenuria suggests a lack of ADH (i.e., central diabetes insipidus), excessive water consumption (primary polydipsia), resistance to ADH (i.e., nephrogenic diabetes insipidus), or loss of medullary tonicity. Primary polydipsia can be caused by hyperthyroidism, hypercalcemia, hypokalemia, or hepatic failure, or it can be “psychogenic.” Resistance to ADH may be caused by secondary nephrogenic diabetes insipidus (e.g., hyperadrenocorticism, hypercalcemia, severe hypokalemia, pyelonephritis, pyometra, *Escherichia coli* septicemia, hypoadrenocorticism). Primary nephrogenic diabetes insipidus is rare and is due to congenital lack of ADH receptor responsiveness. An increased solute load (e.g., glucosuria, post-urethral obstruction, increased salt intake) can also cause polyuria, as can decreased medullary tonicity (e.g., hepatic failure, a very low-protein diet, severe hyponatremia, chronic diuretic therapy).

Urine with a specific gravity of 1.008 to 1.012 is isosthenuric, meaning that the kidneys have not altered the concentration of the glomerular filtrate. Urine with a specific gravity of 1.013 to 1.029 (dog) or 1.013 to 1.034 (cat) has been concentrated, but not enough to document adequacy of renal tubular function.

Urine with a specific gravity of greater than 1.030 (dog) demonstrates concentrating ability sufficient to indicate adequate renal function to maintain normal homeostasis. A patient with a specific gravity of urine greater than 1.030 could still have many of the diseases that cause pu-pd (e.g., hyperadrenocorticism, hepatic insufficiency, hyperthyroidism), as well as renal glomerular disease.

A single urine specific gravity greater than 1.007 and less than 1.030 (dog) or 1.035 (cat) does not imply renal tubular dysfunction or pu-pd unless the patient is clinically dehydrated or azotemic, in which case such a specific gravity reflects abnormal renal tubular function. Otherwise, one must document failure to concentrate urine adequately during water deprivation testing to establish urine concentrating ability as abnormal. Persistently hyposthenuric or isosthenuric urine is an indication for further testing (see Figure 7-1).

Urine pH

Analysis • Urine pH is usually measured with a pH test pad on a urine reagent strip. However, results are not very accurate as compared to pH meters.^{29,34} Portable pH meters should be used when accurate urine pH measurements are needed. Storage of urine in capped containers at room or refrigeration temperatures for at least 24 hours does not affect urine pH as measured with a pH meter.¹

Normal Values • Normal dogs and cats may have a urine pH of 5.0 to 8.5.

Danger Values • None.

Artifacts • Urine pH may be falsely increased if the urine specimen container is allowed to stand open at room temperature, which leads to loss of CO₂; or by contamination by detergents or disinfectants.

Drug Therapy That May Alter Results • Decreased urine pH may be the result of urinary acidifiers such as methionine, mandelate, phosphate salts, or ammonium chloride. Increased urine pH may be caused by acetazolamide, bicarbonate, or potassium citrate.

Causes of Acid or Alkaline Urine • Any urine pH can be normal. Urine pH is a crude index of acid-base balance and is not a reliable index of blood pH (e.g., a vomiting patient with secondary hypochloremia may have aciduria despite systemic alkalosis, because it is conserving bicarbonate as an anion). Causes of acid urine include ingestion of meat, respiratory and metabolic acidosis, severe vomiting with chloride depletion, severe diarrhea, starvation, pyrexia, and administration of urinary acidifiers. Causes of alkaline urine include a recent meal (i.e., postprandial alkaline tide), ingestion of alkali (e.g., bicarbonate or citrate), UTI with urease-producing bacteria (typically *Staphylococcus* or *Proteus* spp.), renal tubular acidosis (RTA), diets rich in vegetables and cereals, and metabolic and respiratory alkalosis.

Persistently alkaline urine is an indication for complete urinalysis and urine culture. If no reason for alkaline urine is found on history, urinalysis, or urine culture, distal RTA may be considered, although it is rare. Both distal and proximal RTA cause hyperchloremic metabolic acidosis with a normal anion gap and often produce hypokalemia (see Chapter 6).

Proteinuria

Analysis • Urine protein is usually measured with semiquantitative tests such as a urine reagent strip (i.e., dipstick) or by precipitation (i.e., sulfosalicylic acid). The dipstick is more sensitive to albumin than to globulins. Although semiquantitative test results correlate with quantitative results, semiquantitative test results should be regarded only as estimates because individual results may differ considerably from quantitative results. Spectrophotometric analysis is much more precise; it is discussed under Urine Protein:Urine Creatinine Ratio later in this chapter.

Proteinuria must be interpreted in light of urine specific gravity. Because screening tests are qualitative, more protein must be lost into diluted urine than into concentrated urine to give the same result (e.g., a trace reaction with a specific gravity of 1.010 means that more protein is being lost into the urine than with the same trace reaction with a 1.030 specific gravity).

Proteinuria associated with hyperglobulinemia due to myeloma (see Chapter 12) may be caused by Bence Jones proteins (free light chains). Bence Jones proteins do not cause a positive dipstick reaction but do cause a positive result on precipitation testing. Serum and urine protein electrophoresis are indicated in such patients, in which case most of the protein is a monoclonal spike in the β or γ regions. Such a finding necessitates a search for osteolytic or lymphoproliferative lesions. Ehrlichiosis occasionally mimics myeloma (i.e., glomerulonephritis, monoclonal-like gammopathy, bone marrow plasmacytosis). A titer may be diagnostic (see Chapter 15).

A semiquantitative test (ERD-HealthScreen; Heska Corporation—see Appendix I) is available to detect low

concentrations (1 to 30 mg/dl) of albumin in urine, with separate tests for dogs and cats. This test is affected by macroscopic hematuria and by inflammation indicated by pyuria. The test is indicative of any illness, not necessarily primary renal disease, and positive results increase with age.^{54–56} Repeatability problems have been reported in cats with this test, and the test did not always detect cats with urine protein:urine creatinine (UPC) ratios greater than 0.5.⁴⁰

Normal Values • A trace of proteinuria or 1+ reaction is probably normal with a specific gravity greater than 1.012 in dogs.⁵⁷ If the urine specific gravity is less than 1.012, any amount of proteinuria may be abnormal. Dipstick and sulfosalicylic acid precipitation tests had such a high level of false-positive results that a normal value was difficult to determine in cats.^{39,40} The dipstick and precipitation tests are not quantitatively accurate. Quantitative tests are needed to precisely determine severity of protein loss, as discussed under Urine Protein:Urine Creatinine Ratio in the next section of this chapter.

Danger Values • None; however marked loss of albumin in urine can lead to hypoalbuminemia.

Artifacts • Urine protein may be falsely decreased on sulfosalicylic acid precipitation tests by very alkaline urine, or on dipstick tests by the presence of Bence Jones proteins. Urine protein may be falsely increased on sulfosalicylic acid precipitation tests by radiographic contrast media; and on dipstick tests by phenazopyridine, chlorhexidine, allowing the test pad to become wet during storage, allowing prolonged contact of the test pad with excessive amounts of urine, or highly alkaline urine (pH $\geq$ 9).

Drug Therapy That May Cause Proteinuria • Any drug that causes renal tubular or glomerular injury can cause proteinuria (Box 7-1).

Causes of Proteinuria • One must first decide if the proteinuria is significant or not by examining the specific gravity, as described earlier (Figure 7-3). If it is insignificant, one may ignore it unless the patient is receiving nephrotoxic drugs (e.g., aminoglycosides). Such drugs should be stopped regardless of the amount of proteinuria, because mild proteinuria may be an early sign of nephrotoxicity and impending acute renal failure. Aminoglycoside nephrotoxicity typically causes proteinuria or other urinalysis changes (e.g., isosthenuria, glucosuria, cylindruria) before azotemia.

One should next determine if abnormal proteinuria could be prerenal (due to hemoglobinuria, myoglobinuria, or Bence Jones proteinuria) by examining serum globulin concentration and the occult blood results on the urinalysis.

The most common causes of abnormal proteinuria are postrenal, associated with hemorrhage or inflammation at or caudal to the renal pelvis. A urinalysis with sediment examination is performed to exclude hemorrhage and inflammation as causes. Proteinuria associated with inflammation or hemorrhage requires resolution of the inflammation or hemorrhage and then rechecking for

BOX 7-1. SELECTED POTENTIALLY NEPHROTOXIC DRUGS*

Aminoglycoside antibiotics such as neomycin, kanamycin, gentamicin, amikacin, and tobramycin (*important*)
 Amphotericin B (*important*)
 Arsenic
 Cephalothin (*uncommon*)
 Cisplatin (*important*)
 Cyclophosphamide (nephrotoxicity is *uncommon*; sterile cystitis is more *common*)
 Dextran (low molecular weight)
 Ethylene glycol (*important*)
 Furosemide (*uncommon*)
 Heavy metals (i.e., gold, lead, mercury)
 Nonsteroidal anti-inflammatory drugs such as aspirin, ibuprofen (*important* when there is preexisting renal disease, hypotension or other nephrotoxic drugs)
 Polymyxin B (*important*)
 Radiographic contrast media (*important* when there is preexisting azotemia and dehydration)
 Sulfonamides (*uncommon* if more soluble sulfonamides are used)
 Tetracyclines (*uncommon*)
 Thallium
 Thiazides (*uncommon*)
 Vancomycin (*uncommon*)
 Zinc

*Not all of these drugs reliably produce nephrotoxicity. Those drugs recognized as the most dangerous are denoted *important*.

persistence of proteinuria. The most common cause of inflammatory proteinuria is UTI. Urine culture should be performed if infection is possible. To exclude extraurinary postrenal causes of proteinuria (e.g., genital tract inflammation), a urine sample collected by cystocentesis should be examined.

If hemorrhage and inflammation are excluded by a normal ("quiet" or "inactive") urine sediment and prerenal causes are unlikely, proteinuria is most likely of renal origin. Renal-origin proteinuria can be transient or persistent and due to glomerular or tubular dysfunction. One should measure serum albumin and repeat the urinalysis to determine if proteinuria is persistent. If hypoalbuminemia is present in association with marked proteinuria, one can assume that the proteinuria is significant and persistent. Transient (i.e., functional) proteinuria has many causes (e.g., strenuous exercise, fever, seizures, venous congestion of the kidneys) and is rarely significant. Persistent proteinuria is defined as abnormal proteinuria on at least three occasions, two or more weeks apart.³⁷ Persistent proteinuria with an inactive urine sediment should prompt determination of a UPC ratio to ascertain its severity.

NOTE: A normal serum albumin concentration does not mean that proteinuria is insignificant.

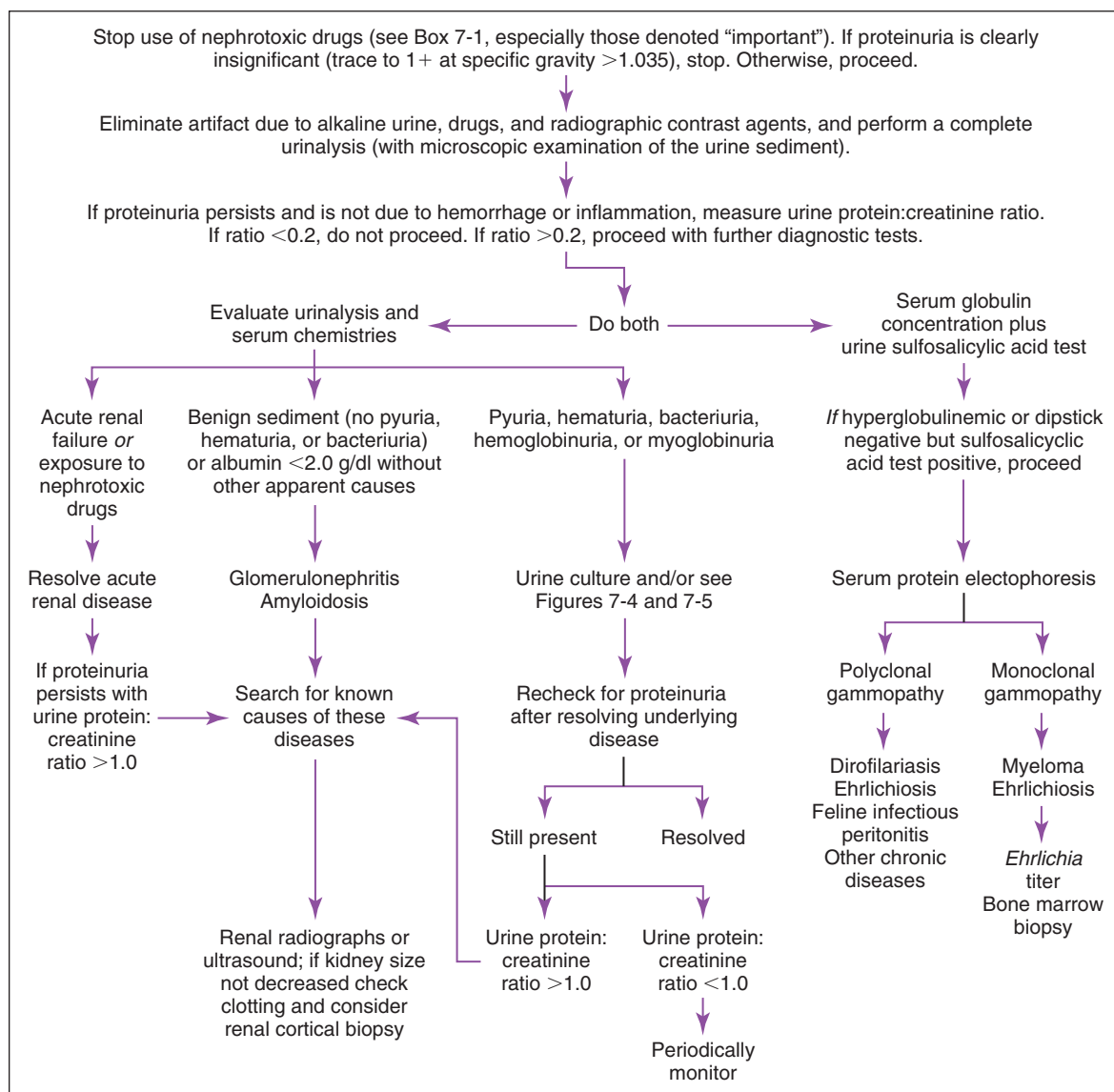


FIGURE 7-3 Diagnostic approach to proteinuria in dogs and cats.

Urine Protein:Urine Creatinine Ratio

Indication • Measurement of the UPC ratio is indicated to determine the magnitude, and therefore the significance of proteinuria.

Advantages • Measurement of the UPC ratio is more accurate than dipstick and precipitation procedures, requires only a single random urine sample, and correlates well with 24-hour determinations. Urine samples can be collected by any method.^{7,25}

Disadvantages • A complete urinalysis should be performed on an aliquot of the same sample to look for macroscopic hemorrhage or inflammation, which would alter assessment of the results. In dogs with abnormal

proteinuria, the UPC ratio varies day-to-day especially in the lower ranges of abnormality.⁴² This variation can be as much as 80% at values near 0.5 and 35% at values about 12 even when the same methodology is used. A single measurement will reliably estimate the UPC ratio at values less than 4, but higher values require two to five determinations. Such variation should be taken into account when following UPC ratios in dogs and cats with chronic renal diseases. The ratio gives no information about the origin of the proteinuria; it only quantifies it.

Analysis • The UPC ratio should not be determined by dipstick estimates of urine protein and urine creatinine because these are inaccurate. Urine protein and creatinine concentrations should be measured spectrophotometrically. Different assay types and equipment are used by

different laboratories, and results vary by methodology, making interlaboratory UPC ratio comparisons problematic. After analysis, the following ratio is calculated: total protein (mg/dl)/creatinine (mg/dl).

Normal Values • Adult dogs and cats, less than 0.2 (higher values are normal in puppies at least up to 2 weeks of age⁴⁶ and in noncastrated male cats); borderline 0.2 to 0.5 (dogs), 0.2 to 0.4 (cats).

Danger Values • None. However, in azotemic and in hypertensive cats, the risk for death or euthanasia increases with increasing UPC ratio.^{50,51} In nonazotemic, geriatric cats, the UPC ratio was predictive of survival and of development of azotemia.³³ In dogs with renal failure, a UPC value greater than 1 at initial evaluation was associated with increased risk of uremic morbidity and mortality.³² Marked loss of albumin in urine can lead to hypoalbuminemia.

Artifacts • See discussions of total protein (see Chapter 12) and of creatinine later in this chapter.

Causes of Increased UPC Ratio • Remember that inflammation and hemorrhage can increase the UPC ratio and that the UPC ratio should only be interpreted on urine with a normal urine sediment examination. In this situation, moderate proteinuria (i.e., UPC ratios of 0.5 to 2) may be associated with renal tubular or glomerular diseases.

Marked proteinuria (UPC ratio > 2) associated with a quiet sediment and normal serum globulins or a polyclonal gammopathy is usually the result of renal glomerular disease (i.e., glomerulonephritis, amyloidosis). One should search for causes of glomerulonephropathy: chronic parasitic disease such as heartworm disease, hepatozoonosis, or leishmaniasis; chronic inflammatory diseases such as systemic lupus erythematosus; chronic infectious diseases such as borreliosis, feline leukemia virus (FeLV) infection, feline immunodeficiency virus (FIV) infection, ehrlichiosis, pyometra, or endocarditis; neoplasia; endocrine diseases such as hyperadrenocorticism^{31,43} and diabetes mellitus⁴⁸; and familial glomerulopathies. Immunosuppressive glucocorticoid therapy leads to mild proteinuria.^{47,53} If no underlying disease is identified or if proteinuria persists despite treatment for an identified disease, renal cortical biopsy should be considered to determine whether glomerulonephritis or amyloidosis is present.

NOTE: Feline amyloidosis usually affects the renal medulla and may not cause proteinuria.

Glucosuria

Analysis • Urine glucose concentration is measured using a test pad on a urine dipstick or a paper test strip (i.e., glucose oxidase method) or with a test for reducing substances in urine (Clinitest).

Normal Values • Dog and cat urine should be negative for glucose by these tests.

Danger Values • None.

Artifacts • Urine glucose may be falsely decreased by refrigerated urine, large amounts of ascorbic acid, tetracycline (due to ascorbic acid in the formulation), formaldehyde (metabolite of methenamine, used as a urinary antiseptic), low urine pH, and increased urine salt concentrations (paper test strip). Urine glucose may be falsely increased by hydrogen peroxide, hypochlorite or bleach, and a nonglucose oxidizing substance in the urine of cats with urethral obstruction (dipstick). Overall, the urine dipstick was found to be more accurate in cats than dogs, although false positives were more common in cats.⁸ In dogs, the test strips had a high percentage of false-negative results and tended to underestimate urine glucose concentrations.⁸

The Clinitest reaction is not specific for glucose. Urine glucose may be falsely increased by galactose, pentose, lactose, fructose, salicylates, penicillins, some cephalosporins, radiographic contrast media, and large amounts of ascorbic acid or sulfonamides. Very strong positive reactions may be read out low because the final color is less orange than that which occurred during the reaction (i.e., “pass-through” phenomenon).

Drug Therapy That May Cause Glucosuria • Glucosuria may be caused by drugs capable of producing hyperglycemia (see Chapter 8), intravenous (IV) infusion of dextrose-containing solutions, and selected nephrotoxins causing proximal renal tubular dysfunction (e.g., aminoglycoside nephrotoxicity).

Causes of Glucosuria • Glucosuria usually occurs because the renal threshold for glucose reabsorption is exceeded because of hyperglycemia (i.e., blood glucose > 180 mg/dl in dogs and > 300 mg/dl in cats). Glucosuria always necessitates measuring blood glucose. The most common cause of glucosuria because of hyperglycemia is diabetes mellitus (Table 7-4). If blood glucose concentration is normal, urine should be re-evaluated with both urine dipstick and Clinitest. If glucosuria is still present, proximal renal tubular dysfunction is likely. History

TABLE 7-4. CAUSES OF GLUCOSURIA IN DOGS AND CATS

Blood glucose concentration exceeding renal threshold	Diabetes mellitus Stress (especially in cats) Infusion of dextrose-containing fluids Hyperadrenocorticism (rarely causes glucose > 180 mg/dl) Pheochromocytoma (rare)
Abnormal proximal renal tubular function	Aminoglycoside toxicity Acute renal failure Fanconi's syndrome Primary renal glucosuria
Contamination	Urinary hemorrhage in a patient with mild hyperglycemia
Artifact	Pseudoglucosuria in cats with urethral obstruction

should be reviewed for nephrotoxins, and urinalysis and BUN and serum creatinine concentrations measured to search for proximal renal tubular disease. Occasionally, multiple tubular defects are found; Fanconi's syndrome occurs genetically in some breeds (e.g., basenji) and can be induced by certain toxins, leading to hyperchloremia, metabolic acidosis, hyperphosphatemia, aminoaciduria, and glucosuria despite normoglycemia.

Ketonuria

Analysis • A test pad on a urine dipstick or a tablet (Acetest) is commonly used to measure ketone concentration. These detect acetoacetate and acetone but not β -hydroxybutyrate (which is responsible for acidosis).

Normal Values • Urine results should be negative for ketones.

Danger Values • Severity of ketoacidosis is not necessarily correlated with the degree of ketonuria. Large amounts of urine ketones plus lethargy and vomiting strongly suggest ketoacidosis and warrant immediate measurement of blood glucose plus evaluation of acid-base status (e.g., total carbon dioxide [Tco_2] determination, blood gas analysis).

Artifacts • Urine ketone concentration may be falsely increased by discolored urine, phenazopyridine, dimercaprol, aspirin, captopril, mesna, *N*-acetylcysteine, and valproic acid.

Drug Therapy That May Cause Ketonuria • Streptozotocin and aspirin intoxication may cause ketonuria.

Causes of Ketonuria • Lipolysis produces ketones. Starvation, fasting, and diabetic ketoacidosis are the most common causes. If ketonuria and glucosuria are present, diabetes mellitus is highly likely and should be confirmed by measurement of blood glucose. The presence of ketonuria, glucosuria, and hyperglycemia confirms a diagnosis of diabetes mellitus, and serum sodium, potassium, phosphorus, and Tco_2 or blood gas determinations are indicated. Ketonuria without glucosuria suggests excessive lipid catabolism and is not generally investigated further in anorexic, nondiabetic patients.

Bilirubinuria

Analysis • A test pad on a urine dipstick (i.e., diazo method) and occasionally the oxidation method (i.e., Harrison's spot test) are used to test for bilirubinuria. The tablet method may be more sensitive than the dipstick.

Normal Values • Dogs (especially males) may have small amounts of bilirubinuria if the urine specific gravity is greater than or equal to 1.030. Normal cats do not have bilirubinuria.

Danger Values • None.

Artifacts • Urine bilirubin may be falsely decreased if the urine sample undergoes prolonged exposure to

ultraviolet light or stands at room temperature exposed to air (bilirubin oxidizes into biliverdin, which is not detected). Substantial hemoglobinuria also may cause a falsely decreased reading by masking the bilirubin-induced color change on dipsticks. Urine bilirubin may be falsely increased by large amounts of phenothiazines.

Drug Therapy That May Cause Bilirubinuria • See Chapters 3 and 9 for causes of hemolytic anemia and icterus, respectively.

Causes of Bilirubinuria • Bilirubin must be conjugated to be excreted into urine. The liver is principally responsible for conjugation, but canine kidneys can also conjugate bilirubin. The most common causes of hyperbilirubinemia in dogs and cats are hepatic disease, post-hepatic bile duct obstruction, and hemolytic diseases. Mild bilirubinuria may result from prolonged anorexia.

Excessive bilirubinuria in a dog or any bilirubinuria in a cat is an indication to determine total serum bilirubin concentration, SAP and ALT activities, and a hematocrit. If the hematocrit is below the lower limit of normal, a CBC plus reticulocyte count is indicated. See Chapter 9 for the diagnostic approach to icterus.

Urobilinogen

Assay • A test pad on a urine dipstick is used to test for urobilinogen.

Normal Values • 0.1 to 1.0 Ehrlich units. One cannot detect total absence of urobilinogen with this test.

Danger Values • None.

NOTE: Urobilinogen urine concentration correlates poorly with hepatobiliary disease. The results of this test have no clinical usefulness and should be ignored.

Occult Blood

Analysis • A test pad for occult blood on most urine dipsticks detects hemoglobin, myoglobin, and, to a lesser extent, intact RBCs. The assay is very sensitive, detecting 0.03 mg hemoglobin/dl and 1 to 2 RBCs/hpf.¹⁴

Normal Values • No hemoglobinuria or myoglobinuria. A few RBCs (i.e., 5 or fewer/hpf) may occur in normal urine. Higher numbers of RBCs are found in voided urine in proestral bitches.

Danger Values • None, although marked hemoglobinuria or myoglobinuria in a dehydrated animal may cause renal tubular injury.

Artifacts • Occult blood test results may be falsely decreased by ascorbic acid and captopril. Results may be falsely increased by flea dirt or oxidizing agents such as disinfectants.

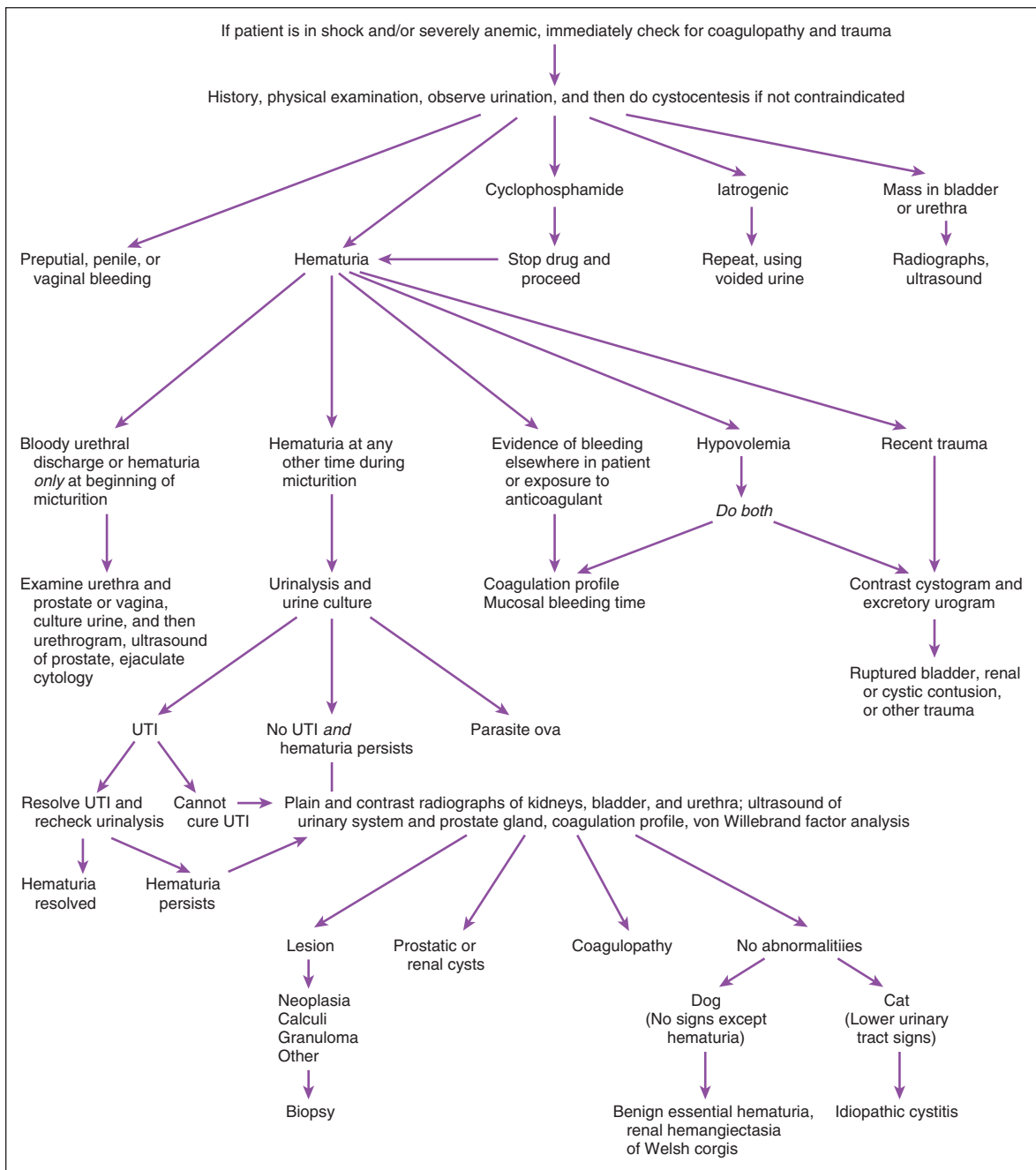


FIGURE 7-4 Diagnostic approach to persistent hematuria (gross or microscopic) in dogs and cats. UTI, Urinary tract infection.

Causes of Positive Occult Blood • Hematuria is the most common cause of a positive dipstick finding of occult blood; therefore, a urine sediment examination should be performed. If RBCs or RBC ghosts are found in the sediment, hematuria is confirmed. Dilute or alkaline urine may cause RBC lysis, and hemolyzed RBCs are not always visible. Hemoglobinuria because of hematuria is followed up as for hematuria (Figure 7-4).

If no RBCs are found, especially if the urine is grossly discolored after centrifugation, one should determine

hematocrit and plasma color. If the plasma is pink or red despite proper venipuncture technique (hemoglobinemuria) (Figure 7-5), hemoglobinuria is present. Hemoglobinuria because of hemoglobinemia indicates hemolytic anemia (see Chapter 3), and a complete CBC is indicated. If no evidence of hematuria or hemolysis exists, myoglobinuria must be considered. One may test for myoglobinuria by requesting urine precipitation with 80% saturated ammonium sulfate. If the urine supernate remains red-brown after centrifugation, 2.8 g

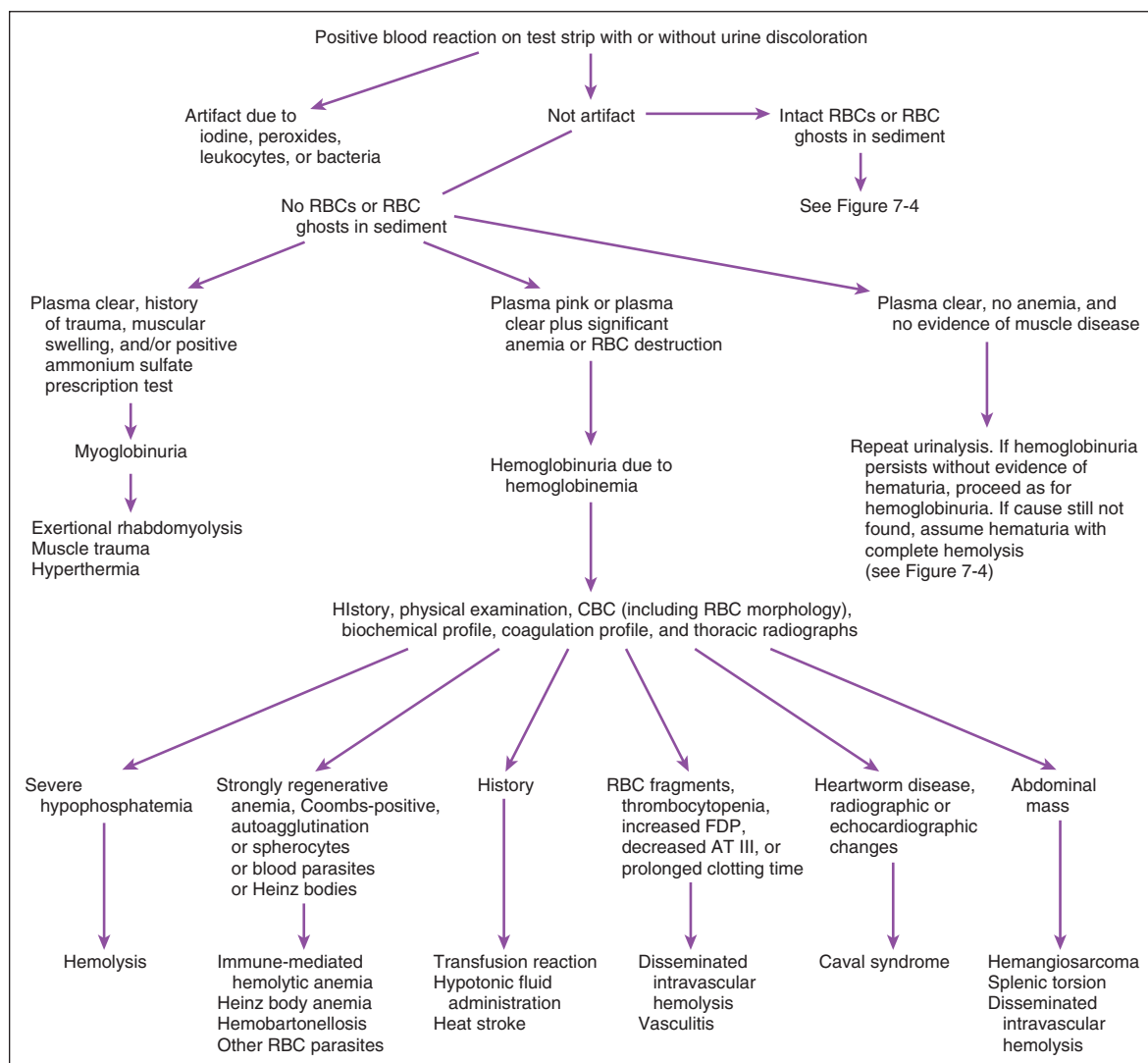


FIGURE 7-5 Diagnostic approach to hemoglobinuria in dogs and cats. AT III, Antithrombin III; CBC, complete blood count; FDP, fibrin degradation products; RBC, red blood cell.

ammonium sulfate should be added to 5 ml of urine with a neutral pH. After centrifuging this mixture, if the supernate remains dark, myoglobin is confirmed. Occasional interpretation problems occur if the urine is colored because of nonprotein pigments. Myoglobinuria requires a search for rhabdomyolysis or myositis (see Chapter 14), and serum creatine kinase activity should be measured.

If no evidence of hematuria, hemoglobinemia, hemolysis, or muscle disease is seen, one should recheck for artifacts. Persistent hemoglobinuria of unknown cause necessitates looking for occult urinary hemorrhage.

Hematuria

Analysis • A test pad on a urine dipstick for occult blood and microscopic examination of urine sediment is used to test for hematuria.

Normal Values • Less than 5 RBCs/hpf. If urine is obtained by cystocentesis or catheterization, iatrogenic trauma in obtaining the sample can cause gross or microscopic hematuria.

Danger Values • None. However, severe or persistent hematuria can result in anemia, either regenerative or, with chronic loss, nonregenerative due to iron deficiency.

Artifacts • Hematuria may be falsely decreased because hemolysis occurs rapidly in hyposthenuric or alkaline urine and may be complete within 2 hours. This causes a positive occult blood reaction with no obvious RBCs in the urine sediment, although RBC ghosts are sometimes visible.

Causes of Hematuria • The clinician should first consider iatrogenic hemorrhage during sampling (see

Figure 7-4). Using voided urine avoids the possibility of iatrogenic hemorrhage during catheterization or cystocentesis.

If gross hematuria is present, timing of the most intense urine discoloration during the urine stream helps localize the bleeding site. Blood independent of urination or most severe at the beginning of urination suggests the urethra, the prostate gland or prepuce in male dogs, or the uterus or vagina of females. Blood at the end of urination suggests the bladder as the site of origin. Bleeding at any site can cause blood throughout urination. If urine collected by cystocentesis has no blood but voided urine has blood, the distal urethra, prepuce, vagina, or uterus is most likely. Blood from prostatic and proximal urethral lesions usually refluxes back into the bladder as well as causing bleeding independent of urination.

Hematuria may result from infection, calculi, other nonseptic inflammation, coagulopathies, trauma (exogenous, iatrogenic), neoplasia, cysts, renal infarcts, chronic passive renal congestion, urinary parasites, strenuous exercise, estrus in females, or glomerulonephritis (rare).

In dogs, UTI is the most common cause of hematuria; therefore, a urine culture is indicated even if pyuria and bacteriuria are absent. Calculi are another common cause, and survey abdominal radiographs or ultrasound are indicated. If the patient has a history of anticoagulant exposure, evidence of coagulopathy on history or physical examination, hypovolemia or anemia because of hemorrhage, or no obvious genitourinary cause of the hemorrhage, coagulation screening tests (see Chapter 5) are indicated. If all these tests are unrevealing, contrast radiography, ultrasonography, or both are used to examine kidneys, ureters, urinary bladder, prostate, and urethra. In male dogs, prostatic fluid (ejaculate, postprostatic massage, or aspiration of ultrasonically visible intraprostatic cysts) should be examined.

In cats, idiopathic cystitis is a common cause of hematuria. Prostatic disease is an uncommon cause of feline hematuria. Other than these, the causes for hematuria in cats are similar to those in dogs.

Nitrituria

Analysis • A test pad on a urine dipstick is used to test for nitrituria.

Normal Values • Negative.

Danger Values • None.

NOTE: This test is inaccurate in dogs and cats and should be ignored.

Pyuria/Leukocytes

Analysis • Microscopic examination of urine sediment is used to test for pyuria/leukocytes. The leukocyte esterase test strip used to evaluate human urine for WBCs is insensitive in dogs, failing to detect pyuria, and has a high false-positive rate in cats. Results from the test strip should not be substituted for urine sediment examination in dogs and cats.⁶

Normal Values • Less than 4 WBCs/hpf; samples obtained by cystocentesis are preferred to avoid distal urethral and reproductive tract contamination.

Danger Values • None.

Artifacts • Pyuria/leukocyte test results may be falsely decreased by alkaline urine, dilute urine, or prolonged exposure of the urine sample to room temperature (causes WBC lysis).

Causes of Pyuria • Pyuria indicates inflammation. WBCs may also enter voided urine via preputial or vaginal secretions. A cystocentesis-obtained urine sample should be used to eliminate these sites as possible sources. UTI is the most common cause of pyuria; calculi and neoplasia are other common causes. Urine culture is indicated.

NOTE: Dilute urine or urine from patients with impaired WBC function (e.g., hyperadrenocorticism, diabetes mellitus) may not have pyuria despite a UTI.

If pyuria persists and bacteria cannot be cultured, survey and contrast radiographs and/or ultrasonography of the urinary tract are indicated to eliminate calculi and neoplasia.

Bacteriuria

Analysis • Microscopic examination of urine sediment or urine culture (see Chapter 15) is used to test for bacteriuria. Examination of urine sediment with modified Wright staining significantly improved accuracy of detection of bacteriuria as compared to unstained wet mounts.⁴⁹ Unstained wet mounts were reasonably accurate when negative, but positive prediction was poor.⁴⁹ Medical technologists were reasonably accurate in determining whether bacteria seen on wet mount were rods or cocci.⁴ Gram-stained slides of canine urine sediment also correlated well with urine culture results.² Cystocentesis-obtained urine is preferred to avoid contamination from the distal urethra and reproductive tract.

Normal Values • Bacteriuria is abnormal in urine obtained by cystocentesis. Quantitative urine culture is needed to determine the significance of bacteria in urine obtained by catheterization. Bacteria in voided urine could be the result of infection or contamination with normal flora of the distal urethra and genital tracts.

Danger Values • None, although in rare instances, UTI can lead to septicemia or renal or prostatic abscessation.

Artifacts • Bacteriuria test results may be falsely decreased by recent antibiotic therapy, diuresis, contamination of urine with oxidants (e.g., bleach), or delay between collection and examination. Bacteriuria test results may be falsely increased by delay in examination with urine remaining at room temperature, or by contaminated centrifuge tubes or stain solutions. Brownian

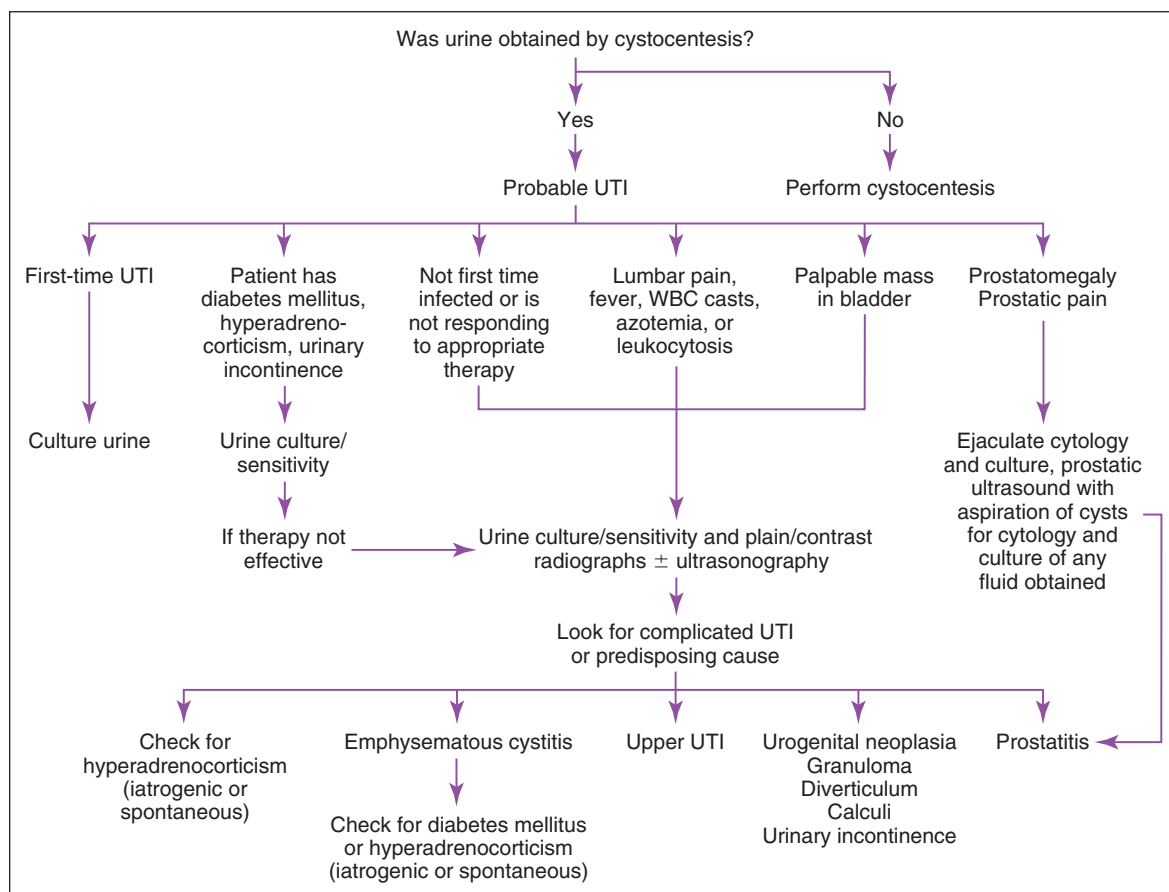


FIGURE 7-6 Diagnostic approach to bacteriuria in dogs and cats. UTI, Urinary tract infection; WBC, white blood cell.

motion of amorphous debris may be confused with bacteriuria in unstained, wet mount preparations.

Causes of Bacteriuria • Once artifacts and contamination are eliminated, bacteriuria allows diagnosis of UTI (Figure 7-6). Bacteriuria, pyuria, and hematuria in properly obtained urine are classic findings for UTI; however, not all UTIs have detectable pyuria, hematuria, or bacteriuria. Dilute urine may have such a low concentration of cells that they cannot be found in the sediment. Greater than 10^4 rods/ml or 10^5 cocci/ml must be present before they can readily be seen in the urine sediment. Patients with hyperadrenocorticism (endogenous or iatrogenic) or diabetes mellitus may not have any evidence of UTI on urinalysis. Therefore, it is reasonable to culture urine from all patients with these conditions.

Urine culture is recommended in all patients with suspected UTI. If a UTI persists or recurs after treatment, urine culture is mandatory. Recurrent UTIs are indications for survey and contrast radiographs, ultrasonography, and ejaculate cytology and culture in intact male dogs. Careful search must be made for underlying causes such as inappropriate therapy (i.e., drug resistance), lack of drug administration by the owner, repeated or indwelling urinary catheterization, neoplasia, partial obstruction,

pyelonephritis, prostatitis, calculi, granulomas, diverticuli, incontinence, polyuria, urine retention, diabetes mellitus, and hyperadrenocorticism (Table 7-5).

Other Cells

Analysis • Microscopic examination of urine sediment is used to look for other cells. A few large and small round cells may be visible in the urine sediment of normal animals. Large numbers of urothelial cells suggest inflammatory or neoplastic disease.⁵ Urothelial cells begin to degenerate within 30 minutes of collection.⁵

Causes of Other Cells • Neoplastic cells are occasionally found in the urine of patients with malignancies (especially transitional cell carcinoma) of the bladder or urethra. If neoplastic cells are being sought in urine, a large volume of fresh urine should be immediately centrifuged, the sediment smeared on a slide and allowed to dry, and the slide stained with new methylene blue (NMB) or Wright-Giemsa. Swelling and early degeneration of cells exposed to urine are common, as are reactive changes because of cystitis. These changes may mimic malignancy (i.e., large or atypical nucleus and nucleolus). Radiographic contrast agents may produce similar

TABLE 7-5. CAUSES OF PERSISTENT OR RELAPSING URINARY TRACT INFECTIONS AND METHODS OF DIAGNOSIS

CAUSE	MEANS OF DIAGNOSIS
Lack of owner compliance in drug administration	History (check for leftover medications)
Upper urinary tract infection	Excretory urogram showing dilated pelvis, culture urine from renal pelvis, white blood cell casts, ultrasonography
Calculi	Survey and/or contrast radiographs, ultrasonography, cystoscopy
Prostatitis	Ejaculate cytology and culture, prostatic aspirate, prostatic biopsy, ultrasonography
Neoplasm	Cytology of urine sediment, contrast radiographs, biopsy, ultrasonography, urethrocystoscopy
Diverticulum	Positive-contrast radiographs
Granuloma	Contrast radiographs, urethrocystoscopy, biopsy
Urinary incontinence or urine retention due to any cause	History, physical examination, determination of residual urine volume
Decreased resistance to infection	Hyperadrenocorticism, diabetes mellitus, hyperthyroidism in cats (see Chapter 8)
Urinary catheterization	History, physical examination

changes. If urethral or cystic neoplasia is suspected but either no abnormal cells are found in the urine or uncertainty exists as to the nature of the cells seen, a catheter biopsy procedure or ultrasound-guided aspirate is preferred to obtain a specimen suitable for cytologic evaluation.

Rarely, funguria occurs in blastomycosis and disseminated aspergillosis. Other fungi and yeasts are usually contaminants, although infection can occur with *Candida albicans* and *Torulopsis* spp., especially in association with prolonged antibiotic therapy, immunosuppressant therapy, and/or indwelling urinary catheterization.

Sperm are normally visible in the urine of intact male dogs.

Cylindruria

Analysis • Microscopic examination of urine sediment is used to check for cylindruria.

Normal Values • 0 to 2 hyaline or granular casts/lpf in moderately concentrated urine is expected in otherwise normal animals.

Danger Values • None.

Artifacts • Casts disintegrate if urine is stored too long (hours) or subjected to vigorous mixing or handling. Casts are less commonly visible in alkaline urine.

Pseudocasts can form from degenerating urothelial cells after 10 hours of urine storage.⁵

Causes of Cylindruria • Casts originate in the kidney, and their presence supports a diagnosis of renal disease; absence of casts does not eliminate renal disease. The type of cast provides some information about the disease process. Number of casts is not correlated with reversibility or irreversibility of underlying disease.

Hyaline casts are colorless and composed of Tamm-Horsfall mucoprotein without cells. They may be found during diuresis, after correcting dehydration, or in patients with proteinuria. Epithelial cell casts contain renal tubular cells that have not yet disintegrated. They occur in renal diseases with tubular epithelial cell injury. Granular casts are composed of degenerating epithelial cells, proteins, and other substances. Distinguishing coarse granular casts from fine granular casts is not useful. These casts are associated with diseases causing degeneration and necrosis of renal tubular epithelium. Waxy casts represent older, degenerate granular casts. They are colorless to gray, refractile, and wider than hyaline casts. Broad casts are wide hyaline, granular, or waxy casts. They are thought to be wide because of formation in collecting ducts or dilated renal tubules. WBC casts signify renal tubulointerstitial inflammation. This type is rare, and few patients with pyelonephritis have them. Therefore, absence of this cast type does not exclude pyelonephritis. RBC casts are rare and signify hemorrhage into renal tubules or severe glomerular injury, allowing RBCs to enter the tubules (e.g., glomerulonephritis, vasculitis, renal infarction).

Crystalluria

Analysis • Crystalluria is identified by microscopic examination of urine sediment. Crystal habit (i.e., the characteristic shapes of mineral crystals) is used as an index of crystal composition (Table 7-6); however, microscopic identification of urine crystals is imperfect because their appearance is altered by numerous factors. Definitive identification of crystal composition requires special analyses. To minimize time and temperature effects on crystal formation, urine should be examined within 60 minutes of collection.¹

Artifacts • Crystalluria means the urine specimen is oversaturated with crystallogenic substances; however, numerous variables influence crystalluria. The significance of crystalluria is easily misjudged if these factors are not considered. *In vivo* variables include urine concentration, urine pH, amount and solubility of crystalloids, presence of crystallization promoters and inhibitors, and excretion of medications or diagnostic agents. *In vitro* variables include temperature, evaporation, pH, and technique of specimen preparation. It is important to note that refrigeration of urine samples leads to precipitation of crystals, a falsely positive result.

Causes of Crystalluria • Crystalluria is usually clinically insignificant, but can be important in limited situations. Crystal type is important in association with current or prior occurrence of calculi and when one suspects a portosystemic shunt, ethylene glycol toxicity (Figure 7-7),

TABLE 7-6. CRYSTALS THAT MAY BE FOUND IN CANINE AND FELINE URINE

NAME	DESCRIPTION	SIGNIFICANCE
Struvite (magnesium ammonium phosphate)	Colorless prisms with 3–6 sides (coffin lid)	Common in mildly acidic to alkaline urine in normal dogs and cats; may be associated with struvite calculi and infection with urease-producing bacteria
Calcium oxalate (monohydrate)	Dumbbells or small spindles	May be normal, due to ethylene glycol intoxication, or associated with oxalate calculi (see Figure 7-7)
Calcium oxalate (dihydrate)	Colorless envelopes or small stars	May be normal, due to ethylene glycol intoxication, or associated with oxalate calculi
Calcium phosphate	Prisms (long) or amorphous	May be normal or associated with calculi
Ammonium urate	Yellow-brown “thorn apples”	Normal in dalmatians and English bulldogs; associated with hepatic insufficiency and portosystemic shunts; may be associated with urate calculi
Uric acid	Yellow to yellow-brown prisms, diamonds, or rosettes	Same as ammonium urate
Bilirubin	Golden-yellow to brown needles or granules	May be present in normal dogs with concentrated urine or due to bilirubinuria
Cystine	Colorless, flat hexagonal plates	Due to cystinuria; may be associated with calculi
Cholesterol	Colorless, flat, notched plates	May be found in normal dogs and cats
Hippuric acid	Prisms (4–6 sides) with rounded corners	Uncertain; have been confused with calcium oxalate monohydrate crystals
Sulfonamide	Clear to brown eccentrically bound needles in sheaves	Associated with sulfonamide administration
Xanthine	Amorphous, spheroid, or ovoid; yellow-brown	Difficult to differentiate from amorphous urates; can form during allopurinol therapy
Crystals associated with melamine toxicity	Yellow-brown spherical crystals with radial striations	Present in urine from animals fed melamine/cyanuric acid-contaminated diets

or melamine intoxication. Whenever crystalluria is considered significant, it is important to examine fresh, unrefrigerated urine specimens. Number, size, and structure of crystals should be evaluated, as well as their tendency to aggregate. Urine pH is important in regard to precipitation of crystals, with some types being less soluble in acid urine (cystine, urate) and others in neutral to alkaline urine (struvite). Whenever crystalluria is detected, urine

pH should be noted. It is important to assess urine pH within 1 hour of urine collection.

Detection of ammonium urate crystals in cats and in dog breeds other than Dalmatians and English bulldogs may suggest hepatic insufficiency (e.g., portosystemic shunt). Calcium oxalate monohydrate crystals in animals in acute renal failure suggest ethylene glycol ingestion (see [Figure 7-7](#)). The presence of cystine crystals indicates cystinuria, which places the animal at risk for cystine calculi.

Crystalluria often causes concern about urolithiasis. Evaluation of urine crystals may aid in detecting conditions that predispose to urolith formation, estimating mineral composition of existing uroliths, and evaluating effectiveness of therapy intended to dissolve uroliths or prevent their reformation. Crystalluria must not be the sole criterion for assessment of stone composition when uroliths are present. Animals with crystalluria do not necessarily form uroliths, and finding crystalluria is not an indication for treatment. For example, dogs and cats normally excrete a large amount of ammonium magnesium phosphate (struvite). With urine pH greater than 6.5, this normal excretion begins to become visible as struvite crystals. The higher the urine pH, the more crystals become evident. Struvite crystalluria is normal in most dogs and cats. Urolithiasis becomes a risk when urine pH remains consistently alkaline, usually from infection with urease-producing bacteria (dogs) or when urine is very concentrated in association with a urine pH greater than 6.5 (cats).



FIGURE 7-7 Calcium oxalate monohydrate crystals. These elongated six-sided crystals in urine of dogs and cats are very suggestive of ethylene glycol toxicity.

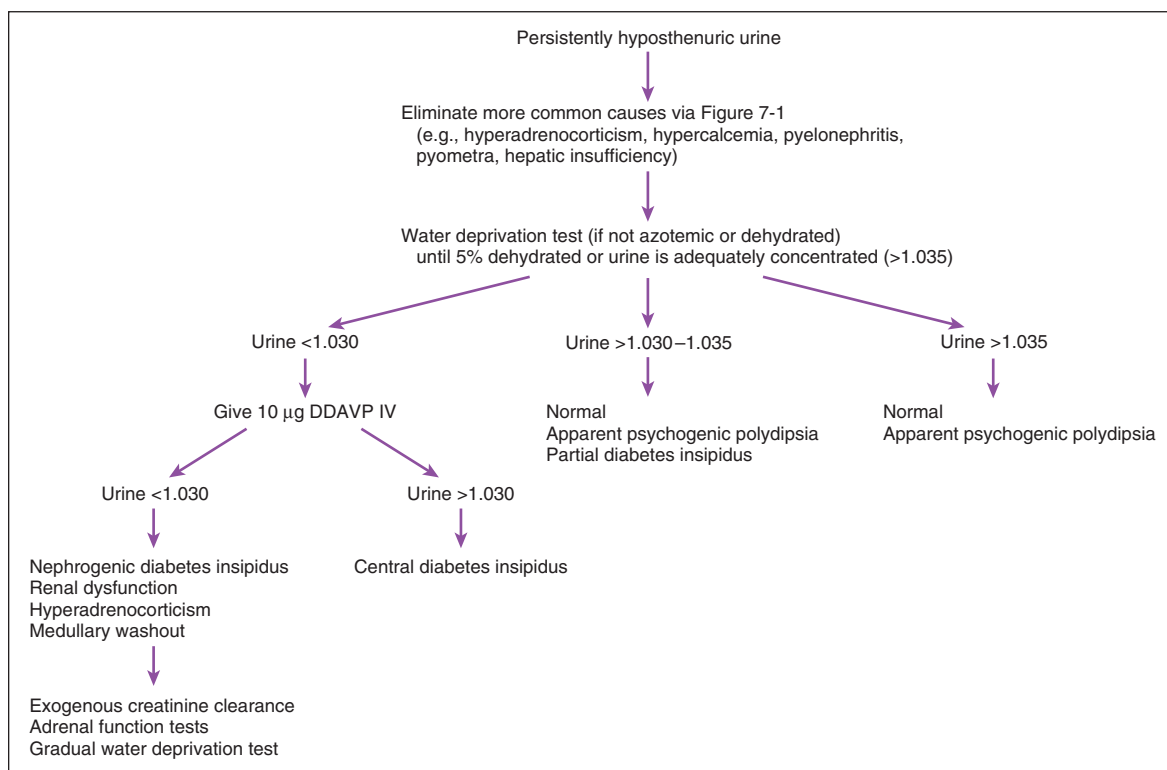


FIGURE 7-8 Use of water deprivation and antidiuretic hormone (ADH) response testing for differentiation of causes of polyuria-polydipsia, especially those that result in hyposthenuric urine.

WATER DEPRIVATION TESTING

Indication • Water deprivation testing is indicated in selected patients with confirmed pu-pd: generally those that are hyposthenuric and in whom most causes of pu-pd have been excluded by history, physical examination, CBC, urinalysis, biochemical profile (i.e., azotemic renal failure, hepatic failure, diabetes mellitus, hypercalcemia, marked hyponatremia, marked hypokalemia), and endocrine testing where indicated (i.e., hyperthyroidism, hyperadrenocorticism, hypoadrenocorticism) (see Table 7-1). After excluding these diseases, water deprivation testing can distinguish apparent psychogenic polydipsia from central diabetes insipidus or nephrogenic diabetes insipidus (Figure 7-8).

Advantage • Water deprivation testing is specific for psychogenic polydipsia when more common causes of pu-pd have been eliminated.

Disadvantages • This test is not able to differentiate between many common medical causes of pu-pd, and close monitoring is needed to avoid morbidity and mortality.

Contraindications • Water deprivation testing should never be performed in a dehydrated animal or one with significant abnormalities on CBC and/or serum biochemical profile.

Analysis • This test is performed only in nonazotemic, euhydrated patients after taking a thorough history, performing a complete physical examination, and evaluating a CBC, biochemical profile, and urinalysis. One must eliminate drugs and diets that cause pu-pd (e.g., diuretics, glucocorticoids, anticonvulsants, excessive thyroid supplementation, low-protein or high-salt diets). Changes in environment are also possible causes of changes in water intake.

An abrupt water deprivation test is one approach. The patient is hospitalized for the test, which is begun with an overnight fast during which water intake is not limited. The first urine voided in the morning is collected and specific gravity is measured because urine is most likely to be concentrated at this time of day. A portion of the urine and a blood sample are saved for measurement of osmolality. The animal is walked to encourage defecation and complete urination. The bladder is palpated to be sure it has been emptied. The animal is then accurately weighed. A 5% decrease in body weight is calculated and becomes the target weight at which point the test will be ended. The test is also ended if the urine specific gravity reaches 1.035. All access to water is removed. Fasting is continued. Urine is collected, specific gravity measured, and the animal weighed every 1 to 4 hours, depending on the rate of weight loss. Once the target weight or specific gravity is reached, samples are collected for urine and plasma osmolality.

A practical problem with water deprivation testing is that the duration of the test is unpredictable and one may be faced with night approaching and an animal that has neither reached 5% dehydration nor concentrated urine to 1.035. In this situation, one can transfer the animal to a facility with overnight care so that sampling can continue or one can provide the animal with a maintenance water amount (calculated at 2.75 ml/kg/hr that the animal is to be unobserved). The next morning the animal is weighed, specific gravity is measured, water is again withdrawn, and the test is continued until the original target weight indicating 5% dehydration or a specific gravity of 1.035 is reached. With a prolonged test, dry food can be fed.

Several methods have been described for performing gradual water deprivation testing. All methods involve first measuring the animal's water intake over 24 hours. Body weight is also measured, and a 5% body weight loss calculated. In one method, the amount of water offered is gradually restricted to 100 ml/kg/day over 3 days and then the animal is completely deprived of water.¹⁸ In another method, volume of water given daily is reduced each day from the initial measured amount by an amount equal to 2% of the initial body weight. For example, if the animal weighed 10 kg and drank 1.5 L/day, the amount offered each day would be reduced by 0.2 L; the animal would be given 1.3 L the first day, 1.1 L the second day, and so forth. Regardless of method, urine specific gravity and the animal's weight should be monitored each day initially and then more frequently as the target weight is approached. The test is interpreted the same way as the abrupt test. The reason for performing a gradual rather than an abrupt test is concern that medullary washout of solute secondary to prolonged polyuria can prevent urine concentration during abrupt water deprivation. Medullary washout is rare unless the animal has been on a low-protein diet or has been receiving diuretics.

Normal Values • Ninety-five percent of normal cats and dogs concentrate urine to a specific gravity of 1.048 before they lose 5% of their body weight. Normal dogs and cats require several days to reach this level of dehydration. Normal animals reach the target specific gravity of 1.035 before they reach the target degree of dehydration. A dog with pu-pd with normal response to water deprivation most likely has psychogenic polydipsia; however, some dogs with hyperadrenocorticism respond to water deprivation. Psychogenic polydipsia has not been described in cats. If urine specific gravity does not reach 1.030 with 5% dehydration, the animal's response is definitively abnormal. Values between 1.030 and 1.035 are a questionable response but may indicate a degree of medullary washout or partial central or nephrogenic diabetes insipidus. Measurement of serum osmolality can confirm that dehydration has occurred by the change from the beginning to the end of the test. A 1% to 2% increase in serum osmolality induces maximal release of ADH. Urine osmolality changes are used to confirm the specific gravity measurements.

Danger Values • **Warning:** Failure to closely monitor patients may result in life-threatening hypernatremic dehydration, especially in small patients or those with

severe pu-pd as the result of hyposthenuric disorders (e.g., diabetes insipidus). Failure to perform a urinalysis, CBC, and biochemical profile before the test can lead to destabilization of a serious medical problem (e.g., azotemic renal failure, hypercalcemia, diabetes mellitus, hepatic failure).

Artifacts • Urine specific gravity may be falsely increased by contamination of urine, suggesting concentration. The accuracy of the refractometer should be confirmed by ensuring that water results in a reading of 1.000.

ANTIDIURETIC HORMONE RESPONSE TESTING

Indication • If urine is not adequately concentrated with water deprivation, ADH response testing is used to differentiate central from nephrogenic diabetes insipidus.

Analysis • The clinician can use several methods to perform this test. One approach is to use a synthetic analog of vasopressin (i.e., deamino D-arginine vasopressin [DDAVP]). The animal should be euhydrated at the time of the test and water is not withheld during testing. Ten micrograms of DDAVP are injected IV, and urine specific gravity is measured 1, 2, 4, 6, 8, 12, and 24 hours later. In another method, the nasal solution (0.1 mg/ml, 1 to 4 drops) is administered into the conjunctival sac q12hr for 5 to 7 days or oral tablets are given (0.1 mg/20 kg q8-12 hr) and the owner monitors response by measurement of water intake each day. If the owner notes a response, urine specific gravity should be measured for confirmation.

Normal Values • Exogenous administration of ADH seems less effective in stimulating a maximal response than water deprivation testing (endogenous release of ADH). Urine specific gravity greater than or equal to 1.030 or urine/plasma osmolality of greater than 3:1 (typically > 5:1) indicates renal responsiveness to ADH. Such a response in an animal that did not respond to water deprivation highly suggests complete or partial central diabetes insipidus (see Figure 7-8). Partial central diabetes insipidus and hyperadrenocorticism can be difficult to differentiate, because both can cause some response (but less than normal) to water deprivation and some responsiveness to exogenous vasopressin. Adrenal function tests may be necessary to differentiate these two conditions.

Animals not responding normally to water deprivation or ADH most likely have preazotemic renal failure or primary or secondary nephrogenic diabetes insipidus. Animals with nephrogenic diabetes insipidus usually have hyposthenuric urine before the test, whereas patients with renal failure usually have isosthenuric or mildly concentrated urine. Note that hyperadrenocorticism causes secondary nephrogenic diabetes insipidus; thus responses of such animals to water deprivation and ADH response testing can mimic the responses in other diseases causing pu-pd (see Chapter 8).

Danger Values • Warning: Unlimited access to water in a dehydrated animal to which ADH is administered could lead to water intoxication if the patient responds to ADH.

ANURIA-OLIGURIA

Anuria and oliguria necessitate aggressive diagnostic efforts because the prognosis is guarded to poor unless appropriate therapy is quickly begun. The clinician's immediate diagnostic aims are to simultaneously determine the presence of life-threatening secondary changes and the cause of the oliguria (Figure 7-9). One must first determine from history and physical examination if the patient has a urinary obstruction or rupture, severe dehydration, or any likelihood of nephrotoxins. Often passing a urethral catheter and checking for abdominal fluid can eliminate obstruction and rupture. If abdominal fluid is present, its creatinine concentration should be compared with that of the serum (see Chapter 10). Urine for urinalysis and blood for CBC and chemistry profile should be obtained (at least serum sodium, potassium, calcium, phosphorus, TCO_2 , anion gap, glucose, BUN, and creatinine). The clinician should check for hyperkalemia (chemistry analysis or electrocardiogram [ECG], although the ECG is not as sensitive or specific) and severe acidosis (blood gas preferred, but TCO_2 is useful). Severe azotemia, hyperkalemia, and acidosis should be treated as soon as they are identified. Dehydration should be corrected, but first the clinician should try to collect a urine sample to determine specific gravity.

If UTI is present, a urine culture should be obtained. If an indwelling urinary catheter is being used for patient management, antibiotic therapy for the UTI should be avoided unless the clinician finds evidence of systemic or renal infection.³

Ethylene glycol intoxication must be considered in any dog or cat with acute oliguric renal failure, regardless of "lack of exposure." Calcium oxalate crystalluria often occurs within the first 24 hours of ingestion. The elongated crystals found in association with ethylene glycol toxicity are calcium oxalate monohydrate. In early stages of intoxication, patients have severe metabolic acidosis, central nervous system (CNS) signs (i.e., ataxia, seizures), and hypocalcemia. Hyperosmolality and increased osmolal and anion gaps (see Chapter 6) suggest ethylene glycol intoxication. If ethylene glycol is even a remote possibility, a blood test for ethylene glycol should be performed (see Chapter 17). Anuria-oliguria and uremia usually do not occur for 1 to 4 days after ingestion, depending on the amount ingested. By that time, calcium oxalate crystalluria and hypocalcemia may have resolved. Hyperkalemia is not invariable. Ultrasonography may detect marked renal hyperechogenicity because of deposition of calcium oxalate crystals. Renal biopsy may be needed to confirm the diagnosis once uremia has developed.

Hypoadrenocorticism may mimic acute renal failure and hypercalcemic nephropathy. Hyponatremia, hyperkalemia, and decreased Na:K ratio that occur in hypoadrenocorticism can occur in acute renal failure, and some hypoadrenal patients do not have normal renal concentrating ability. Therefore, if serum electrolyte

concentrations suggest hypoadrenocorticism, one should begin appropriate fluid therapy and perform an ACTH stimulation test. Most patients with hypoadrenocorticism produce urine in response to IV fluids. If hypoadrenocorticism is strongly suspected, glucocorticoid therapy should commence after the ACTH test is completed. Most dogs with hypoadrenocorticism will respond rapidly to fluid and glucocorticoid support. Glucocorticoids should not be used indiscriminately in azotemic animals, because the drugs can worsen both azotemia and uremic enteritis.

Renal ischemia from any cause (including dehydration and hypoadrenocorticism) may produce oliguria. Most affected dogs and cats produce urine once they are properly rehydrated. It can be difficult to distinguish if severe, prolonged ischemia has caused significant renal parenchymal destruction. If the cause of the oliguria is uncertain and urine production remains inadequate despite appropriate fluid therapy, fractional urine sodium excretion (see Chapter 6) may help determine if renal tubular necrosis has occurred.

If oliguria persists despite appropriate IV fluid administration and cause is still uncertain, bilateral ureteral obstruction or unilateral ureteral obstruction in an animal with only one functional kidney must be considered, especially in cats due to calcium oxalate ureteroliths. Abdominal ultrasonography is useful for eliminating these possibilities. Excretory urograms are contraindicated in markedly azotemic animals because insufficient dye is excreted, and the contrast agent may worsen renal failure.

Hyperosmolar diabetes mellitus uncommonly causes acute oliguric renal failure. The blood glucose level in these patients is often greater than 1000 mg/dl.

Patients with anuric and oliguric renal failure of unknown cause not responding well to initial therapy or requiring prolonged, expensive therapy should undergo a clotting screen and a renal biopsy for diagnosis and prognosis. Biopsy is usually performed using laparoscopy, ultrasonography, or keyhole technique in dogs and percutaneously in cats.

AZOTEMIA-UREMIA

Azotemia (i.e., above-normal BUN or serum creatinine concentrations) and uremia (marked azotemia plus clinical signs such as lethargy, depression, reduced appetite, and vomiting) are caused by markedly decreased glomerular filtration. The clinician must remember three things. First, a mild increase in BUN or serum creatinine concentration signifies a substantial decrease in glomerular filtration (>75% decrease in GFR). Second, such a substantial decrease in GFR can be caused by prerenal (e.g., severe dehydration) and postrenal (e.g., urethral obstruction) causes and by renal diseases. Third, factors unrelated to GFR can also mildly affect these tests (especially BUN).

There can be significant renal disease without azotemia. A complete urinalysis may document renal disease (i.e., proteinuria, glucosuria with normoglycemia, casts, reduced concentrating ability) before azotemia occurs (e.g., aminoglycoside nephrotoxicity typically causes

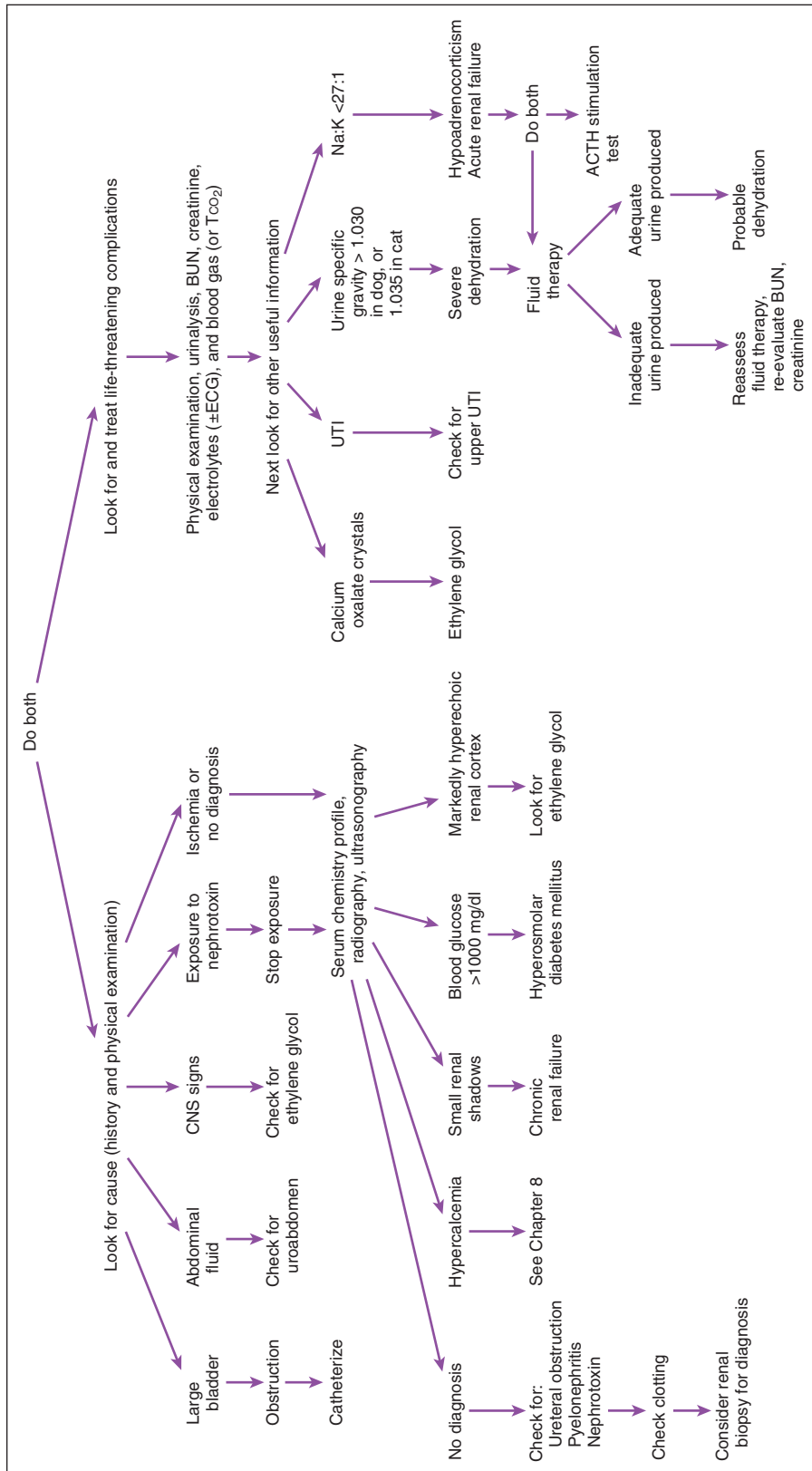


FIGURE 7-9 Diagnostic approach to anuria or oliguria in dogs and cats. ACTH, Adrenocorticotropic hormone; BUN, blood urea nitrogen; CNS, central nervous system; ECG, electrocardiogram; TCO₂, total carbon dioxide; UTI, urinary tract infection.

TABLE 7-7. DISTINGUISHING CHARACTERISTICS OF PRERENAL, RENAL, AND POSTRENAL AZOTEMIA IN DOGS AND CATS

Prerenal azotemia	Urine specific gravity > 1.030 (dogs); no definitive specific gravity for cats (see below). NOTE: Significant proteinuria with a benign sediment may be due to primary glomerular disease, in which case a concentrated urine specific gravity does not rule out primary renal disease.
Renal azotemia	Urine specific gravity 1.008–1.030 (dogs) or 1.008–1.035 (cats); some cats in early renal failure have urine specific gravity of >1.035, whereas dogs in renal failure may have urine specific gravity of 1.006–1.007. Patient may be polyuric, oliguric, or anuric.
Postrenal azotemia	Animal cannot urinate because of urethral obstruction or urine is emptying into the abdomen because of a ruptured urinary tract; ureteral or renal pelvic obstruction, either bilateral or unilateral, if there is only one functional kidney. Urine specific gravity may be any value.

isosthenuria, proteinuria, glucosuria, and cylindruria before causing azotemia). Therefore, patients treated with nephrotoxic drugs should be periodically evaluated with both urinalysis and serum creatinine.

A simultaneous urinalysis must be performed to allow accurate evaluation of serum creatinine and BUN. The first step in evaluating azotemia is to decide whether it is prerenal, renal, or postrenal (Figure 7-10 and Table 7-7); see the discussion of azotemia in the next section on Blood Urea Nitrogen.

BLOOD UREA NITROGEN

Indications • BUN can be used to screen for renal function as part of a general health profile or in any ill animal (especially those with vomiting, weight loss, chronic non-regenerative anemia, pu-pd, anuria-oliguria, chronic UTI, proteinuria, or dehydration). Serum creatinine should be measured simultaneously.

Advantages • Tests for BUN concentration are readily available and easy to use.

Disadvantages • BUN concentration is affected by extrarenal factors (sometimes this is an advantage when assessing owner compliance with recommended reduced-protein diets). In addition, concentration is inversely affected by rate of urine flow.

Analysis • BUN is measured in serum or plasma (heparin or ethylenediaminetetraacetic acid [EDTA]) by spectrophotometric, “dry reagent” reflectance meter, and ammonia-sensitive electrode methods, as well as by placing one drop of fresh whole blood on a dipstick. Different methods give comparable results, except for the

dipstick, which provides an estimate. The dipstick is reasonably accurate for determining whether azotemia is present or not in an emergency situation, but results should be confirmed.⁹

Normal Values • Dogs and cats, generally 10 to 30 mg/dL.

Danger Values • Urea itself is nontoxic; however, marked increases are associated with uremia in which acid-base, fluid, and electrolyte disorders may become life-threatening.

Artifacts • See Chapter 1.

Drug Therapy That May Alter Results • Decreased BUN may be the result of drugs causing marked pu-pd. Increased BUN may be caused by corticosteroids, arginine, and nephrotoxic drugs (see Box 7-1).

Causes of Decreased BUN • BUN is decreased by decreased production (i.e., hepatic insufficiency, dietary protein restriction) or increased excretion (i.e., polyuric conditions, overhydration, late pregnancy). Decreased BUN may be an indication for hepatic function tests (see Chapter 9).

Causes of Increased BUN • Increased BUN requires concurrent pretreatment urinalysis for proper interpretation. Serum creatinine concentration should also be measured. If the serum creatinine concentration is normal, extrarenal factors affecting BUN must be considered (Table 7-8). These extrarenal factors usually cause only mild changes unless underlying renal disease is present.

TABLE 7-8. CAUSES OF INCONGRUITIES BETWEEN BUN AND SERUM CREATININE CONCENTRATIONS

INCREASED BUN PLUS NORMAL SERUM CREATININE	INCREASED SERUM CREATININE PLUS NORMAL TO LOW BUN
Early prerenal azotemia (decreased urine flow rate)	
Increased BUN	Decreased BUN
High-protein diet	Hepatic insufficiency
Gastrointestinal hemorrhage	Polyuria-polydipsia
Tetracycline or corticosteroid administration	Low-protein diet
Fever	
Severe tissue trauma (?)	
Decreased Creatinine	Increased Creatinine
Decreased muscle mass (severe cachexia needed to cause significant changes)	Myositis/muscle trauma (unlikely)
	Cooked meat diet (mild, transient changes)
	Ketonemia (falsely increased)

BUN, Blood urea nitrogen.

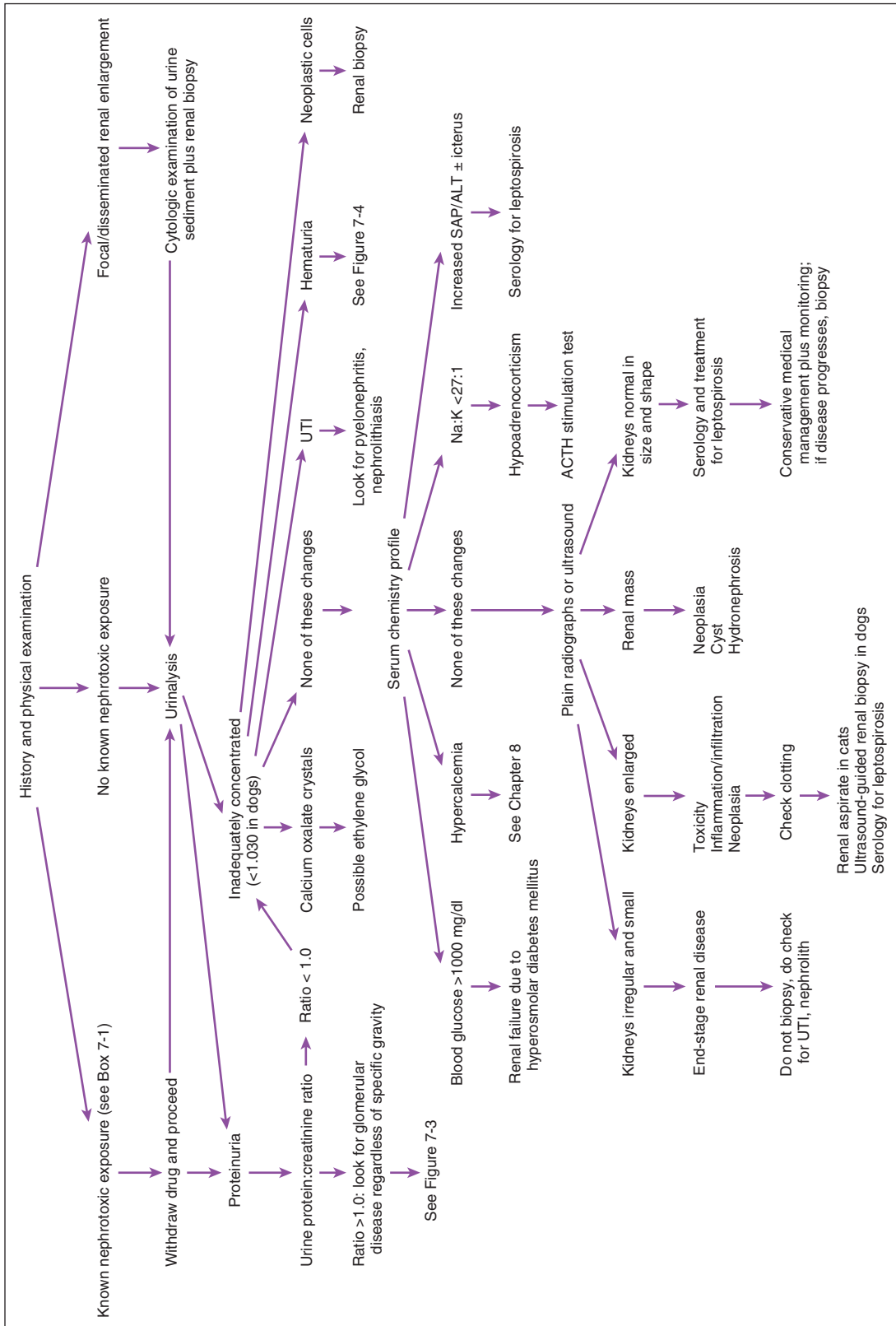


FIGURE 7-10 Diagnostic approach to nonoliguric renal azotemia in dogs and cats without blocked or ruptured urinary tracts. *ACTH*, Adrenocorticotrophic hormone; *ALT*, alanine aminotransferase; *SAP*, serum alkaline phosphatase; *UTI*, urinary tract infection.

If both BUN and creatinine are increased, decreased GFR is established. Decreased filtration, however, may be the result of prerenal causes (e.g., inadequate renal perfusion because of shock, dehydration, poor cardiac output), renal parenchymal disease, or postrenal causes (e.g., urethral or ureteral obstruction; see Table 7-7).

Prerenal azotemia is typically associated with urine specific gravities of greater than 1.030 in dogs and 1.035 in cats and similar increases in urine osmolality. However, cats with early renal disease may have azotemia and urine specific gravities of greater than 1.035. It is important to obtain pretreatment urine for analysis. If a patient is receiving fluid therapy or drugs that alter renal concentrating ability (e.g., diuretics, corticosteroids) or has another disease inhibiting renal tubular function (e.g., hypercalcemia), urine specific gravity may be inappropriately decreased, making it appear that renal azotemia is present when in fact *prerenal* disease is present. Occasionally, a clue may be the finding of hyposthenuric urine (i.e., specific gravity <1.008). The ability to dilute the glomerular filtrate indicates normal renal tubular function up to the distal tubule and collecting duct. However, some dogs with renal failure may have mildly hyposthenuric urine (i.e., 1.006 to 1.007).

A special case involves glomerular diseases. Glomerular lesions may impair glomerular filtration and cause azotemia with significant proteinuria, while urine can still be concentrated. Called *glomerulotubular imbalance*, the urine is concentrated because glomerular lesions have not yet resulted in sufficient tubular injury to impair renal concentrating ability.

Inadequately concentrated urine (urine specific gravity of 1.008 to 1.029) plus an increased BUN and serum creatinine concentration (i.e., renal azotemia) suggest primary renal disease; however, other diseases that can result in dehydration and decreased renal concentrating ability may produce similar results (Box 7-2). Hypoadrenocorticism may produce identical urine

BOX 7-2. DISEASES/CONDITIONS THAT MAY RESULT IN AZOTEMIA WITH A URINE SPECIFIC GRAVITY BETWEEN 1.008 AND 1.029

Acute or chronic renal insufficiency
E. coli septicemia/pyometra/prostatic abscessation
 Pyelonephritis
 Hypoadrenocorticism
 Hypercalcemia
 Marked hyponatremia
 Marked hypokalemia
 Ketoacidotic or hyperosmolar diabetes mellitus
 Hyperadrenocorticism with dehydration
 Diabetes insipidus with dehydration (rare)
 Hepatic failure
 Urinary tract obstruction or rupture (post-renal azotemia)
 Treatment of any prerenal cause of azotemia with fluids or diuretics

specific gravity, BUN, and serum creatinine values that are not caused by morphologic renal lesions but are reversible with proper therapy. Although many patients with renal azotemia have chronic renal disease of unknown cause, hypercalcemia, pyelonephritis, drug nephrotoxicity (e.g., aminoglycoside, amphotericin B), leptospirosis, and hyperosmolar diabetes mellitus are causes of renal azotemia that, although potentially life-threatening, may be resolved with early diagnosis and appropriate therapy. Therefore, renal azotemia is an indication for carefully reviewing history, physical examination, urinalysis, CBC, and biochemical profile (e.g., serum sodium, potassium, calcium, total protein, albumin, glucose, TCO₂ values; see Figure 7-10). Although of poor sensitivity, survey abdominal radiographs may reveal focal or diffuse renomegaly, decreased renal size, or nephroliths. Ultrasonography can also be used to evaluate the kidneys. Hyperechogenicity is common in both acute and chronic renal diseases; however, marked hyperechogenicity suggests ethylene glycol toxicity. Excretory urography may be useful in mild to moderate renal azotemia to document pyelonephritis, nephrolithiasis, ureterolithiasis, and renal size and shape; however, care must be taken to avoid exacerbating renal disease.

Blood Urea Nitrogen in Abdominal Fluids • Finding a significantly higher urea concentration in abdominal fluid than in blood suggests urinary tract rupture; however, urea readily diffuses across the peritoneal membrane. Forty-eight hours after bladder rupture, urea concentrations may be similar between abdominal fluid and serum. Therefore, measurement of fluid creatinine is preferred.

CREATININE

Indications • Measurement of creatinine concentration is indicated for the same reasons as for BUN.

Advantages • Serum creatinine concentration is not altered by as many extrarenal factors as BUN or by urine flow rate.

Analysis • Serum creatinine concentration is measured in serum or plasma (heparin) by spectrophotometric or dry reagent reflectance meter methods. These give comparable results.

Normal Values • Dogs and cats, generally less than 1.7 mg/dl, although normal values may be affected by breed.³⁰ Normal values are lower in kittens and puppies 2 to 6 months of age due to less muscle mass and higher GFR.^{12,35,36,38,46} Normal values are 5% to 10% higher in serum than in plasma.¹² Creatinine was formerly measured by the nonspecific Jaffé reaction, which measured noncreatinine chromogens as well as creatinine; the Jaffé reaction has been largely replaced by specific enzymatic techniques that produce normal values approximately 20% to 45% lower.¹²

Danger Values • Same as for BUN.

Artifacts • See Chapter 1.

Drugs That May Alter Results • Nephrotoxic drugs (see Box 7-1) and drugs that decrease GFR hemodynamically may increase serum creatinine concentration.

Causes of Decreased Serum Creatinine • Decreased serum creatinine may be the result of significant loss of muscle (see Table 7-8) or pregnancy (which increases cardiac output and subsequently GFR) or portocaval shunts in dogs.¹²

Causes of Increased Serum Creatinine • Feeding cooked meat may increase serum creatinine by less than 1 mg/dl within a few hours of eating (see Table 7-8). Acute myositis and severe muscle trauma are potential causes, but their significance is uncertain. Decreased glomerular filtration is the major cause of increased serum creatinine concentrations. As for BUN, decreased filtration may be prerenal, renal, or postrenal in origin. Urine specific gravity is essential for differentiating renal and prerenal causes, as described for BUN. An increased serum creatinine concentration is an indication for urinalysis and measurement of BUN. An increased serum creatinine concentration and BUN plus inadequately concentrated urine is an indication for a CBC, biochemical profile (e.g., serum sodium, potassium, calcium, phosphorus, total protein, albumin, glucose, TCO₂), and renal imaging (see Figure 7-10).

Creatinine Concentration in Abdominal Fluid • Abdominal fluid creatinine concentration is useful in diagnosing uroabdomen. An abdominal fluid creatinine concentration substantially greater than serum creatinine is highly suggestive of uroabdomen and an indication for a positive-contrast cystogram or excretory urography.

URINE CREATININE

Indications • Urine creatinine measurement is indicated for calculation of clearance or fractional excretion and in assessing significance of proteinuria (i.e., UPC ratio).

Analysis • Urine creatinine is measured by spectrophotometric methods.

URINE FRACTIONAL EXCRETION

Indications • Determination of urine fractional excretion is indicated to assess renal clearance of various substances (i.e., sodium, potassium, calcium, phosphorus, or albumin). Single-sample measurements ("spot" tests) often do not correlate well with 24-hour excretion.¹⁹ Clinical usefulness of spot tests is not established and likely to be limited.

Analysis • The clinician can use the following formula, in which all values are determined on simultaneous blood and urine samples:

$$\frac{\text{Urine substance}}{\text{Plasma substance}} \times \frac{\text{Plasma creatinine}}{\text{Urine creatinine}} \times 100$$

MEASUREMENT OF GLOMERULAR FILTRATION RATE

Indications • Measurement of GFR is indicated in nonazotemic patients with suspected renal disease or patients with nonazotemic or borderline azotemic renal disease (International Renal Interest Society [IRIS] stages 1 and 2¹⁶) who are in need of serial monitoring. It is important to note that patients must be hemodynamically stable and normally hydrated for meaningful GFR measurement.

Analysis • Three tests are readily available and reasonably accurate for the measurement of GFR: (1) endogenous creatinine clearance, (2) exogenous creatinine clearance, and (3) iothexol clearance.^{13,27}

Creatinine Clearance

Analysis • For endogenous clearance, a timed urine collection plus a serum sample taken approximately midway through the urine collection is required. Total volume of urine produced during the timed period is measured, and creatinine concentrations are determined on the serum and a 3-ml aliquot of pooled urine. For accurate GFR measurements, the Jaffé reaction should not be used to measure serum creatinine. An enzymatic assay specific for creatinine should be used.²² The clearance is calculated as follows:

$$\frac{\text{Urine volume (ml)} \times \text{Urine creatinine (mg/dl)}}{\text{Time (min)} \times \text{Serum creatinine (mg/dl)} \times \text{Wt (kg)}}$$

which gives a value in ml/min/kg.

For exogenous clearance, a sterile creatinine solution (50 mg/ml) is administered subcutaneously at 2 ml/kg for patients up to 20 kg and 1.5 ml/kg for those over 20 kg after an 8-hour fast. Only 10 ml should be injected per site. Time of injection is noted. A stomach tube is passed, and water equal to 3% of body weight is administered. A urinary catheter is inserted and left in place. The bladder is carefully emptied and rinsed with saline twice at 38 to 40 minutes after the subcutaneous injection. At 40 minutes after injection, a 20-minute urine collection is begun and a serum sample is obtained at the beginning and end of the urine collection. At 60 minutes, a second 20-minute collection is begun with a final blood sample collected at 80 minutes. Volume of urine for each 20-minute collection plus the three serum and two urine creatinine concentrations (from an aliquot of each 20-minute collection) are determined. GFR is calculated separately for each 20-minute collection period. The same formula is used as described for endogenous creatinine clearance, except that the serum creatinine concentration used in the calculation is the mean of the two measured serum values for each collection period.

Iothexol Clearance

The patient is fasted for 12 hours, but allowed free access to water. The patient is weighed (in kg). The test is performed by administering a single dose of iothexol at

300 mg iodine/kg IV and recording the time to the nearest minute. At 2, 3, and 4 hours after administration, a 3- to 4-ml blood sample is collected, the blood is allowed to clot, and the serum is transferred to a plastic vial (at least 1.2 ml of serum is needed). The exact sampling time within the nearest minute must be recorded. The serum samples are then shipped chilled or frozen with frozen gel packs in an insulated container to the Animal Health Diagnostic Laboratory at Michigan State University (See Appendix I). The laboratory will then report the GFR.

Normal Values • If the laboratory used reports normal ranges, the clinician should ask how those were obtained and use those, if they were appropriately determined, rather than the values listed below.

Endogenous creatinine clearance: Dogs, 2.5 to 3.5 ml/min/kg²¹; cats, 1.8 to 2.8 ml/min/kg.⁴⁵

Exogenous creatinine clearance: Dogs, 3.5 to 4.5 ml/min/kg²⁰; cats, 2.3 to 3.3 ml/min/kg.⁴⁴

Iohexol clearance: Dogs, 2.5 to 3.5 ml/min/kg¹⁰; cats, 3.2 to 6.2 ml/min/kg.⁴¹

Danger Values • None, although very low values are associated with azotemia.

Artifacts • Factors that affect measurement of creatinine affect creatinine clearance tests. Failure to collect all urine produced during the time period causes significant error in creatinine clearance tests. Measurement of noncreatinine chromogens in plasma as creatinine (e.g., Jaffé method of creatinine measurement) can falsely lower endogenous creatinine clearance values. Inaccurate recording of dose, time of administration, and actual times of sample collection yields inaccurate calculation of GFR by iohexol clearance. Hydration status affects results, so tested animals should be euhydrated. A significant negative linear relationship was found between body weight and estimated GFR using iohexol, suggesting that normal values should consider body weight.^{10,28}

Drug Therapy and Other Factors That May Alter Results • Decreased GFR may be caused by nephrotoxic drugs (see Box 7-1). State of hydration affects GFR; thus fluid therapy may affect results.

Causes of Decreased Glomerular Filtration Rate • Decreased GFR can be the result of prerenal, renal, and postrenal causes. Because of this, it is important to rule out prerenal (e.g., dehydration, decreased cardiac output) and postrenal (e.g., urinary tract obstruction, rupture) causes before measuring GFR. If these causes are excluded, renal dysfunction is the most likely cause of decreased GFR. Administration of a water load at the beginning of exogenous creatinine clearance avoids subclinical dehydration as a factor. Decreased GFR in a patient without prerenal or postrenal problems and without any evident reason for renal dysfunction on renal imaging is reason for blood pressure measurement and renal biopsy.

Causes of Increased Glomerular Filtration Rate • Not significant.

PHOSPHORUS

Serum phosphorus concentrations increase in patients with markedly decreased GFR as indicated by the presence of azotemia. In dogs with chronic renal failure, severity of hyperphosphatemia correlates with severity of azotemia.¹⁵ Evaluation of serum phosphorus is discussed in Chapter 8.

URINE CULTURE

Indications • Culture of urine is indicated whenever bacteria are suspected based on urinalysis (i.e., bacteriuria, pyuria, proteinuria, and/or hematuria) or when an identified disease process is one potentially associated with UTIs.³ These include hyperadrenocorticism, diabetes mellitus, hyperthyroidism, chronic renal failure, urolithiasis, and other structural or functional urinary tract abnormalities (e.g., ectopic ureters; incontinence; presence of tubes, catheters, or stents). UTIs are also common in animals receiving immunosuppressive therapy, especially with glucocorticoid therapy.

Advantages • Isolation and identification of the organism causing UTI helps differentiate relapses from reinfections. Antimicrobial sensitivity testing allows more rational therapy.

Disadvantages • Depending on the laboratory, urine culture and sensitivity testing can be relatively expensive. Quantitative as well as qualitative cultures are required on samples collected by catheterization. In addition, voided samples are often contaminated by organisms distal to the bladder, making interpretation difficult; and bacterial numbers in urine change significantly upon storage, necessitating culture within a relatively short time frame. Sensitivity testing based on expected serum concentrations of antimicrobials do not reflect efficacy to urine concentrations of antimicrobials.

Contraindications • Coagulopathies and the possibility of pyometra are contraindications for cystocentesis. There are no contraindications for culture of urine.

Analysis • Any bacteria isolated from a properly collected cystocentesis sample indicates UTI. In samples collected by catheterization, greater than 10⁴ bacteria/ml in male dogs and greater than 10³ bacteria/ml in male and female cats indicate infection. After collection, urine should be refrigerated and cultured within 6 hours unless placed in tubes with preservatives. Before culture, urine should not be incubated, kept at room temperature, or frozen. If a practice cannot deliver urine samples to a laboratory within this time frame, the practice can inoculate blood agar plates with a calibrated loop and incubate them at 37° C for 24 hours. If bacteria grow, the plates can be sent to a commercial laboratory for species identification and antimicrobial sensitivity testing.

Normal Values • Urine collected from the bladder should be sterile. Note that contamination from the lower tract can occur with other methods of urine collection.

Danger Values • None.

Artifacts • If urine is stored at room temperatures, bacterial numbers will increase after 2 hours. With storage at room or refrigeration temperatures longer than 6 hours, bacterial numbers can increase or decrease and bacteria can die, leading to a falsely negative culture. Contamination of a sample during or after collection can lead to a falsely positive sample.

URINE DRUG SCREENING

Canine urine samples can be reliably screened for barbiturates, opiates, benzodiazepines, and amphetamines/methamphetamines using an on-site test designed for humans that is rapid, available, and affordable.⁵² Such a test may be useful in an emergency room setting where cases with signs possibly related to such drugs are seen. The test did not detect marijuana or methadone.

URINE INFECTIOUS DISEASE TESTING

Antigen Testing for Blastomycosis

Antigen testing of urine for blastomycosis is a very accurate diagnostic method, more accurate than using serum, and urine antigen testing correlated better with clinical remission than serum testing.²³ See Chapter 15.

CALCULI

Urinary calculi may cause urethral obstruction (e.g., anuria and oliguria, azotemia, uremia), cystitis and urethritis (e.g., dysuria, hematuria), ureteral or renal pelvic obstruction (e.g., azotemia, uremia), and destruction of renal tissue (e.g., azotemia, uremia). Calculi should be considered in any patient with urinary obstruction, persistent or recurrent UTI, hematuria, or renal failure of unknown cause. Uroliths are diagnosed by physical examination (i.e., bladder or urethral palpation, urinary catheterization), survey or contrast radiographs (some calculi are radiolucent), or ultrasonography. All calculi removed or spontaneously passed should be analyzed quantitatively, and urine should be cultured. Many canine uroliths are struvite and form secondary to urine alkalization by urease-producing bacteria (principally *Staphylococcus* or *Proteus* spp.), whereas other types cause UTI secondary to tissue injury. Therefore, a diagnosis of UTI and urolithiasis does not mean an infection has caused a struvite calculus unless the urine is alkaline and a urease-producing organism is cultured. Even in this situation, calculus identity is only an educated guess. If therapy does not proceed as expected, calculi should be retrieved and analyzed. Accurate determination of the crystalloid composition of calculi is essential for prevention and appropriate medical dissolution.

Calculi Analysis

Indications • Urinary calculi obtained from a cat or dog by any method (routine voiding, urinary hydropropulsion, catheter-assisted retrieval, cystoscopy, or surgery) should be analyzed for mineral content. If a urine culture is negative, bacterial culture of the urolith and a bladder mucosal sample are also recommended,^{24,26} especially in dogs in which struvite uroliths are suspected. The entire urolith(s) should be submitted in a dry, clean, unbreakable container. Refrigeration is not required.

Advantages • Calculi analysis determines the type of calculi, which allows appropriate approaches to prevention of recurrence. Culture of calculi permits recognition of the role of infection in calculus formation when urine culture is negative.

Disadvantage • The clinician needs to send calculi to laboratories equipped for proper analysis.

Analysis • Calculi are most commonly analyzed by optic crystallography, x-ray diffraction, and chemical analysis (e.g., the spot test). Less commonly used methods include scanning electron microscopy, electron microprobe, and infrared spectroscopy. Crystallography and diffraction give accurate results, but chemical analysis does not.¹¹ Chemical analysis should *not* be used.

Artifacts • Chemical or qualitative analysis (e.g., the spot test) is fraught with inaccuracies.¹¹

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8

Endocrine, Metabolic, and Lipid Disorders

Richard W. Nelson

CALCIUM

Commonly Indicated • Common indications for serum calcium concentration include patients with lethargy, anorexia, vomiting, constipation, weakness, polydipsia, polyuria (i.e., signs of hypercalcemia), facial pruritus, restlessness, muscle tremors, fasciculations, cramping of rear legs, tetany, or seizures (i.e., signs of hypocalcemia). Other indications include azotemia, diffuse bone disease, and selected abnormalities on an electrocardiogram (ECG), such as prolonged QT interval with a normal QRS complex or unexplained premature ventricular contractions.

Analysis • Calcium can be measured in serum (preferred), heparinized plasma, and urine by photometric assays that use colorimetric reactions or potentiometry using ion-specific calcium electrodes. Oxalate, citrate, and ethylenediaminetetraacetic acid (EDTA) anticoagulants should not be used, because calcium is bound to these chemicals and becomes unavailable for analysis. Most automated and in-house serum chemistry analyzers measure total serum calcium concentration, which consists of biologically active, ionized calcium (55%), protein-bound calcium (35%), and calcium complexes (10%). In dogs, alterations in the plasma protein concentration may alter the total serum calcium concentration, yet the ionized calcium levels remain normal. Simple quantitative changes in the albumin and total plasma proteins do not cause hypocalcemia or hypercalcemia in dogs, even though the total serum calcium levels may “appear” low or high on the biochemistry panel. The following formulas can be used to determine the corrected total serum calcium concentration²²:

$$\begin{aligned}\text{Adjusted calcium (mg/dl)} &= \text{measured calcium (mg/dl)} - \text{albumin (g/dl)} + 3.5 \\ \text{Adjusted calcium (mg/dl)} &= \text{measured calcium (mg/dl)} \\ &\quad - [0.4 \times \text{serum protein (g/dl)}] + 3.3\end{aligned}$$

The formula based on albumin is preferred because of the stronger relationship between serum albumin and total calcium concentrations. The formulas should not be

used in dogs less than 24 weeks of age because high values may be obtained, nor are they used in cats because there is no linear relationship between serum total calcium and serum albumin and total protein concentrations in cats.¹¹ These formulas yield a rough estimate of the corrected total serum calcium concentration and were developed without verification by serum ionized calcium measurements. Unfortunately, the correlation between “corrected” serum calcium concentration and ionized calcium concentration is weak,²⁴ suggesting that corrected total serum calcium concentrations may not be reliable indicators of calcium homeostasis.

The biologically active, ionized fraction of calcium can be determined directly, thus bypassing the influence of plasma proteins on the total serum calcium concentration. Ionized calcium measurements are generally superior to serum total calcium measurements for assessing calcium in dogs and cats. Automated equipment that uses a calcium ion-selective electrode allows accurate measurement of ionized calcium in blood, plasma, or serum. Ionized calcium results can be affected by many variables, including method of sample collection (samples collected anaerobically provide more precise results); the amount and type of heparin, if used (may underestimate or overestimate ionized calcium results); and change in sample pH (ionized calcium increases as pH decreases). Protocols established by the clinical chemistry laboratory for submitting blood samples for ionized calcium determination should be followed to ensure accurate results.

Normal Values for Total and Ionized Serum Calcium • Adults:

Total Ca, 9.0 to 11.5 mg/dl
Ionized Ca, 1.12 to 1.42 mmol/L

NOTE: An estimate of total Ca = ionized Ca × 8. To convert from mg/dl to mmol/L, multiply by 0.25.

Immature: The serum total and ionized calcium concentration can be as much as 1 mg/dl and 0.1 mmol/L higher in young dogs (i.e., <12 months old), especially

in the large and giant breeds, than in adults. The serum total calcium concentration does not fluctuate with age in cats, but the serum ionized calcium concentration may be as much as 0.1 mmol/L higher in cats less than 2 years of age, compared with results in older cats.

Danger Values • Total serum Ca less than 7.0 mg/dl (tetany).

NOTE: This value depends on the blood pH. The lower the blood pH (i.e., the more acidemic), the lower the calcium can be without causing clinical signs and vice versa.

Total serum Ca greater than 16 mg/dl (depending on serum albumin concentration) can result in acute renal failure (especially in the presence of hyperphosphatemia) and cardiac toxicity.

Artifacts • Serum Ca may be falsely decreased by increased bilirubin concentrations or laboratory error. Serum Ca may be falsely increased by laboratory error, dehydration (mild increase), and lipemia (see Chapter 1).

Drug Therapy That May Alter Serum Calcium Concentration • Mithramycin, EDTA, glucagon, anti-convulsants, citrate, fluoride, glucocorticoids, phosphate-containing enemas, and intravenous (IV) phosphate administration (i.e., potassium phosphate) may cause hypocalcemia.

Vitamin D, cholecalciferol rodenticides, estrogen, progesterone, testosterone, anabolic steroids, acetaminophen, hydralazine, parenteral calcium administration, and excess oral phosphate binders may cause hypercalcemia.

Causes of Hypercalcemia • In dogs, nonparathyroid malignancy (i.e., humoral hypercalcemia of malignancy [HHM]), most notably lymphosarcoma, is the most common cause of hypercalcemia (Box 8-1). Other hemolymphatic malignant tumors (i.e., lymphocytic leukemia, multiple myeloma, myeloproliferative diseases), anal sac apocrine gland carcinoma, and soft tissue tumors metastasizing to bone (e.g., mammary gland adenocarcinoma) may also cause hypercalcemia. Less frequent causes include primary hyperparathyroidism, chronic renal failure, hypoadrenocorticism, and hypervitaminosis D (i.e., cholecalciferol rodenticide toxicity, ingestion of toxic plants containing glycosides of calcitriol). In the cat, idiopathic hypercalcemia, hypercalcemia of malignancy (especially lymphoma and squamous cell carcinoma), chronic renal failure, and primary hyperparathyroidism are the most common diagnoses. Calcium oxalate urolithiasis and consumption of acidifying diets are commonly identified in cats with hypercalcemia; however, their role, if any, in causing hypercalcemia is unknown.

Hypercalcemia should always be reconfirmed, preferably from a nonlipemic blood sample obtained from the dog or cat after a 12-hour fast, before embarking on an extensive diagnostic evaluation. Results of a complete blood count (CBC), serum biochemistry panel, and urinalysis, in conjunction with the history and

BOX 8-1. CAUSES OF HYPERCALCEMIA IN DOGS AND CATS

- Hypercalcemia of malignancy (*very common*)
 - Humoral hypercalcemia
 - Lymphoma
 - Apocrine gland adenocarcinoma
 - Carcinoma (squamous cell, mammary, bronchogenic, prostate, thyroid, nasal cavity)
 - Local osteolysis
 - Lymphoma
 - Multiple myeloma
 - Squamous cell carcinoma
 - Osteosarcoma
 - Fibrosarcoma
- Primary hyperparathyroidism (*uncommon but important*)
- Hypoadrenocorticism (*uncommon but important*)
- Chronic and acute renal failure (*common*)
- Hypervitaminosis D (*uncommon but important*)
 - Excess supplementation
 - Plants containing glycosides of calcitriol (e.g., jasmine)
 - Cholecalciferol rodenticides
- Idiopathic hypercalcemia of cats (*common*)
- Granulomatous disease
 - Systemic mycosis
 - Feline infectious peritonitis
- Nonmalignant skeletal lesions
 - Osteomyelitis
 - Hypertrophic osteodystrophy
- Iatrogenic disorders
 - Excessive calcium supplementation
 - Excessive oral phosphate buffers
 - Doronex ointment (calcipotriene)
- Dehydration
- Factitious
 - Lipemia
 - Postprandial measurement
- Young dog (<6 months), large or giant breed
- Laboratory error

physical examination findings often provide clues to the diagnosis (Figure 8-1). Special attention should be paid to the serum electrolytes and renal parameters. Hypoadrenocorticism-induced hypercalcemia occurs in conjunction with mineralocorticoid deficiency; hyponatremia, hyperkalemia, and prerenal azotemia should usually be present. The serum phosphorus concentration is in the lower half of the normal range or low with HHM and primary hyperparathyroidism (Figure 8-2). If the serum phosphorus concentration is increased and renal function is normal, hypervitaminosis D or bone osteolysis from metastatic or primary bone neoplasia are the primary differentials. Measurement of serum ionized calcium concentration may help identify dogs and cats with renal failure-induced hypercalcemia; serum ionized calcium concentrations are typically normal or decreased

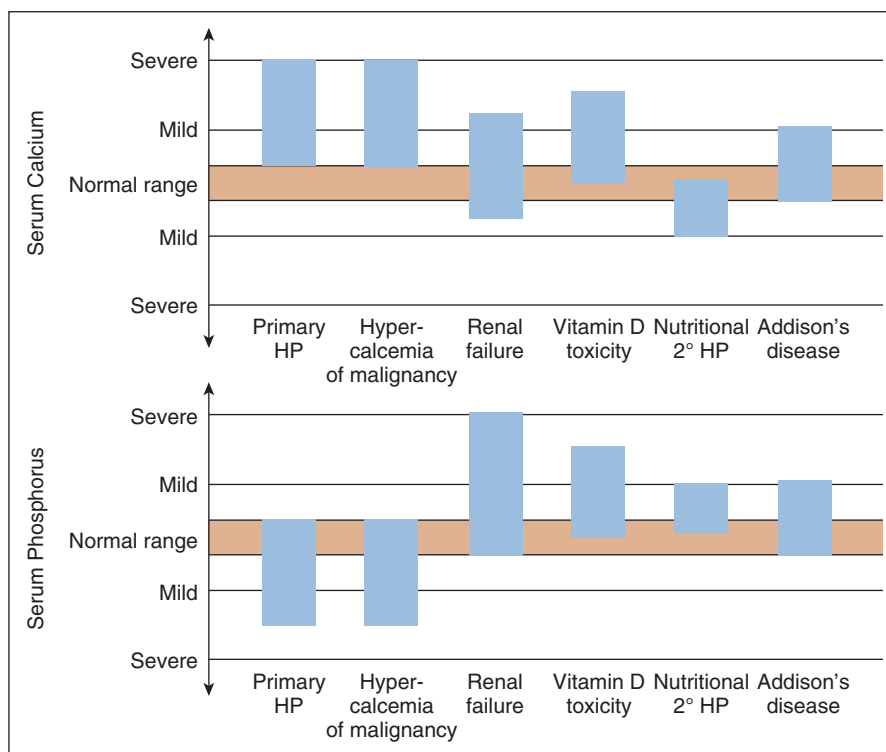


FIGURE 8-2 The range of serum calcium and phosphorus concentrations for the more common causes of hypercalcemia and for hyperparathyroidism in dogs. HP, Hyperparathyroidism; 2°, secondary. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, ed 2, Philadelphia, 1996, WB Saunders.)

in renal failure and increased in hypercalcemia caused by other disorders.

Hypercalcemia of malignancy and primary hyperparathyroidism are the primary differentials when hypercalcemia and normal-to-low serum phosphorus concentrations are identified. The most common malignancy is lymphoma. A careful review of the history and physical examination findings may provide clues to the diagnosis. Systemic signs of illness suggest HHM. Dogs and cats with primary hyperparathyroidism are usually healthy, and clinical signs are mild. The appendicular skeleton, peripheral lymph nodes, abdominal cavity, and rectum should be carefully palpated for masses, lymphadenopathy, hepatomegaly, splenomegaly, or pain on digital palpation of the long bones. Diagnostic tests that are helpful in identifying the underlying malignancy include thoracic and abdominal radiographs; abdominal ultrasound; cytologic evaluation of aspirates of the liver, spleen, lymph nodes, and bone marrow; determination of serum ionized calcium, parathyroid hormone (PTH), and parathyroid hormone-related peptide (PTHrP) concentrations; and cervical ultrasound.

Sternal and hilar lymphadenopathy is common with lymphoma-induced hypercalcemia and can be readily identified with thoracic radiographs. Radiographs of the thorax and abdomen can also be used to evaluate bones; discrete lytic lesions in the vertebrae or long bones suggest multiple myeloma. Hyperproteinemia, proteinuria, and plasma cell infiltration in the bone marrow

suggest multiple myeloma (see also Ehrlichia, Chapter 15). Cytologic evaluation of peripheral lymph node, bone marrow, and splenic aspirates can be helpful in identifying lymphoma; involvement of the peripheral lymph nodes or spleen by lymphoma can be present without causing their enlargement. Ideally the largest lymph node should be evaluated. Normal lymph node, bone marrow, and splenic aspirates do not rule out lymphoma.

Measurement of serum ionized calcium, PTH, and PTHrP from the same blood sample is helpful in differentiating primary hyperparathyroidism from HHM (Figure 8-3). Excessive secretion of biologically active PTHrP plays a central role in the pathogenesis of hypercalcemia in most forms of HHM. Increased serum ionized calcium concentration, detectable serum PTHrP concentration, and nondetectable serum PTH concentration is diagnostic for HHM. Lymphoma is the most common cause for detectable PTHrP concentrations, but other tumors, including apocrine gland adenocarcinoma and various carcinomas (e.g., mammary gland, squamous cell, bronchogenic), can also cause hypercalcemia by this mechanism. In contrast, increased serum ionized calcium, normal-to-increased serum PTH, and nondetectable PTHrP concentrations are diagnostic of primary hyperparathyroidism. Ultrasonographic examination of the thyro-parathyroid complex may reveal enlargement of one or more parathyroid glands. Most parathyroid adenomas measure 4 to 8 mm in diameter, although some can exceed 2 cm.¹⁰ In contrast, the parathyroid glands

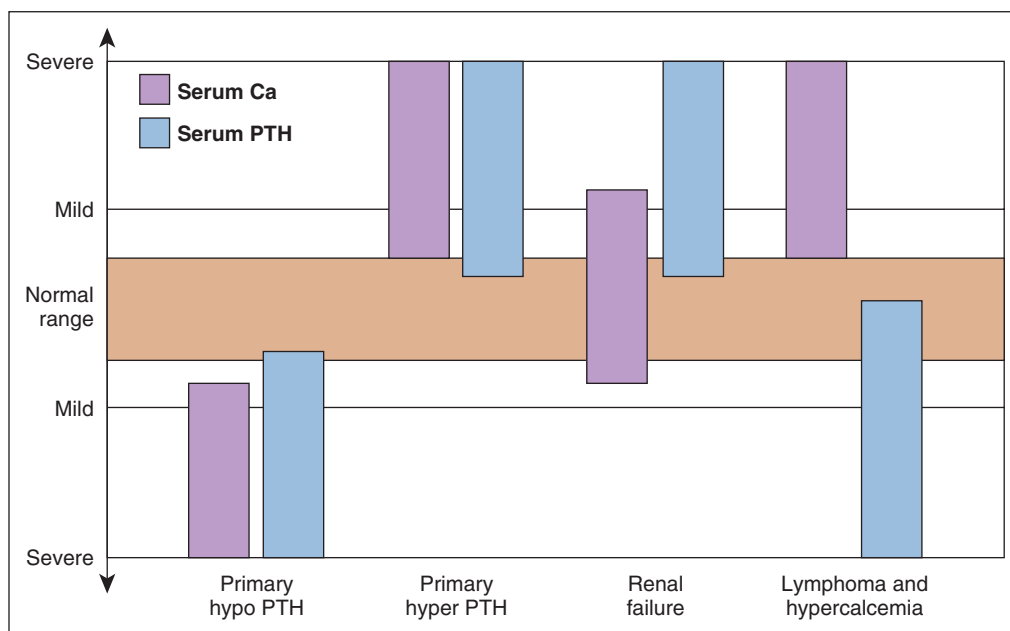


FIGURE 8-3 Range of serum calcium (Ca) and parathyroid hormone (PTH) concentrations for the more common disorders causing alterations in serum calcium or parathyroid gland function. (From Nelson RW, Couto CG: *Essentials of small animal internal medicine*, St. Louis, 1992, Mosby.)

will be small (<2 mm in diameter) or undetectable with HHM. Evaluation of the change in serum calcium concentration after L-asparaginase administration should be considered for the patient with hypercalcemia of undetermined cause to rule out occult lymphoma. A marked reduction in serum calcium within 48 hours, usually into the normal range, is strongly suggestive of occult lymphoma.

Idiopathic hypercalcemia is an increasingly common diagnosis in young to middle-aged cats.²³ Hypercalcemia is usually mild (<13 mg/dl) and asymptomatic. Serum phosphorus concentration and renal parameters are normal. The cause is unknown. Results of a complete diagnostic evaluation as described previously are unremarkable. Serum PTH concentrations are in the normal range or low; primary hyperparathyroidism has not been confirmed in any of these cats. Excessive serum PTHrP, 25-hydroxyvitamin D, or calcitriol concentrations have not been identified. Nephrocalcinosis and urolithiasis may develop, presumably secondary to increased urinary calcium excretion. Effective treatment has not been identified, primarily because the pathogenesis of this problem remains unknown. Serum calcium concentrations have decreased in some cats following a dietary change to a high-fiber diet, a diet designed for renal failure, or a diet designed to prevent calcium oxalate urolithiasis, or after prednisone treatment (initial dose, 5 mg q24hr) was initiated, but the response has been unpredictable and often short lived.

Causes of Hypocalcemia • The most common causes of hypocalcemia in dogs and cats are puerperal tetany, acute and chronic renal failure, malassimilation

BOX 8-2. CAUSES OF HYPOCALCEMIA IN DOGS AND CATS

Primary hypoparathyroidism (*uncommon but important*)
 Naturally acquired
 Post-thyroidectomy (bilateral)
 Puerperal tetany (eclampsia) (*uncommon but important*)
 Acute and chronic renal failure (*more common in acute*)
 Ethylene glycol toxicity
 Critical illness (e.g., systemic inflammatory response syndrome, sepsis)
 Acute pancreatitis (*rare*)
 Intestinal malabsorption syndromes
 Hypoproteinemia or hypoalbuminemia (*common*)
 Hypomagnesemia
 Rhabdomyolysis (*rare*)
 Tumor lysis syndrome (*rare*)
 Nutritional secondary hyperparathyroidism (*rare*)
 Hypovitaminosis D (*rare*)
 Phosphate-containing enemas
 Anticonvulsant medications
 Sodium bicarbonate administration
 Laboratory error

syndromes, and primary hypoparathyroidism (especially after thyroidectomy in hyperthyroid cats (Box 8-2).

The serum total calcium concentration is typically decreased in animals with concurrent hypoalbuminemia for reasons discussed in the Analysis section on calcium.

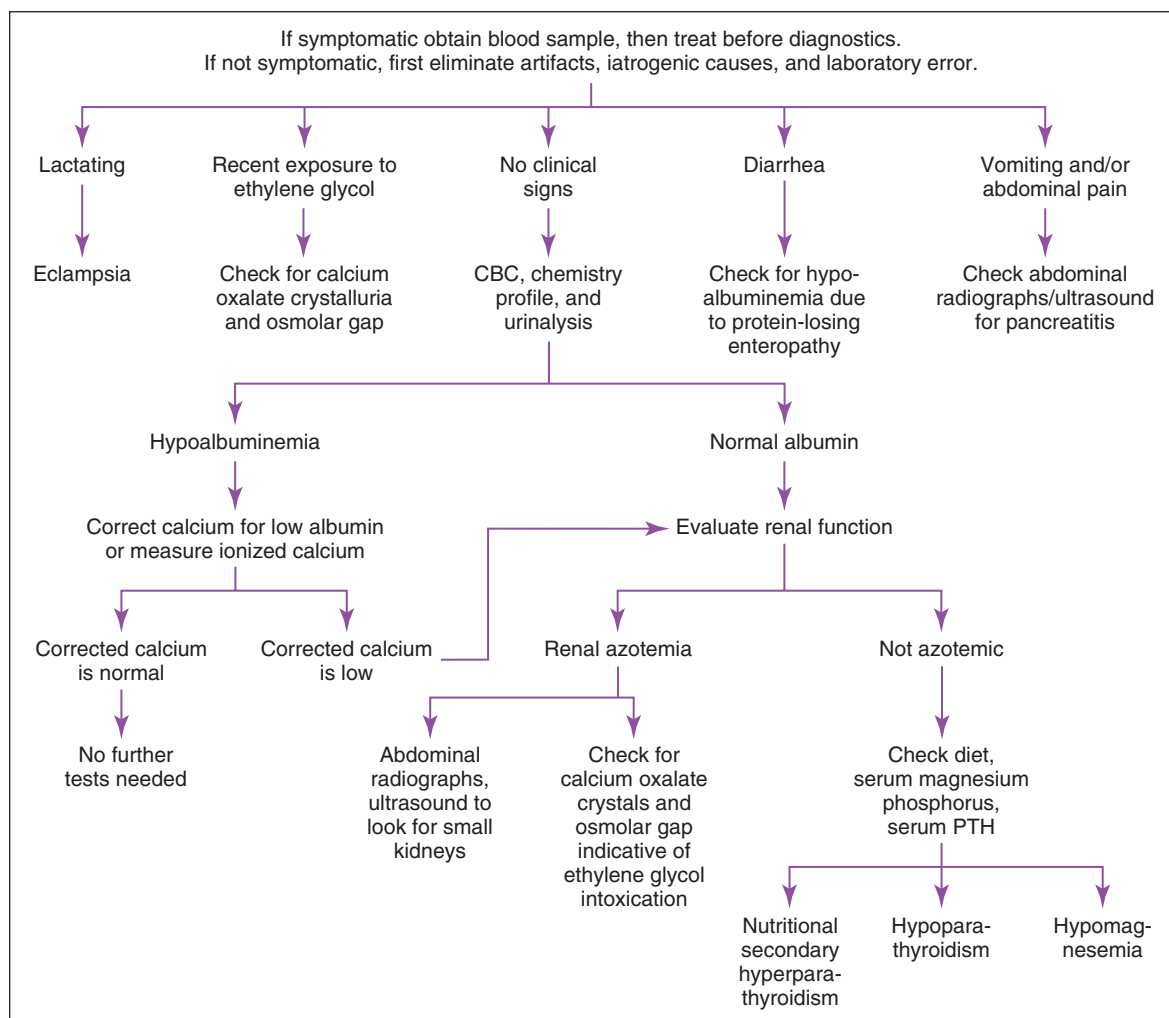


FIGURE 8-4 Diagnostic evaluation of hypocalcemia in dogs and cats. CBC, Complete blood count; PTH, parathyroid hormone.

Depending on the underlying etiology, the serum ionized calcium concentration may or may not be decreased. Measurement of serum ionized calcium should be done before rendering a diagnosis of hypocalcemia in an animal with decreased serum total calcium and albumin concentrations.

Hypocalcemia should be confirmed before initiating diagnostic tests to identify the cause. The list of differential diagnoses for hypocalcemia is relatively short, and the history, physical examination findings, CBC, serum biochemistry panel, and urinalysis usually provide the clues necessary to establish the diagnosis (Figure 8-4). Measurement of serum pancreatic lipase immunoreactivity and evaluation of an abdominal ultrasound should be done if pancreatitis is suspected (see Chapter 9). Primary hypoparathyroidism is the most likely diagnosis in the nonazotemic, nonlactating dog or cat with clinical signs of hypocalcemia. Documentation of a low baseline serum PTH concentration confirms this diagnosis (see Figure 8-3).

PHOSPHORUS

Commonly Indicated • Measurement of serum phosphorus concentration is commonly indicated in patients with any indications mentioned for calcium, plus unexplained hemolysis, anorexia, weakness, ataxia, or seizures.

Analysis • Phosphorus can be measured in serum (preferred), heparinized plasma, and urine as inorganic phosphorus by photometric assays that use colorimetric reactions.

Normal Values • Adult: 3.0 to 6.0 mg/dl.

Immature: Young dogs (i.e., <12 months) especially of the large and giant breeds, and young cats (i.e., <6 months) have higher serum phosphorus concentrations (dog, 4 to 9 mg/dl; cat, 4 to 8 mg/dl) than do adults. Serum phosphorus concentration should decrease to adult values by 12 months of age.

To convert from mg/dl to mmol/L, multiply by 0.323.

BOX 8-3. CAUSES OF ALTERED SERUM PHOSPHORUS IN DOGS AND CATS**HYPERPHOSPHATEMIA**

Young growing animal (*common*)
 Renal failure (*common*)
 Prerenal and postrenal azotemia (*common*)
 Endocrine
 Primary hypoparathyroidism
 Nutritional secondary hyperparathyroidism
 Hyperthyroidism (cats)
 Acromegaly
 Hyperadrenocorticism
 Hypervitaminosis D
 Excess supplementation
 Cholecalciferol rodenticides
 Jasmine ingestion
 Osteolytic bone lesions (neoplasia)
 Rhabdomyolysis (*rare*)
 Trauma
 Necrosis
 Tumor cell lysis syndrome (*rare*)
 Metabolic acidosis
 Hemolysis
 Drugs—see text
 Iatrogenic
 Intravenous phosphorus supplementation
 Phosphate-containing enemas
 Diuretics
 Laboratory error

HYPOPHOSPHATEMIA

Decreased intestinal absorption
 Decreased dietary intake
 Malabsorption, steatorrhea
 Vomiting, diarrhea
 Phosphate-binding antacids
 Hypovitaminosis D
 Increased urinary excretion
 Primary hyperparathyroidism (*uncommon but important*)
 Humoral hypercalcemia of malignancy (*important*)
 Diabetic ketoacidosis
 Eclampsia
 Fanconi's syndrome
 Diuretics
 Transcellular shifts
 Hypothermia recovery
 Aggressive parenteral fluid therapy
 Insulin administration, esp. for DKA (*common and important*)
 Sodium bicarbonate administration
 Parenteral glucose administration
 Hyperalimentation
 Respiratory, metabolic acidosis
 Laboratory error

Danger Values • Less than 1.5 mg/dl (hemolysis, neurologic signs).

Artifacts • Serum phosphorus may be falsely increased: by postprandial protein intake (mild change), hemolysis, hyperlipidemia, hyperproteinemia, and thrombocytosis. The effect is dependent on the methodology used to measure phosphorus. Serum phosphorus may be falsely decreased: by postprandial carbohydrate intake (mild change; see Chapter 1).

Drug Treatments That May Alter Phosphorus Values •

Phosphate-binding antacids, anesthetic agents, anticonvulsants, bicarbonate, diuretics, insulin, parenteral glucose administration, hyperalimentation, mithramycin, and salicylates may cause hypophosphatemia. Hyperphosphatemia may be caused by phosphate-containing enemas in cats or obstipated small dogs, IV potassium phosphate supplementation, anabolic steroids, vitamin D supplements, furosemide, hydrochlorothiazide, and minocycline. Tetracyclines have a variable effect on phosphorus.

Causes of Hyperphosphatemia • Hyperphosphatemia can result from increased intestinal phosphate absorption, decreased phosphate excretion in urine, or a shift in

phosphate from the intracellular to the extracellular compartment. Translocation of phosphate between the intracellular and extracellular compartments is similar to that of potassium. The most common cause for hyperphosphatemia is decreased renal excretion secondary to renal failure (Box 8-3). History, physical examination, and routine clinical pathologic assessment (e.g., CBC, serum biochemical panel, urinalysis) usually enable the clinician to identify the cause. Azotemic patients may require additional tests to distinguish between prerenal, renal, and postrenal azotemia (see Chapter 7). (Evaluation for primary hypoparathyroidism is discussed under [Causes of Hypocalcemia](#) earlier in this chapter.) Serum thyroxine concentration should be determined in the nonazotemic cat with signs of hyperthyroidism (i.e., weight loss, polyphagia, restlessness). Survey skeletal radiographs may identify osseous neoplasia.

Causes of Hypophosphatemia • Hypophosphatemia results from decreased phosphate absorption in the intestinal tract, increased urinary phosphate excretion, or a shift from the extracellular to the intracellular compartment. Hypophosphatemia is commonly associated with HHM (i.e., lymphosarcoma), primary hyperparathyroidism, and aggressive therapy for diabetic ketoacidosis (DKA; see Box 8-3).¹⁰ Translocation of phosphate between

the intracellular and extracellular compartments is similar to that of potassium. Factors that promote a shift of potassium into the intracellular compartment (e.g., alkalosis, insulin, glucose infusion) also promote a similar shift in phosphate.

When evaluating hypophosphatemia, one should eliminate artifacts and iatrogenic causes first. Mild hypophosphatemia (i.e., >2.0 mg/dl) without hypercalcemia is often ignored unless the animal is ketoacidotic (see [Causes of Hyperglycemia](#) later in this chapter). If concurrent hypercalcemia is present, diagnostic evaluation for HHM and primary hyperparathyroidism should be performed (see [Causes of Hypercalcemia](#) earlier in this chapter).

MAGNESIUM

Occasionally Indicated • Serum magnesium should be measured in dogs and cats with disorders and predisposing factors associated with hypomagnesemia and hypermagnesemia ([Box 8-4](#)), especially those with unexplained hypocalcemia (hypomagnesemia may inhibit the secretion and actions of PTH and promote calcium uptake into bone); hypokalemia resistant to parenteral supplementation (hypomagnesemia may cause potassium-losing nephropathy); DKA (hypomagnesemia may develop during the initial 24 hours of therapy); cardiac arrhythmias refractory to conventional therapy; and unexplained muscle weakness (including dysphagia and dyspnea), muscle fasciculations, or seizures.

Analysis • Magnesium can be measured in serum (preferred), heparinized plasma, or urine by photometric assays that use colorimetric reactions. Measurement of serum ionized magnesium concentration via an ion-selective electrode more accurately assesses total body magnesium.

Normal Values • 1.5 to 2.5 mg/dl; ionized magnesium, 0.4 to 0.6 mmol/L.

Danger Values • Development of clinical signs of hypomagnesemia varies widely, but treatment is probably indicated if serum magnesium concentration is less than 1.0 mg/dl. Serum magnesium concentrations greater than 10 mg/dl are associated with respiratory depression, apnea, coma, and cardiac arrest in people.

Artifacts • See Chapter 1.

Drug Therapy That May Alter Serum Magnesium Concentration • Drug-induced renal tubular injury (e.g., cisplatin, aminoglycosides, amphotericin B), diuretics (e.g., furosemide, thiazides), digitalis, insulin, glucose, amino acids, massive blood transfusion, and total parenteral nutrition solutions may cause hypomagnesemia. Magnesium-containing drugs (especially oral antacids and laxatives), chronic aspirin therapy, lithium, and progestagens may cause hypermagnesemia.

Causes of Hypomagnesemia • Hypomagnesemia results from decreased oral intake or gastrointestinal

BOX 8-4. CAUSES OF ALTERED SERUM MAGNESIUM IN DOGS AND CATS

HYPOMAGNESEMIA

Gastrointestinal Causes (*common*)

- Inadequate intake
- Chronic diarrhea and vomiting
- Malabsorption syndromes
- Acute pancreatitis
- Cholestatic liver disease
- Nasogastric suction

Renal Causes

- Glomerulonephritis
- Acute tubular necrosis
- Postobstructive diuresis
- Drug-induced tubular injury (e.g., aminoglycosides, cisplatin)
- Prolonged intravenous fluid therapy
- Diuretics
- Digitalis administration
- Hypercalcemia
- Hypokalemia

Endocrine Causes

- Diabetic ketoacidosis (*common*)
- Hyperthyroidism
- Primary hyperparathyroidism
- Primary hyperaldosteronism

Miscellaneous Causes

- Acute administration of insulin, glucose, amino acids
- Sepsis
- Hypothermia
- Massive blood transfusion
- Peritoneal dialysis, hemodialysis
- Total parenteral nutrition

HYPERMAGNESEMIA

Renal insufficiency, failure (*common*)

- Excessive oral intake (e.g., antacids, laxatives)
- Excessive parenteral administration (e.g., Mg^{2+} -containing fluids)

absorption of magnesium (e.g., small intestinal disease causing malabsorption), increased gastrointestinal loss (e.g., protracted vomiting, diarrhea), increased urinary magnesium excretion (e.g., interstitial nephritis, diuretics), or a shift of the cation from the extracellular to the intracellular compartment. The most common causes of clinically significant hypomagnesemia include disorders causing small intestinal malabsorption, renal disorders with high urine output, osmotic diuresis of DKA, and shift of potassium, phosphate, and magnesium from the extracellular to the intracellular compartment occurring within the first 24 hours of therapy for DKA (see [Box 8-4](#)). Magnesium is predominately an intracellular cation. Translocation of magnesium between the intracellular and extracellular compartments is similar to that of potassium. Factors that promote a shift of potassium into the

intracellular compartment (e.g., alkalosis, insulin, glucose infusion) also promote a similar shift of magnesium. During therapy for DKA, serum magnesium concentration can severely decline (i.e., <1 mg/dl) because of the dilutional effects of fluid therapy and intracellular shift of magnesium after initiation of insulin and bicarbonate therapy.

Identifying hypomagnesemia is problematic because no simple, rapid, and accurate laboratory test is available to identify total body magnesium status. Serum total magnesium represents 1% of the body's magnesium stores, and serum ionized magnesium represents 0.2% to 0.3% of total body magnesium stores. As a result, serum total and ionized magnesium concentrations do not always reflect total body magnesium status. A normal serum magnesium concentration can occur despite total body magnesium deficiency. A low serum magnesium concentration, however, supports a total body magnesium deficiency, especially when clinical signs or concurrent electrolyte abnormalities are consistent with hypomagnesemia. A serum-ionized magnesium concentration determined using an ion-selective electrode more accurately assesses total body magnesium content than measurement of serum total magnesium and is recommended.²⁷ In animals with low serum magnesium concentration, a review of history, physical examination, and routine clinical pathologic assessment (e.g., CBC, serum biochemistry panel, urinalysis) usually provides clues to the underlying cause (see [Box 8-4](#)).

Causes of Hypermagnesemia • Hypermagnesemia occurs in dogs and cats with renal failure and postrenal obstruction (see [Box 8-4](#)) or is iatrogenically induced after excessive magnesium intake (e.g., IV administration, antacids, laxatives). Because the healthy kidney rapidly excretes excess magnesium, iatrogenically induced hypermagnesemia usually reflects underlying renal insufficiency. Hypermagnesemia has also been reported in cats with thoracic neoplasia and pleural effusion, although the mechanism involved with the development of hypermagnesemia in these cats is unknown.⁴² Measurement of serum magnesium concentration identifies hypermagnesemia. Evaluation of history, physical examination, and routine clinical pathologic assessments (e.g., CBC, serum biochemistry panel, urinalysis) usually identifies the cause of hypermagnesemia.

PARATHYROID HORMONE

Occasionally Indicated • Serum PTH concentration is required to diagnose primary hyperparathyroidism and hypoparathyroidism in animals with hypercalcemia and hypocalcemia, respectively.

Advantages • Serum PTH measurement can establish a diagnosis of primary parathyroid disease without surgical intervention.

Disadvantages • Validated PTH assays are limited, samples must be frozen during transit, and azotemia may interfere with interpretation.

Analysis • PTH concentration is measured in serum by immunoradiometric and chemiluminescent immunoassays.¹² The sample should be centrifuged as soon as possible after clotting, frozen, and shipped frozen to the laboratory. Different PTH assays measure different parts of the PTH molecule and may give different results in the same patient. The “two-site” PTH assay system uses two different polyclonal antibodies to measure midregion and C-terminal 39 to 84 amino acids and N-terminal 1 to 34 amino acids of PTH simultaneously, is valid for measurement of dog and cat PTH, and is currently used by most veterinary laboratories.

Normal Values • 2 to 13 pmol/L. Normal values may differ depending on the laboratory used.

Danger Values • None.

Artifacts • Prolonged storage or transport at temperatures above freezing may produce erroneous results.

Drug Treatment That May Alter PTH Concentrations • Any drug therapy affecting serum calcium concentration can affect serum PTH concentration (see [Drug Therapy That May Alter Serum Calcium Concentration](#) earlier in this chapter). Drugs that decrease serum calcium may increase serum PTH concentration and vice versa.

Causes of Increased Serum PTH Concentration • Disorders that cause increased serum PTH concentration include primary hyperparathyroidism, secondary renal hyperparathyroidism, secondary nutritional hyperparathyroidism, secondary adrenal hyperparathyroidism^{31,39} and nonparathyroid causes of hypocalcemia (see [Causes of Hypocalcemia](#) earlier in this chapter). A midnormal or increased serum PTH concentration in a hypercalcemic patient with normal renal function strongly suggests primary hyperparathyroidism (see [Figure 8-3](#)).¹⁰ Animals with nonparathyroid-induced hypercalcemia have low to undetectable serum PTH concentrations. Serum PTH concentrations can be increased in animals with renal failure because of concurrent secondary renal hyperparathyroidism. Serum calcium concentration in these animals is usually in the normal range but may be decreased or, less commonly, increased with chronic end-stage renal failure. Serum ionized calcium concentration is usually normal in dogs and cats with secondary renal hyperparathyroidism. Serum calcium is in the low-normal or low range in animals with secondary nutritional hyperparathyroidism.

Causes of Decreased Serum PTH Concentration • Nondetectable serum PTH concentration in a hypocalcemic animal strongly suggests primary hypoparathyroidism. Patients with nonparathyroid-induced hypocalcemia should have normal or increased serum PTH concentrations. Nonparathyroid disorders causing hypercalcemia (see [Causes of Hypercalcemia](#) earlier in this chapter) also have low to undetectable serum PTH concentrations. The notable exception is hypercalcemia of chronic renal failure.

GLUCOSE

Commonly Indicated • Measurement of blood glucose concentration is commonly indicated in patients with polyuria, polydipsia, weakness, coma, behavioral change, or seizures (partial or complete). It should also be determined in patients with known hepatic or adrenal insufficiency, severe sepsis, pancreatic neoplasia, or glucosuria and in patients receiving insulin or total parenteral nutrition.

Advantages • The test is easily performed and readily available.

Disadvantages • A single blood glucose concentration may not detect disorders causing clinically significant but episodic hypoglycemia in some patients. Prolonged storage of blood before separation of serum or plasma may cause artifacts (i.e., low glucose results).

Analysis • Glucose can be measured in whole blood, serum, or plasma (heparin, sodium fluoride, or EDTA). One can measure blood glucose in two main ways: (1) reagent strips and a reflectance meter (portable blood glucose meter [PBGM]) and (2) standard laboratory methods. The latter usually entail photometric assays that use glucose oxidase or hexokinase to initiate colorimetric reactions. PBGMs use whole blood, and are quick, simple, inexpensive, and readily available. Correlation between results obtained with PBGMs versus standard laboratory methods varies widely.⁵

Differences between these methods are more pronounced at higher glucose concentrations (i.e., >300 mg/dl). Blood glucose concentrations measured by PBGMs are usually lower than corresponding results with standard laboratory methods. The exception is a PBGM designed for use in dogs and cats (AlphaTrak; Abbott Laboratories, Abbott Park, IL), with which results may be higher or lower than corresponding results with standard laboratory methods.⁵

Normal Values • 70 to 120 mg/dl. To convert from mg/dl to mmol/L, multiply by 0.056.

Danger Values • Less than 40 mg/dl (coma or seizures) or greater than 1000 mg/dl (hyperosmotic diabetes with central nervous system [CNS] dysfunction and possible coma).

Artifacts • See Chapter 1.

Serum or plasma must be separated from red blood cells (RBCs) and white blood cells (WBCs) within 30 minutes after collection to minimize consumption of glucose by cells. At 22° C, glucose concentration decreases approximately 10% every 30 to 60 minutes, and this may occur more rapidly if large concentrations of metabolically active cells (e.g., leukocytosis, leukemia) are present.

PBGMs are less accurate at high glucose concentrations (i.e., >300 mg/dl). Inadequate or excessive coverage of the reagent pad with blood may cause erroneous results. Expired reagent strips, meters that have not been properly calibrated, and inaccurate timing may also cause erroneous results.

Drug Therapy That May Alter Blood Glucose Concentration • Hypoglycemia may be caused by insulin, oral hypoglycemic drugs (e.g., sulfonylureas, biguanides), propranolol, atenolol, and ethylene glycol. Hyperglycemia (especially in prediabetic patients) may be caused by beta-adrenergic agonist drugs, corticosteroids, diazoxide, carbonic anhydrase inhibitors (e.g., acetazolamide, methazolamide), thiazides, glucagon, levothyroxine, progestagens, estrogens, and xylitol. Megestrol acetate may cause transient or persistent hyperglycemia in cats.

Causes of Hypoglycemia • Hypoglycemia is typically the result of excessive glucose use by normal (e.g., with hyperinsulinism) or neoplastic cells, impaired hepatic gluconeogenesis and glycogenolysis (e.g., hepatic insufficiency), a deficiency in diabetogenic hormones (e.g., hypocortisolism), inadequate dietary intake of glucose and other substrates required for hepatic gluconeogenesis (e.g., starvation in neonates), or a combination of these mechanisms (e.g., sepsis; Box 8-5). Iatrogenic hypoglycemia is a common problem with overzealous insulin administration to diabetics.

Hypoglycemia should always be confirmed before beginning diagnostics to identify the cause. Careful evaluation of history, physical findings, and routine clinical pathologic assessments (e.g., CBC, serum biochemical panel, urinalysis) usually provides clues to the underlying cause (Figure 8-5). Starvation, hepatic insufficiency (i.e., portosystemic shunt), sepsis, or idiopathic hypoglycemia are the usual causes in the puppy or kitten. In young adults, hepatic insufficiency, hypoadrenocorticism, or sepsis usually causes hypoglycemia. In older animals, hepatic insufficiency, beta cell neoplasia, extrapancreatic neoplasia, hypoadrenocorticism, and sepsis are the most common causes.

Hypoglycemia tends to be mild (i.e., >45 mg/dl) and is often an incidental finding in animals with hypoadrenocorticism and hepatic insufficiency. Additional alterations in clinical pathologic assessments (e.g., hyponatremia or hyperkalemia [hypoadrenocorticism], increased alanine aminotransferase [ALT] activity, hypoalbuminemia [hepatic insufficiency]) are usually present. An adrenocorticotrophic hormone (ACTH) stimulation test or hepatic function test (see Chapter 9) may be required to confirm the diagnosis. Severe hypoglycemia (i.e., <35 mg/dl) may develop in neonates and juvenile kittens and puppies (especially toy breeds) and with sepsis, beta cell neoplasia, and extrapancreatic neoplasia (especially hepatic adenocarcinoma and leiomyosarcoma). Sepsis is readily identified by physical findings and CBC abnormalities, including a neutrophilic leukocytosis (typically >30,000/μl), a shift toward immaturity, neutropenia, and/or toxic neutrophils (see Chapter 4). Extrapancreatic neoplasia can usually be identified on physical examination or on abdominal or thoracic imaging. Dogs with beta cell neoplasia typically have normal physical examination results and lack abnormalities except hypoglycemia. Measurement of baseline serum insulin concentration when blood glucose is less than 60 mg/dl (preferably <50 mg/dl) is necessary to confirm a beta cell tumor (see next section on Insulin).

BOX 8-5. CAUSES OF ALTERED BLOOD GLUCOSE IN DOGS AND CATS**HYPOGLYCEMIA**Beta cell tumor (insulinoma) (*uncommon but important*)

Extrapaneacretic neoplasia

Hepatocellular carcinoma, hepatoma

Leiomyosarcoma, leiomyoma

Hemangiosarcoma

Hepatic insufficiency (*uncommon but important*)

Portocaval shunts

Chronic fibrosis, cirrhosis

Sepsis (*important*)Hypoadrenocorticism (*uncommon but important*)

Hypopituitarism

Idiopathic hypoglycemia

Neonatal hypoglycemia

Juvenile hypoglycemia (esp. toy breeds)

"Hunting dog hypoglycemia"

Renal failure (*uncommon*)

Exocrine pancreatic neoplasia

Glycogen storage diseases (*rare*)

Severe polycythemia

Prolonged starvation

Prolonged sample storage (*common*)

Iatrogenic

Insulin therapy (*common*)

Sulfonylurea therapy

Ethanol

Ethylene glycol

Artifact

Portable blood glucose meters

Laboratory error

HYPERGLYCEMIADiabetes mellitus (*common*)"Stress," aggression, excitement, fear (esp. cats) (*common*)

Postprandial (propylene glycol, corn syrup, xylitol sweetener)

Hyperadrenocorticism

Acromegaly (cat)

Diestrus (bitch)

Pheochromocytoma (dog)

Pancreatitis

Exocrine pancreatic neoplasia

Renal insufficiency

Head trauma

Drug therapy

Glucocorticoids

Progestagens

Megestrol acetate

Thiazide diuretics

Parenteral nutrition solutions

Dextrose-containing fluids (*common*)

Causes of Hyperglycemia • Hyperglycemia results from insulin deficiency, impairment of insulin's action in peripheral tissues (i.e., decreased glucose use), increased hepatic gluconeogenesis and glycogenolysis, or a combination of these (see Box 8-5). Iatrogenic causes of hyperglycemia include IV infusion of dextrose-containing fluids and parenteral nutritional solutions, and administration of diabetogenic drugs (e.g., glucocorticoids, megestrol acetate). Infusion of fluids containing as little as 2.5% dextrose may cause hyperglycemia, depending on infusion rate and concurrent disorders interfering with carbohydrate tolerance. Severe hyperglycemia (typically without glucosuria) occurs commonly in "stressed" cats, presumably from epinephrine secretion. Many diseases also cause carbohydrate intolerance and mild hyperglycemia, primarily by interfering with insulin action in peripheral tissues.

Hyperglycemia between 130 and 180 mg/dl does not cause glucosuria, polyuria, or polydipsia. Hyperglycemia in this range is clinically silent and often an unsuspected finding. If the patient with mild hyperglycemia (i.e., <180 mg/dl) also has polyuria-polydipsia, a disorder

other than insulin-requiring diabetes mellitus should be sought. Mild hyperglycemia can occur in some animals shortly after consumption of foods containing large amounts of monosaccharides, disaccharides, or xylitol; in stressed cats and dogs; with administration of diabetogenic medications; in early stages of diabetes mellitus; and with disorders decreasing insulin's effectiveness (see Box 8-5). A diagnostic evaluation for disorders causing insulin ineffectiveness is indicated if mild hyperglycemia (i.e., <180 mg/dl) persists in the fasted, unstressed animal in which diabetogenic medications have been discontinued.

All hyperglycemic animals should be checked for glucosuria. Persistent hyperglycemia and glucosuria plus polyuria-polydipsia are diagnostic of diabetes mellitus. Persistence of diabetes mellitus depends, in part, on pathologic findings in the pancreatic islets, functional status of beta cells, and reversibility of concurrent diabetogenic diseases. Diabetogenic drugs (see [Drug Therapy That May Alter Blood Glucose Concentration](#) earlier in this section) should be stopped or adjusted; concurrent inflammatory, infectious, hormonal, or

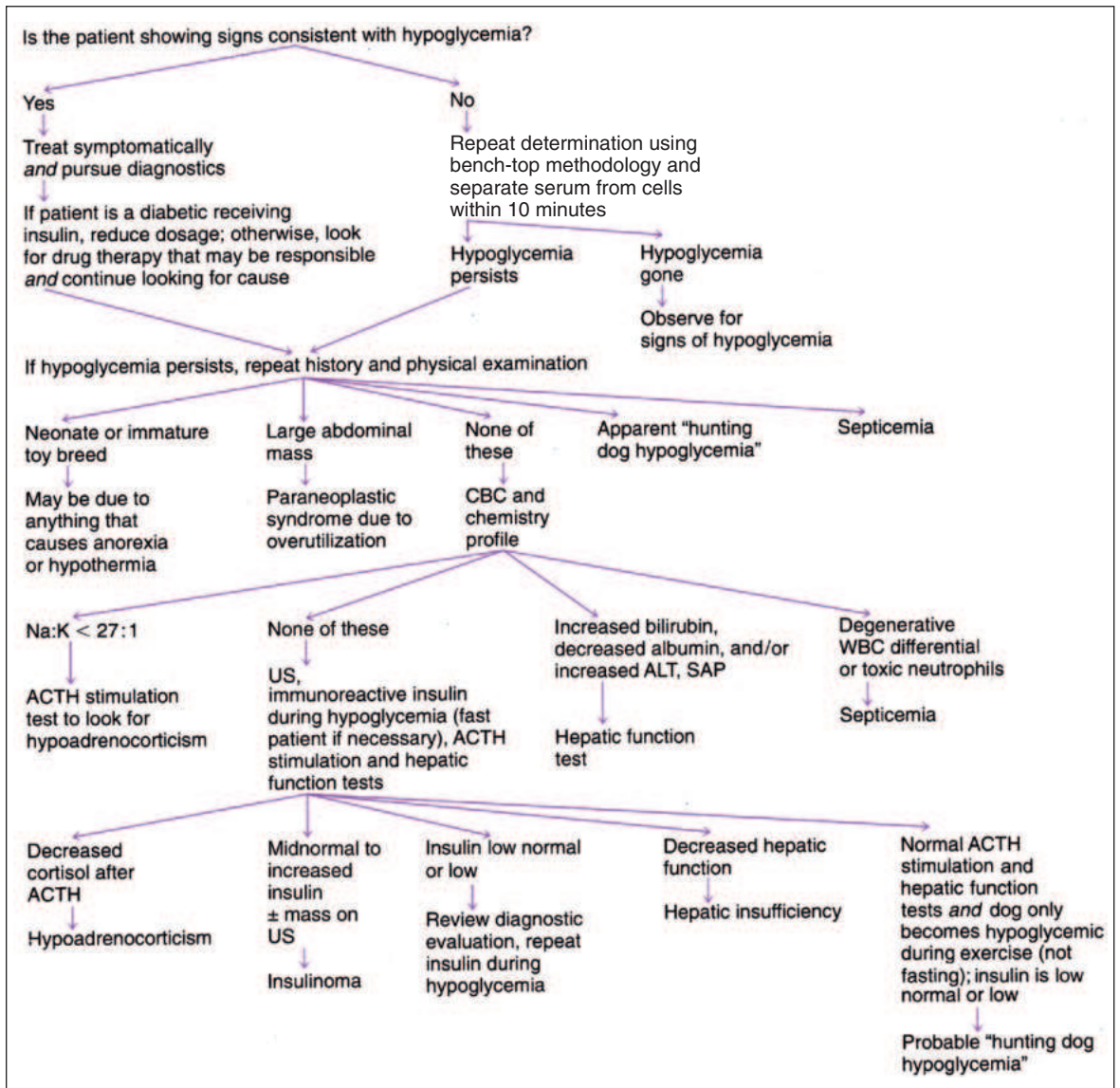
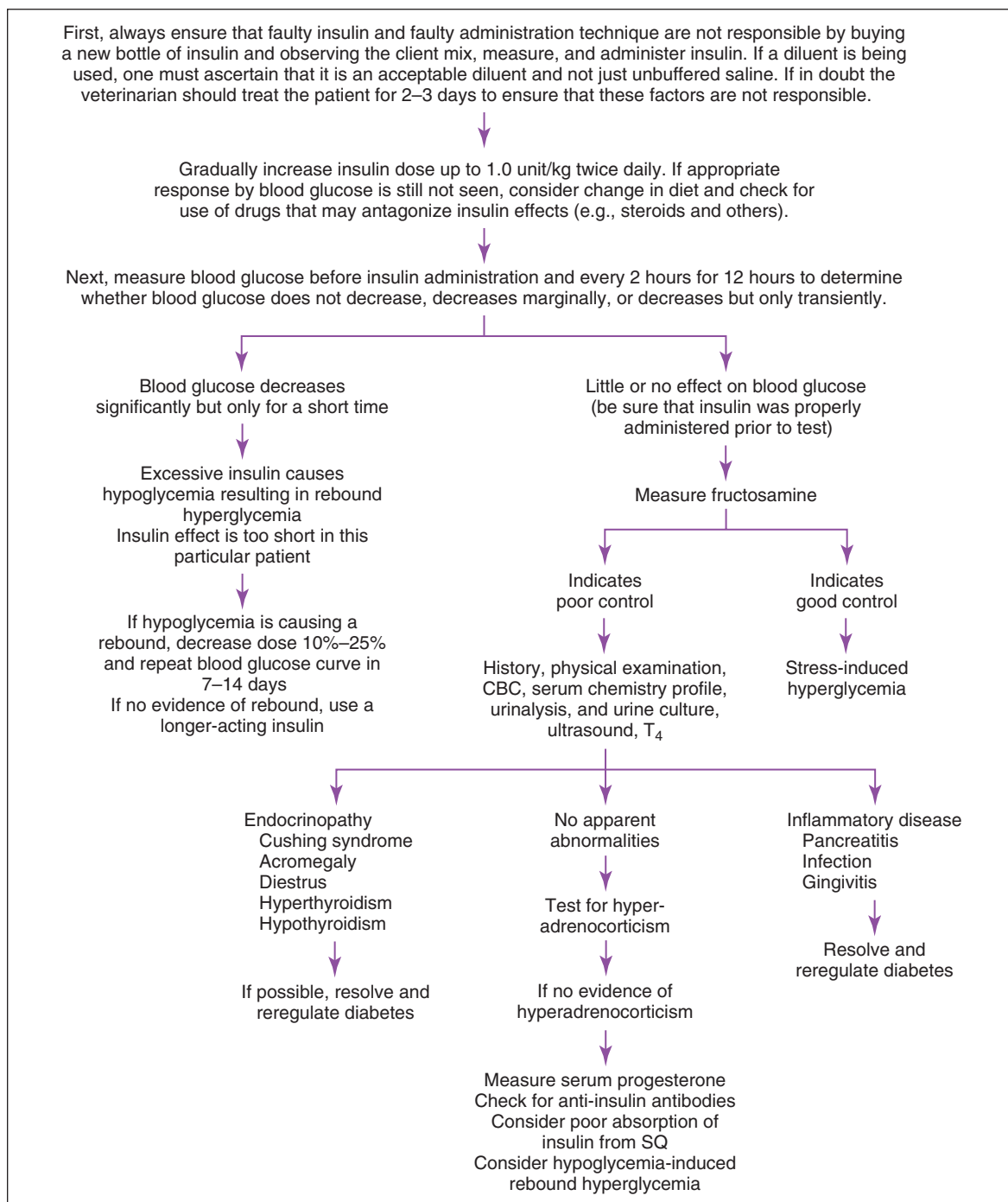


FIGURE 8-5 Diagnostic approach to hypoglycemia in dogs and cats. ACTH, Adrenocorticotrophic hormone; ALT, alanine aminotransferase; CBC, complete blood count; SAP, serum alkaline phosphatase; US, ultrasound; WBC, white blood cell.

neoplastic disorders controlled or eliminated; diets designed for treatment of diabetes initiated; and effects on blood glucose re-evaluated in animals with suspected diabetes mellitus. Concurrent diabetogenic disorders are usually suggested from the history, physical examination, evaluation of routine clinical pathologic assessments (e.g., CBC, serum biochemical panel, urinalysis), and ease of glycemic regulation with insulin therapy. Concurrent diabetogenic disorders should always be suspected when glycemic control is difficult to attain with insulin therapy. The most common disorders interfering with glycemic regulation in dogs are obesity; chronic inflammation such as chronic pancreatitis, gingivitis, and inflammatory bowel disease; hyperadrenocorticism; diestrus; and concurrent infection. In

cats, the most common are obesity, chronic inflammation (especially pancreatitis), hyperadrenocorticism, acromegaly, occult hyperthyroidism, and infection (Figure 8-6). Additional diagnostic tests (e.g., plasma lipase immunoreactivity, low-dose dexamethasone suppression test (LDDS), serum thyroxine concentration) may be necessary. Problems with insulin therapy itself (e.g., poor absorption, Somogyi response, short or prolonged duration of insulin effect) should also be considered in the animal with poorly regulated diabetes. Assessment of insulin therapy by measuring blood glucose concentrations at the time of and every 2 hours for 10 to 12 hours after insulin administration is the first diagnostic step in identifying problems with insulin therapy.



Disadvantages • Insulin assay must be validated in dogs and cats. Many variables can affect serum insulin concentration. Interpretation must be done in conjunction with corresponding blood glucose concentration.

Analysis • Insulin is measured in serum by radioimmunoassay (RIA) or chemiluminescent immunometric assay. Insulin concentrations measured in plasma tend to be higher than corresponding values in serum. Fasting samples are preferred to minimize the stimulatory effects of a meal on insulin secretion. Serum samples should be separated from cellular elements of blood and frozen before submission to the laboratory.

Normal Fasting Values • 5 to 20 $\mu\text{U/ml}$.

Fasting serum insulin concentration greater than 20 $\mu\text{U/ml}$ in an untreated diabetic patient suggests type 2 diabetes or diabetes induced by concurrent insulin antagonistic disease.

To convert from $\mu\text{U/ml}$ to pmol/L , multiply by 7.18.

Danger Values • None unless accompanied by hypoglycemia.

Artifacts and Effect of Drugs on Insulin Concentration • Serum insulin concentration is increased for several hours after a meal. In addition, many drugs and disorders affecting blood glucose concentration also affect serum insulin concentration. Insulin derived from an insulin injection may be measured in blood samples for up to 24 hours after the insulin injection. Chronic exogenous insulin therapy in diabetics may cause circulating insulin-binding antibody formation, which can interfere with some RIAs and enzyme-linked immunosorbent assays (ELISAs), causing spuriously increased (i.e., $>400 \mu\text{U/ml}$) or nondetectable values.⁶

Interpretation During Hypoglycemia • Confirmation of an insulin-secreting neoplasm requires documentation of inappropriate insulin secretion during hypoglycemia. If blood glucose concentration is less than 60 mg/dl (preferably $<50 \text{ mg/dl}$) and serum insulin concentration is increased (i.e., $>20 \mu\text{U/ml}$) in the same blood sample, an insulin-secreting neoplasm is likely. If serum insulin is in the high-normal range (i.e., 10 to 20 $\mu\text{U/ml}$), an insulin-secreting tumor remains possible. Insulin values in the low-normal range (i.e., 5 to 10 $\mu\text{U/ml}$) may be found with other causes of hypoglycemia, as well as insulin-secreting tumors. Careful assessment of history, physical findings, clinical pathologic assessments, abdominal ultrasound, and possibly repeated serum glucose and insulin values usually identifies the cause of hypoglycemia. A serum insulin concentration that is below the normal range (i.e., $<5 \mu\text{U/ml}$) is inconsistent with an insulin-secreting tumor.

Interpretation During Hyperglycemia • Serum insulin concentration should be increased (i.e., $>20 \mu\text{U/ml}$) during periods of hyperglycemia in normal animals. Documenting serum insulin concentration greater than 20 $\mu\text{U/ml}$ in newly diagnosed diabetics suggests residual beta cell function and either type 2 diabetes or diabetes induced by concurrent insulin antagonistic disease. Most

animals with type 1 (insulin-dependent) diabetes mellitus have serum insulin concentrations less than 10 $\mu\text{U/ml}$. Markedly increased serum insulin concentration (i.e., $>400 \mu\text{U/ml}$) in blood obtained more than 24 hours after the last insulin injection in an insulin-treated diabetic suggests insulin-binding antibodies. Serum insulin concentration is typically less than 50 $\mu\text{U/ml}$ 24 hours after the previous insulin injection in the diabetic without antibodies causing interference with the insulin assay. Nondetectable serum insulin concentrations in an insulin-treated diabetic, especially when measured 2 to 8 hours after insulin administration, also suggests insulin-binding antibodies causing interference with the insulin assay.

INSULIN SECRETAGOGUE TESTING

Rarely Indicated • Insulin secretagogue testing can aid in differentiating type 1 from type 2 diabetes mellitus, help identify carbohydrate intolerance in animals with suggested preclinical diabetes mellitus, and possibly identify occult insulin-secreting tumors. The glucagon stimulation test can identify hepatic glycogen storage disease. The most common insulin secretagogue tests are the IV glucose tolerance test (IVGTT), the IV glucagon stimulation test (IVGST), and the IV arginine stimulation test.

Advantages • None.

Disadvantages • The tests are labor intensive, expensive, often ineffective in differentiating type 1 from type 2 diabetes mellitus, and not recommended for diagnosis of insulin-secreting tumor. Insulin secretagogue testing is primarily indicated in a research setting. The reader is referred to the references for more information on performing these tests.^{13, 14, 18, 26}

FRUCTOSAMINE

Commonly Indicated • Measurement of serum fructosamine concentration is used to monitor glycemic control in diabetic dogs and cats and occasionally to document persistent hyperglycemia and possible diabetes mellitus in animals with conflicting clinical signs and results of blood and urine glucose tests, and persistent hypoglycemia in insulin-treated diabetic animals and dogs with suspected beta cell tumor.

Analysis • Fructosamine is measured in serum by automated colorimetric assay using the nitroblue tetrazolium chloride reduction method. Serum should be kept frozen until assayed for fructosamine.

Normal Values • 225 to 375 $\mu\text{mol/L}$. Normal values may differ depending on the laboratory used.

Artifacts • Serum fructosamine concentration may be affected by hypoalbuminemia (decreased), hyperlipidemia (mild decrease in dogs), azotemia (mild decrease in dogs), hyperthyroidism (decrease in cats), and storage at room temperature (decreased).

Drug Therapy That May Alter Serum Fructosamine Concentration • Any drugs that cause a persistent and prolonged increase (e.g., glucocorticoids) or decrease (e.g., glipizide, glyburide) in blood glucose concentration can increase or decrease serum fructosamine concentrations, respectively.

Interpretation in Diabetic Dogs and Cats • Fructosamines result from an irreversible, nonenzymatic, insulin-independent binding of glucose to serum proteins. The extent of glycosylation of serum proteins is directly related to the blood glucose concentration; the higher the average blood glucose concentration during the preceding 2 to 3 weeks, the higher the serum fructosamine concentration, and vice versa. Serum fructosamine concentrations increase when glycemic control of the diabetic dog or cat worsens and decrease when glycemic control improves. Serum fructosamine concentration is not affected by acute increases in the blood glucose concentration, as occurs with stress- or excitement-induced hyperglycemia. Measurement of a single serum fructosamine concentration as part of the routine evaluation of the insulin-treated diabetic dog or cat provides information on the status of glycemic control during the month prior to the evaluation. Serum fructosamine concentrations can be measured to clarify the effect of stress or excitement on blood glucose concentrations, to clarify discrepancies between the history and physical examination findings and results of serial blood glucose concentrations, and to assess the effectiveness of changes in insulin therapy.

The normal reference range for serum fructosamine is determined in healthy dogs and cats with persistently normal blood glucose concentrations. Interpretation of serum fructosamine in a diabetic dog or cat must take into consideration the fact that hyperglycemia is common, even in well-controlled diabetic animals. Most owners are happy with their pet's response to insulin treatment if serum fructosamine concentrations can be kept between 350 and 450 $\mu\text{mol/L}$ (reference range, 200 to 375 $\mu\text{mol/L}$). Values greater than 500 $\mu\text{mol/L}$ suggest inadequate control of the diabetic state, and values greater than 600 $\mu\text{mol/L}$ indicate serious lack of glycemic control. Serum fructosamine concentrations in the lower half of the normal reference range (i.e., <300 $\mu\text{mol/L}$) or below the normal reference range should raise concern for significant periods of hypoglycemia in the diabetic dog or cat or remission of the diabetic state. Increased serum fructosamine concentrations (i.e., >500 $\mu\text{mol/L}$) suggest poor control of glycemia and a need for insulin adjustments. However, increased serum fructosamine concentrations do not identify the underlying problem.

Evaluation of the change in serum fructosamine can be used to assess response to changes in insulin therapy in fractious or stressed diabetic dogs and cats in which reliability of blood glucose results are questionable. Because serum proteins have a relatively short half-life, serum fructosamine concentration changes relatively quickly (i.e., 2 to 3 weeks) in response to a change in glycemic control. This short period for change in serum fructosamine concentration is advantageous for detecting improvement or deterioration of glycemic control quickly. As such, serum fructosamine concentrations can be

measured before and 3 to 4 weeks after changing insulin therapy to assess the effectiveness of the change. If changes in insulin therapy are appropriate, serum fructosamine concentration should decrease by at least 50 $\mu\text{mol/L}$. If the serum fructosamine concentration is the same or has increased, the change was ineffective in improving glycemic control, another change in therapy should be tried, and the serum fructosamine measured again 3 to 4 weeks later.

HYPERLIPIDEMIA

Hyperlipidemia is an increase in serum concentrations of cholesterol, triglycerides, or both. In the clinical setting, veterinarians typically use the term *hypercholesterolemia* when there is an increase in serum cholesterol without lipemia or hypertriglyceridemia, and they use the term *hyperlipidemia* when there is an increase in triglycerides and the presence of gross lipemia with or without concurrent hypercholesterolemia. In the fasted state, increased serum cholesterol, triglycerides, or both is an abnormal finding that represents either accelerated production or delayed degradation of lipoproteins. Lipoproteins function as a carrier system to transport water-insoluble triglycerides and cholesterol through the aqueous environment of blood. Four major classes of lipoproteins are recognized: (1) chylomicrons, (2) very-low-density lipoproteins (VLDLs), (3) low-density lipoproteins (LDLs), and (4) high-density lipoproteins (HDLs). Chylomicrons and VLDLs are predominantly involved in triglyceride metabolism, whereas HDLs and LDLs are primarily involved in cholesterol metabolism. Chylomicrons are derived from dietary fat. Triglycerides produced from free fatty acids by the liver are packaged into VLDL particles and subsequently secreted into the circulation. LDLs result from removal of the triglyceride core of VLDL by endothelial lipoprotein lipase in tissues and subsequent removal of residual triglyceride in the remnant VLDL particle by hepatic lipase in the liver. LDLs function to transport cholesterol to tissues. HDLs are produced in the liver, and act to scavenge excess unesterified cholesterol from cells and other lipoproteins and return it to the liver for excretion in bile. HDLs are the primary lipoprotein in the dog and cat.

Hyperlipidemia is often initially recognized by the finding of gross lipemia (i.e., milky plasma or serum) in a blood sample. Postprandial hyperlipidemia is normal; however, hyperlipidemia in a dog or cat that has been fasted 12 hours or longer is abnormal. Clear plasma or serum does not rule out hypercholesterolemia in the absence of hypertriglyceridemia (which does not cause lipemia). Lipemia is visible when serum triglycerides are greater than 200 mg/dl. Gross lipemia implies hypertriglyceridemia and an increase in chylomicrons, VLDLs, or both. Hyperlipidemia is also diagnosed after measurement of serum cholesterol and triglyceride concentrations. Reference intervals for serum triglyceride concentration are typically 50 to 150 mg/dl for adult dogs and 20 to 110 mg/dl for adult cats. Reference intervals for serum cholesterol concentration are typically 125 to 300 mg/dl for the adult dog and 95 to 130 mg/dl for the adult cat. Hyperlipidemia should be suspected when

BOX 8-6. CAUSES OF HYPERLIPIDEMIA IN DOGS AND CATS**POSTPRANDIAL HYPERLIPIDEMIA****SECONDARY HYPERLIPIDEMIA**

Hypothyroidism (*common and important*)
 Diabetes mellitus (*common and important*)
 Hyperadrenocorticism (*common and important*)
 Pancreatitis
 Cholestasis
 Hepatic insufficiency (*rare*)
 Nephrotic syndrome (*common and important*)

PRIMARY HYPERLIPIDEMIA

Idiopathic hyperlipoproteinemia (miniature schnauzers) (*common and important*)
 Idiopathic hyperchylomicronemia (cat)
 Lipoprotein lipase deficiency (cat)
 Idiopathic hypercholesterolemia

DRUG-INDUCED HYPERLIPIDEMIA

Glucocorticoids
 Megestrol acetate (cat)

serum concentrations of cholesterol, triglycerides, or both exceed the reference interval.

Hyperlipidemia may be idiopathic, a primary defect in lipoprotein metabolism, or a consequence of systemic disease (Box 8-6). Postprandial hyperlipidemia is the most common cause of hyperlipidemia and should be eliminated before one performs more costly tests. The transient increase in serum triglycerides typically resolves within 10 hours after eating. Postprandial plasma cholesterol concentration usually does not exceed the upper limit of the species-specific reference range.

For most animals, persistent hyperlipidemia is caused by an endocrine or metabolic disorder. The patient may be presented because of signs related to the underlying disorder (see Box 8-6) or signs caused by hyperlipidemia, or it may be asymptomatic. Persistent hyperlipidemia is an indication for further tests to eliminate potential secondary causes. History, physical examination, CBC, serum biochemistry panel, serum pancreatic lipase immunoreactivity (see Chapter 9), thyroxine concentration, and urinalysis make up the initial diagnostic approach to hyperlipidemia (Figure 8-7). Additional diagnostic tests (e.g., abdominal ultrasound, LDDs, serum free thyroxine and thyroid-stimulating hormone concentration) may be indicated, depending on initial test results.

Idiopathic hyperlipidemias include idiopathic hyperlipoproteinemia of miniature schnauzers, idiopathic hyperchylomicronemia, feline familial hyperlipidemia caused by lipoprotein lipase deficiency, and idiopathic hypercholesterolemia.⁴⁵ Idiopathic or primary hyperlipidemia is diagnosed after eliminating secondary causes of persistent hyperlipidemia. Idiopathic and primary hyperlipidemias can be further characterized by lipoprotein electrophoresis or combined ultracentrifugation and precipitation techniques. These techniques require specialized equipment and are not widely available commercially. An idea of the nature of lipoprotein alterations can also

be gained by measuring serum cholesterol and triglyceride concentrations and performing the chylomicron test. In the chylomicron test, the lipemic plasma or serum sample is refrigerated at 4° C overnight (i.e., 12 hours) and then assessed visually. Chylomicrons form a cream layer at the top of the sample, whereas persistent lactescence of plasma or serum is caused by VLDLs.

CHOLESTEROL

Occasionally Indicated • Measurement of serum cholesterol concentration is indicated in dogs and cats with hyperlipidemia and as a screening test for hypothyroidism, hyperadrenocorticism, hepatic insufficiency, and protein-losing enteropathy. Hypercholesterolemia by itself does not cause gross lipemia.

Analysis • Cholesterol is measured in serum or heparinized plasma by spectrophotometric, chromatographic, automated direct, and enzymatic methods. Automated direct techniques may slightly overestimate serum cholesterol concentration.

Normal Values • Dogs, 125 to 300 mg/dl; cats, 95 to 130 mg/dl. Normal values may differ depending on the laboratory used. To convert from mg/dl to mmol/L, multiply by 0.026.

Danger Values • None.

Artifacts • See Chapter 1.

Drug Therapy That May Alter Serum Cholesterol Concentration • L-Asparaginase, azathioprine, colchicine, cholestyramine, and oral aminoglycosides may cause hypocholesterolemia. Corticosteroids, methimazole, phenytoin, prochlorperazine, thiazides, and phenothiazines may cause hypercholesterolemia.

Causes of Hypocholesterolemia • Hypocholesterolemia primarily occurs with protein-losing enteropathy (e.g., intestinal lymphangiectasia), hepatic insufficiency (e.g., portocaval shunt and cirrhosis), selected malignancies, and severe malnutrition.

Causes of Hypercholesterolemia • Diet or spontaneous disease (see Box 8-6) may cause hypercholesterolemia. Feeding a very-high-fat diet or sampling blood shortly after eating may cause minor elevations in serum cholesterol concentration. The diagnostic approach to persistent hypercholesterolemia in the fasted animal is as outlined for hyperlipidemia (see Figure 8-7). Primary differentials are diabetes mellitus, hypothyroidism, hyperadrenocorticism, and protein-losing disorders, most notably involving the kidney.

TRIGLYCERIDES

Occasionally Indicated • Occasionally, measurement of serum triglyceride concentration is indicated in patients with hyperlipidemia or hypercholesterolemia, especially

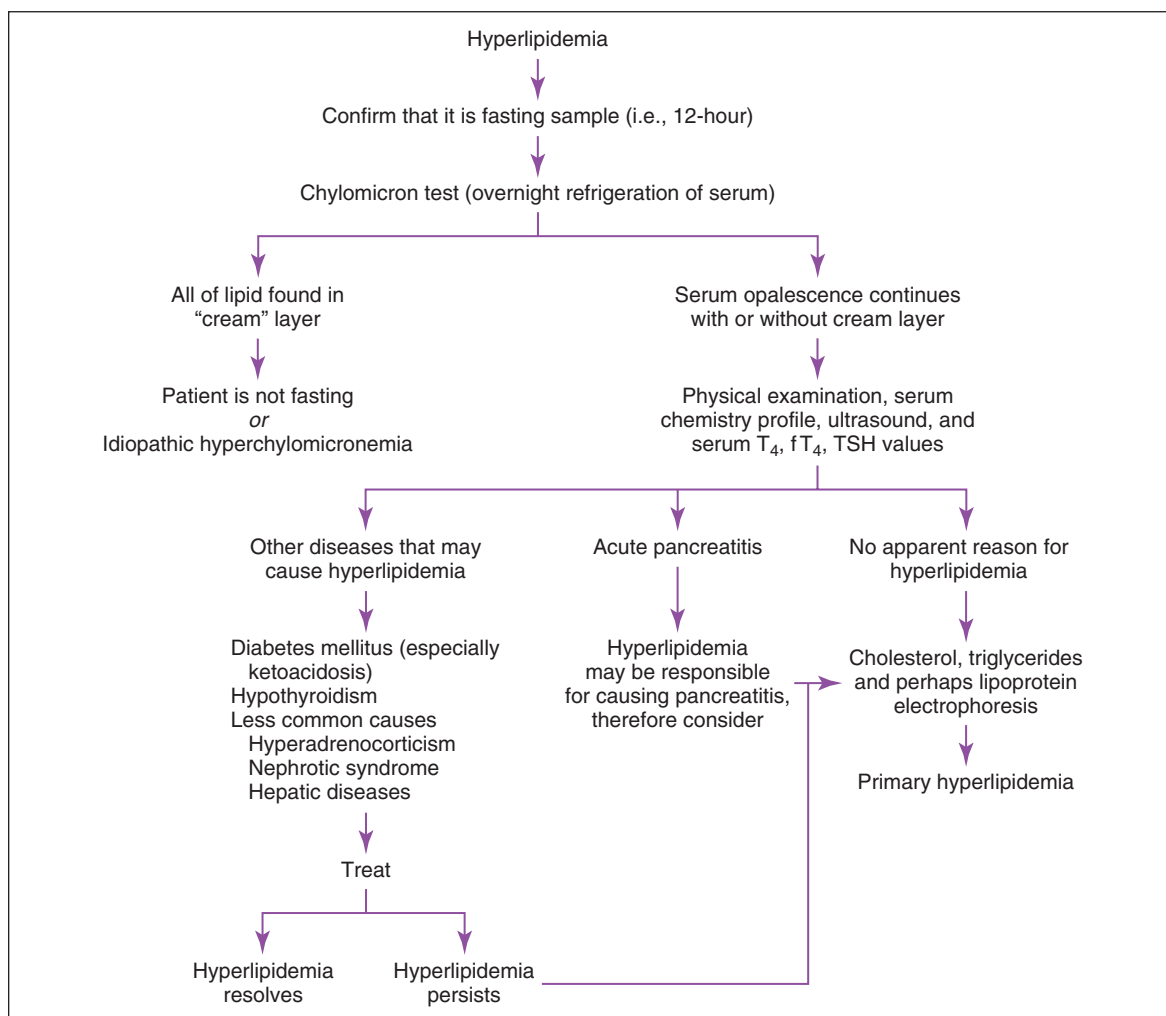


FIGURE 8-7 Diagnostic approach to hyperlipidemia in the dog and cat. fT_4 , free thyroxine; T_4 , thyroxine; TSH, thyroid-stimulating hormone.

if clinical signs associated with hypertriglyceridemia are present (i.e., gastrointestinal signs, abdominal pain, seizures, ataxia, weakness, behavioral changes, lipemia retinalis, and xanthomata). Gross lipemia implies hypertriglyceridemia.

Analysis • Triglycerides are measured in serum or EDTA plasma by spectrophotometric or enzymatic methods. Triglyceride values tend to be slightly less in plasma than in serum. Enzymatic methods may give slightly greater values than spectrophotometric methods. Point-of-care triglyceride meters used in humans may be suitable for identifying hypertriglyceridemia in dogs.¹⁷

Normal Values • Dogs, 50 to 150 mg/dl; cats, 20 to 110 mg/dl. Normal values may differ depending on the laboratory used.

Danger Values • Greater than 1000 mg/dl (neurologic signs, seizures).

Artifacts • Lipemia may interfere with several routine biochemistry tests (Box 8-7), depending upon the species evaluated (i.e., dog versus cat), analytical instrumentation, laboratory methods, and severity of hypertriglyceridemia (see Chapter 1).

Drug Therapy That May Alter Serum Triglyceride Concentration • Ascorbic acid, L-asparaginase, and heparin may cause hypotriglyceridemia. Different anabolic steroids have different effects. Estrogens, cholestyramine, phenobarbital, and bromide may cause hypertriglyceridemia.¹⁶

Causes of Hypotriglyceridemia • Hypotriglyceridemia is not clearly associated with any disease.

Causes of Hypertriglyceridemia • Hypertriglyceridemia may be idiopathic, develop as a primary defect in lipoprotein metabolism, or be a consequence of an underlying systemic disease (see Box 8-6). Postprandial

BOX 8-7. EFFECT OF GROSS LIPEMIA ON CLINICAL CHEMISTRY ANALYTES* IN CANINE AND FELINE SERA**FALSE INCREASE IN VALUES****CANINE SERA**

Total bilirubin
Phosphorus
Alkaline phosphate (SAP)[†]
Glucose[‡]
Total protein[‡]
Lipase
Alanine aminotransferase (ALT)

FELINE SERA

Total bilirubin
Phosphorus
Alkaline phosphate (SAP)[†]
Glucose[‡]
Total protein[‡]

FALSE DECREASE IN VALUES**CANINE SERA**

Creatinine
Total CO₂
Cholesterol
Urea nitrogen

FELINE SERA

Creatinine
Total CO₂
ALT

*Analytes were measured using Coulter DACES (Coulter Diagnostics, Hialeah, FL).

[†]Interference only occurs at very high concentrations of lipid.

[‡]When measured using refractometer.

Adapted from Jacobs RM et al: Effects of bilirubinemia, hemolysis, and lipemia on clinical chemistry analytes in bovine, canine, equine, and feline sera. *Can Vet J* 33:605, 1992.

hypertriglyceridemia is the most common cause of hyperlipidemia and should be eliminated before more costly tests are performed (see [Hyperlipidemia](#) earlier in this chapter). The diagnostic approach for persistent hypertriglyceridemia in the fasted animal is as outlined for hyperlipidemia (see [Figure 8-7](#)). Primary differentials are diabetes mellitus, hypothyroidism, acute pancreatitis, and idiopathic hyperlipoproteinemia.

THYROXINE

Commonly Indicated • Measurement of serum thyroxine (T₄) concentration is performed to diagnose hypothyroidism and hyperthyroidism and to monitor sodium levothyroxine therapy for hypothyroidism and methimazole therapy for hyperthyroidism. Clinical signs suggestive of hypothyroidism include mental dullness, lethargy, exercise intolerance or unwillingness to exercise, gain in weight without a corresponding increase in appetite or food intake, endocrine alopecia, seborrhea, pyoderma, weakness, neurologic signs (e.g., facial nerve paralysis, head tilt, ataxia, seizures), failure to cycle (bitch), and failure to grow (i.e., cretinism). Clinical signs suggestive of hyperthyroidism include weight loss (which may progress to cachexia), polyphagia, restlessness or hyperactivity, haircoat changes (e.g., patchy alopecia, matted hair, lack of or excessive grooming behavior), polyuria, polydipsia, vomiting, diarrhea, and aggressive behavior. Hyperthyroidism should also be considered in an older cat with a palpable cervical nodule.

Advantages • Tests to measure serum T₄ are readily available, including point-of-care ELISAs¹⁵; T₄ is a stable hormone and the primary hormone secreted by the thyroid gland.

Disadvantages • A myriad of variables can affect serum T₄ concentration, resulting in misinterpretation.

Analysis • T₄ concentration is measured in serum by RIA or enzyme, chemiluminescent, particle-enhanced, turbidimetric, or dry chemistry immunoassay systems. T₄ concentration in serum is stable for days at room temperature. Hemolysis, freezing, and thawing does not affect serum T₄ concentrations. Despite stability, serum samples should be frozen and sent to the laboratory on cool packs. The laboratory must use an assay validated for the species being tested. When evaluating levothyroxine sodium therapy, serum ideally should be obtained 4 to 6 hours after levothyroxine sodium administration for dogs on twice-a-day therapy and before and 4 to 6 hours after levothyroxine sodium administration for dogs on once-a-day therapy (see Chapter 18). A single serum sample can be obtained at any time when evaluating methimazole therapy in cats.³⁵

Normal Values • Dogs, 0.8 to 3.5 µg/dl; cats, 1.0 to 4.0 µg/dl.

NOTE: Different laboratories have different normal ranges. To convert from µg/dl to nmol/L, multiply by 12.87.

Interpretation for hypothyroidism in dogs: A normal serum T₄ concentration establishes normal thyroid gland function. The exception is a very small number (<1%) of hypothyroid dogs with lymphocytic thyroiditis that have serum T₄ autoantibodies that interfere with some RIAs used to measure T₄.⁴⁰ A serum T₄ concentration less than 0.5 µg/dl in conjunction with appropriate clinical signs, physical findings, and results of routine blood tests supports the diagnosis of hypothyroidism, especially if systemic illness is not present. Additional diagnostic tests of thyroid gland function are indicated if the serum T₄ concentration is less than the reference range but clinical signs, physical examination findings, and results of routine blood work are not strongly supportive of the

disease; if severe systemic illness is present; or if drugs known to decrease serum T_4 concentration are being administered.

Interpretation for hyperthyroidism in cats: A serum T_4 concentration greater than the reference range is diagnostic for hyperthyroidism, assuming appropriate clinical signs and a thyroid nodule are present. A serum T_4 concentration less than 2 $\mu\text{g/dl}$ rules out hyperthyroidism, except in extremely uncommon situations where concurrent severe nonthyroidal illness is present. Serum T_4 concentrations in the upper half of the reference range are identified in some cats in the early stages of hyperthyroidism. Hyperthyroidism should not be excluded on the basis of one "normal" T_4 test result, especially in a cat with appropriate clinical signs and a palpable nodule in the neck.

Danger Values • None.

NOTE: The clinician must always consider breed of dog, clinical signs, physical findings, clinical pathologic changes, effects of concurrent drugs and illness, and index of suspicion when interpreting serum T_4 results.

Variables That Affect Serum T_4 Concentration • Variables affecting serum T_4 concentration can be divided into physiologic, pharmacologic, and systemic illness (Tables 8-1 and 8-2). Serum T_4 concentration is higher in young dogs (i.e., <1 year of age) and decreases with advancing age.³² Smaller breeds have higher T_4 concentrations than large or giant breeds. Certain breeds (e.g., sight hounds and Nordic breeds) have lower serum T_4 values than other breeds. Estrus, pregnancy, and obesity increase serum T_4 concentration, whereas hypoproteinemia may decrease serum T_4 concentration. Anti-thyroid hormone antibodies may develop in dogs with lymphocytic thyroiditis and cause spuriously increased or decreased serum T_4 values.⁴⁰ The effect of anti-thyroid hormone antibodies on the serum T_4 value depends on the type of assay being used by the laboratory. Drugs that have been documented to alter serum T_4 concentration in dogs are listed in Table 8-2; the most clinically relevant are concurrent glucocorticoid, phenobarbital, and sulfonamide administration. Many nonthyroidal illnesses are associated with decreased serum T_4 concentration (Box 8-8). This phenomenon is called the *euthyroid sick syndrome*. The severity of illness has a direct correlation with the severity of suppression of serum T_4 concentration.

Causes of Decreased Serum T_4 Concentration • The primary disorder causing decreased serum T_4 concentration is hypothyroidism, which must be differentiated from all the variables listed earlier that can also suppress serum T_4 concentration (Box 8-9). In the dog, lymphocytic thyroiditis, idiopathic atrophy, or neoplastic destruction of the thyroid gland may cause primary hypothyroidism. Secondary hypothyroidism results from a deficiency of pituitary gland thyrotropin (thyroid-stimulating hormone [TSH]) and may be caused by pituitary malformation, destruction, or suppression (e.g., glucocorticoid therapy). Feline hypothyroidism is usually

TABLE 8-1. VARIABLES THAT MAY AFFECT BASELINE SERUM THYROID HORMONE FUNCTION TEST RESULTS IN DOGS

Age	Inversely proportional effect
Neonate (<3 mo)	Increased T_4
Aged (>6 yr)	Decreased T_4
Body size	Inversely proportional effect
Small (<10 kg)	Increased T_4
Large (>30 kg)	Decreased T_4
Breed	
Sight hounds (e.g., Greyhound)	T_4 and free T_4 lower than normal range established for dogs; no difference for TSH
Nordic breeds (e.g., Huskies)	
Other breeds (?)	
Gender	No effect
Time of day	No effect
Weight gain/obesity	Increased
Weight loss/fasting	Decreased T_4 , no effect on free T_4
Strenuous exercise	Increased T_4 , decreased TSH, no effect on free T_4
Estrus (estrogen)	No effect on T_4
Pregnancy (progesterone)	Increased T_4
Surgery/anesthesia	Decreased T_4
Concurrent illness*	Decrease T_4 and free T_4 ; depending on illness, TSH may increase, decrease or not change
Moderate/severe osteoarthritis	No effect on T_4 , free T_4 , or TSH
Drugs	See Table 8-2
Dietary iodine intake	If excessive, decreased T_4 and free T_4 ; increased TSH
Thyroid hormone autoantibodies	Increased or decreased T_4 ; no effect on free T_4 or TSH

T_4 , Thyroxine; TSH, thyroid-stimulating hormone.

*There is a direct correlation between severity and systemic nature of illness and suppression of serum T_4 and free T_4 concentration.

From Nelson RW, Couto GC: *Small animal internal medicine*, ed 4, St. Louis, 2009, Elsevier.

iatrogenic, resulting from bilateral thyroidectomy, excessive methimazole, or radioactive iodine (^{131}I) therapy for hyperthyroidism. Naturally occurring feline hypothyroidism is rare and is usually identified in kittens. Congenital hypothyroidism results in a cretin.

Diagnosis of hypothyroidism must consider history, physical findings, clinical pathologic assessments, serum T_4 concentration, and the clinician's index of suspicion for the disease. Fasting lipemia, hypercholesterolemia and, less commonly, mild normocytic normochromic anemia (i.e., hematocrit 30% to 35%) are the most common abnormalities identified on routine clinical pathologic assessments. Mild hypercalcemia may occur in some dogs with congenital hypothyroidism, and skeletal survey radiographs may identify delayed epiphyseal ossification, epiphyseal dysgenesis (i.e., irregularly formed, fragmented, or stippled epiphyseal centers), short broad

TABLE 8-2. DRUGS THAT MAY AFFECT BASELINE SERUM THYROID HORMONE FUNCTION TEST RESULTS IN DOGS

Amiodarone	Increased T ₄ ; decreased T ₃
Aspirin	Decreased T ₄ , free T ₄ ; no effect on TSH
Carprofen	Decreased T ₄ , free T ₄ , and TSH
Deracoxib	No effect on T ₄ , free T ₄ , or TSH
Etodolac	No effect on T ₄ , free T ₄ , or TSH
Meloxicam	No effect on T ₄ , free T ₄ , or TSH
Glucocorticoids	Decreased T ₄ and free T ₄ ; decrease or no effect on TSH
Clomipramine	Decreased T ₄ , free T ₄ ; no effect on TSH
Furosemide	Decreased T ₄
Methimazole	Decreased T ₄ and free T ₄ ; increased TSH
Phenobarbital	Decreased T ₄ and free T ₄ ; delayed increase in TSH
Phenylbutazone	Decreased T ₄
Potassium bromide	No effect on T ₄ , free T ₄ , or TSH
Progestagens	Decreased T ₄
Propranolol	No effect on T ₄ , free T ₄ , or TSH
Propylthiouracil	Decreased T ₄ and free T ₄ ; increased TSH
Stanozol	No effect on T ₄ or free T ₄
Cephalexine	No effect on T ₄ , free T ₄ , or TSH
Sulfonamides	Decreased T ₄ and free T ₄ ; increased TSH
Iopodate	Increased T ₄ , decreased T ₃

T₃, 3,5,3'-Triiodothyronine; T₄, thyroxine; TSH, thyroid-stimulating hormone. From Nelson RW, Couto GC: *Small animal internal medicine*, ed 4, St. Louis, 2009, Elsevier.

skulls, shortened vertebral bodies, and decreased length of diaphyses of long bones.³⁶ Additional diagnostic tests, including baseline serum free thyroxine (fT₄) and endogenous TSH concentration, and clinical response to trial therapy with levothyroxine sodium are usually required to establish the diagnosis.

Monitoring serum concentrations during levothyroxine sodium therapy allows the clinician to evaluate the dose, frequency of administration, and adequacy of intestinal absorption of levothyroxine sodium (see Chapter 18). If the dose and dosing schedule are appropriate, the serum T₄ concentration should be between 2.0 and 4.5 µg/dl. Serum TSH concentration should also be in the normal range. Post-dosing serum T₄ concentrations measured at times other than 4 to 6 hours after levothyroxine sodium administration should be interpreted with the realization that serum T₄ may not be at peak concentrations. When the post-pill serum T₄ concentration is less than 2.0 µg/dl, the decision to adjust therapy is based on the history, physical examination, time of the blood test in relation to levothyroxine sodium administration, and probability the dog consumed the medication. An increase in the dose or frequency of administration of levothyroxine sodium is

BOX 8-8. COMMON CAUSES OF THE "EUTHYROID SICK SYNDROME"**ACUTE DISEASES**

Bacterial bronchopneumonia
Sepsis
Distemper
Autoimmune hemolytic anemia
Systemic lupus erythematosus
Intervertebral disk disease
Polyradiculoneuritis
Acute renal failure
Acute hepatitis
Acute pancreatitis

CHRONIC DISEASES

Generalized demodicosis
Generalized bacterial furunculosis
Systemic mycoses
Lymphosarcoma
Chronic renal failure
Diabetes mellitus
Congestive heart failure
Cardiomyopathy
Chronic hepatitis, cirrhosis
Gastrointestinal disturbances
Megaeosophagus

Data from Feldman EC, Nelson RW: Hypothyroidism. In *Canine and feline endocrinology and reproduction*, Philadelphia, 1996, WB Saunders.

BOX 8-9. CAUSES OF ALTERED SERUM T₃ AND T₄ VALUES IN DOGS AND CATS**DECREASED SERUM THYROID HORMONE VALUES**

Hypothyroidism (primary and secondary)
Nonthyroidal illness (euthyroid sick syndrome) (see Box 8-8)

Drugs (see Table 8-2)

Iatrogenic

Post-thyroidectomy (bilateral)

INCREASED SERUM THYROID HORMONE VALUES

Hyperthyroidism

Drugs (see Table 8-2)

Thyroid hormone autoantibodies

Normal dog or cat

Iatrogenic

Excess thyroid hormone supplementation

indicated if clinical manifestations of hypothyroidism persist, the serum TSH concentration remains increased, or both, but is not necessarily indicated if the clinical response to treatment is good and the serum TSH concentration is in the reference range. The dose of levothyroxine sodium should be decreased whenever the

post-pill serum T_4 concentration is greater than 5 $\mu\text{g/dl}$ regardless of the presence of thyrotoxicosis.

Causes of Increased Serum T_4 Concentration • The primary disorder causing increased serum T_4 concentration is hyperthyroidism (see Box 8-9). Spontaneous feline hyperthyroidism is usually caused by multinodular adenomatous goiter. Less common are thyroid adenomas and malignant thyroid carcinomas. Functional thyroid tumors causing hyperthyroidism are uncommon in dogs but, when present, may be adenoma or carcinoma.

Oversupplementation of levothyroxine sodium to dogs with hypothyroidism and spurious increase caused by antithyroid hormone antibody interference with RIAs for serum T_4 measurement can also cause increased serum T_4 concentrations. Oversupplementation is diagnosed by history; antithyroid hormone antibodies should be suspected in dogs with clinical signs of hypothyroidism but increased serum T_4 concentration. Serum T_4 concentration above the reference range also occurs in normal dogs; 5% of the normal population of dogs falls outside 2 standard deviations (SDs) from the mean or the 95% confidence interval used to establish the normal range. Evaluation of serum TSH is useful in differentiating the causes of increased serum T_4 concentration in dogs.

History and physical examination are the basis for suspecting hyperthyroidism. Results of a CBC are usually normal in hyperthyroid cats. The most common abnormalities are a mild increase in the packed cell volume (PCV) and mean corpuscular volume (MCV). Common serum biochemical abnormalities include an increase in serum activities of ALT, alkaline phosphatase, and aspartate aminotransferase; the increase is typically in the mild to moderate range (i.e., 100 to 400 IU/L). One or more of these hepatic enzymes is increased in approximately 90% of hyperthyroid cats. Additional evaluation of the liver should be considered if hepatic enzyme activities are greater than 500 IU/L. Increased serum urea nitrogen and creatinine concentrations are identified in approximately 10% of hyperthyroid cats, findings that have important implications from the standpoint of the initial treatment implemented.⁴⁴ The urine specific gravity ranges from 1.008 to greater than 1.050. Most hyperthyroid cats have urine specific gravities greater than 1.035. Unfortunately, documentation of a concentrated urine specific gravity is not helpful in differentiating primary renal insufficiency from prerenal azotemia in hyperthyroid cats.³³ ECG abnormalities include tachycardia, increased R wave amplitude in lead II and, less commonly, right bundle branch block, left anterior fascicular block, widened QRS complexes, and atrial and ventricular arrhythmias. Thoracic radiographs may reveal cardiomegaly, pulmonary edema, or pleural effusion. ECG abnormalities depend on the form of thyrotoxic cardiomyopathy. The reader is referred to a text on ultrasonography for details of echocardiographic abnormalities.

Measurement of baseline serum T_4 concentration is reliable in diagnosing hyperthyroidism in most cats (Figure 8-8). Cats with mild or occult hyperthyroidism and hyperthyroid cats with significant nonthyroidal illness can have serum T_4 concentrations in the upper half of the reference range. If the serum T_4 test result is not definitive, measurement of serum T_4 should be repeated

and fT_4 measured in the same blood sample. If the diagnosis is still not established, a critical review of the case is warranted, the serum T_4 and fT_4 tests can be repeated in 4 to 8 weeks, a radionuclide (technetium) thyroid scan can be performed if available, or a triiodothyronine suppression test completed.

3,5,3'-TRIIODOTHYRONINE

Indications • Measurement of serum 3,5,3'-triiodothyronine (T_3) concentration is done during the T_3 suppression test for hyperthyroidism in cats. Although theoretical indications for the measurement of serum T_3 concentration are the same as those for serum T_4 , baseline serum T_3 concentration offers little additional diagnostic information beyond that obtained with serum T_4 concentration in identifying feline hyperthyroidism. It offers minimal to no value in assessing canine thyroid gland function, in part because the majority of circulating T_3 comes from conversion of T_4 to T_3 in peripheral tissues. Measurement of serum T_3 concentration is not currently recommended for assessment of thyroid gland function.

Advantages • None.

Disadvantages • Measurement of serum T_3 concentration is of questionable diagnostic usefulness; a myriad of variables can affect serum T_3 concentration.

Analysis • Same as for serum T_4 ; point-of-care ELISAs are not available for T_3 .

Normal Values • Dogs, 0.5 to 1.8 ng/ml; cats, 0.4 to 1.6 ng/ml.

NOTE: Different laboratories have different normal ranges.

Danger Values • None.

Variables That Affect Serum T_3 Concentration • The variables are the same as for serum T_4 concentration. The incidence of anti- T_3 antibodies is greater than anti- T_4 antibodies in dogs with lymphocytic thyroiditis.

Causes of Decreased Serum T_3 Concentration • Same as for serum T_4 concentration.

Causes of Increased Serum T_3 Concentration • Same as for serum T_4 concentration; spurious increase as the result of anti-thyroid hormone antibody interference with RIAs is more common with T_3 than T_4 .

FREE THYROXINE

Indications • Measurement of serum fT_4 concentration is usually reserved for those dogs with suspected hypothyroidism and nondiagnostic serum T_4 concentration, severe concurrent illness, or both, and cats with occult hyperthyroidism.

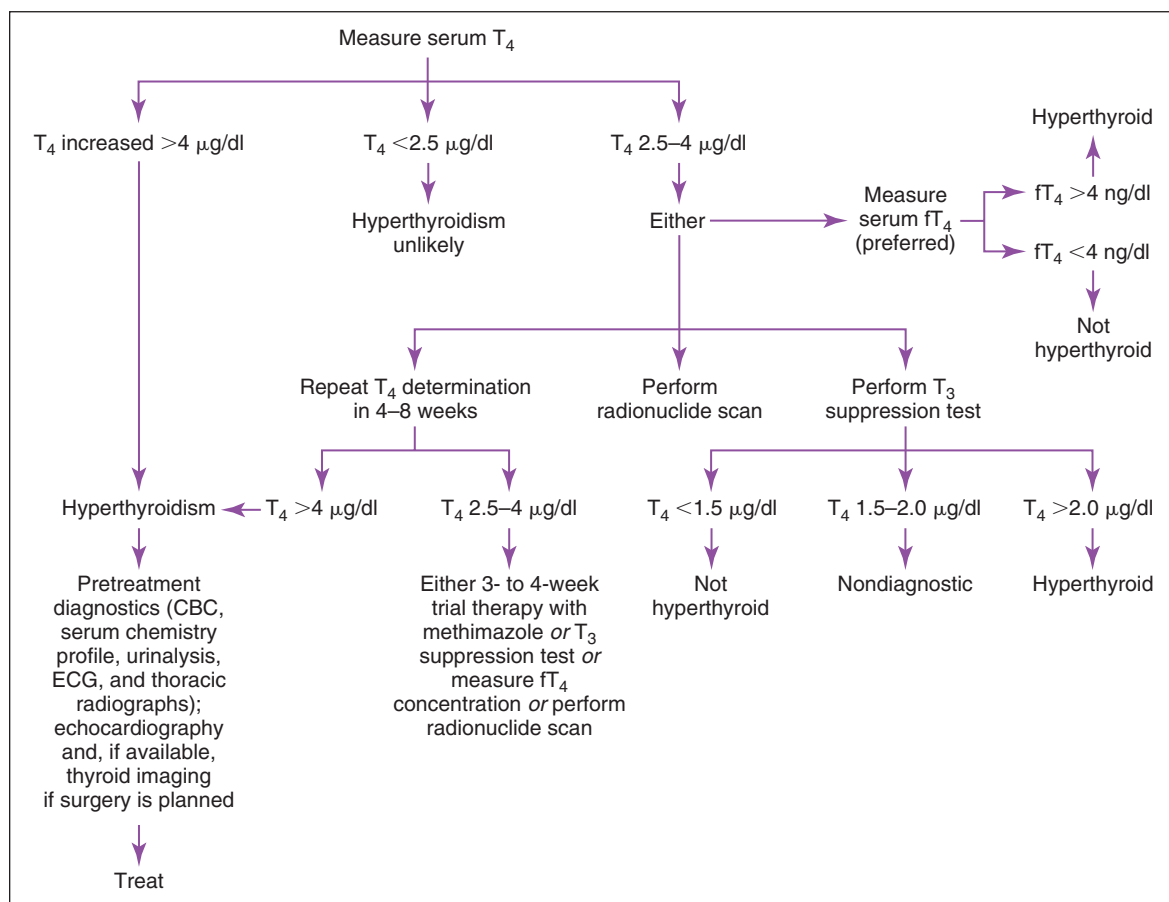


FIGURE 8-8 Evaluation of cats with suspected hyperthyroidism based on history, clinical signs (i.e., weight loss, polyphagia, polyuria-polydipsia, hyperactivity, cervical mass) or laboratory findings (i.e., increased serum alkaline phosphatase [SAP], increased serum phosphorus). CBC, Complete blood count; ECG, electrocardiogram; fT_4 , free thyroxine; T_3 , triiodothyronine; T_4 , thyroxine.

Advantages • The test is readily available and provides a more accurate assessment of thyroid gland function than serum T_4 concentration when measured by modified equilibrium dialysis (MED) techniques.²⁹ Studies evaluating newer fT_4 assays that do not utilize equilibrium dialysis have not been published, although preliminary findings suggest comparable results with ELISA assays and assays that use two incubation temperatures to separate free and protein-bound T_4 before measuring fT_4 by RIA. The hormone is stable during handling and shipping.

Disadvantages • Variables may affect serum fT_4 concentration, resulting in misinterpretation of results. In addition, the test is relatively expensive.

Analysis • Serum fT_4 is most commonly measured by RIA following separation of fT_4 from protein-bound T_4 using equilibrium dialysis, or by one- or two-step immunoassays.

Normal Values • Dogs, 0.8 to 3.5 ng/dl; cats, 1.0 to 4.0 ng/dl.

NOTE: Different fT_4 assays have different normal ranges. Different laboratories have different normal ranges. To convert from ng/dl to pmol/L, multiply by 12.87.

Danger Values • None.

Variables That Affect Serum fT_4 Concentration • Concurrent illness can suppress serum fT_4 concentrations, although alterations in serum concentrations of fT_4 are more variable than with serum T_4 and probably depend in part on the pathophysiologic mechanisms involved in the illness. In general, serum fT_4 concentrations tend to be decreased in dogs with concurrent illness but to a lesser extent than total T_4 concentrations. However, fT_4 concentrations can be less than 0.5 ng/dl if severe illness is present. Drugs and hormones that suppress serum T_4 can also suppress serum fT_4 concentrations (see Table 8-2). Unlike serum T_4 , circulating anti-thyroid hormone antibodies do not affect fT_4 results. The impact of many of the other variables known to affect serum T_4 concentrations have not yet been reported for serum fT_4 . Until

clinical studies indicate otherwise, the clinician should assume that variables affecting serum T_4 may have a similar effect on serum fT_4 concentration.

Causes of Decreased Serum fT_4 Concentration •

Causes are the same as for serum T_4 concentration. In the author's laboratory, serum fT_4 values less than 0.8 ng/dl (especially <0.5 ng/dl) suggest hypothyroidism, assuming that history, physical examination, and clinicopathologic abnormalities are also consistent with the disorder, and severe concurrent illness is not present.

Causes of Increased Serum fT_4 Concentration •

Causes are the same as for serum T_4 concentration. Measurement of serum fT_4 concentration provides a more accurate assessment of thyroid gland function than serum T_4 in some cats with occult hyperthyroidism.³⁰ Because of cost, measurement of fT_4 concentration is often reserved for cats with suspected hyperthyroidism where T_4 values are borderline. Occasionally, concurrent illness causes an increase in serum fT_4 concentration in cats (an increase that can exceed the reference range). For this reason, serum fT_4 concentration should always be interpreted in conjunction with serum T_4 concentration measured from the same blood sample. An increased serum fT_4 concentration in conjunction with high-normal or increased serum T_4 concentration is supportive of hyperthyroidism. An increased serum fT_4 concentration in conjunction with a low-normal or low serum T_4 concentration is supportive of euthyroid sick syndrome rather than hyperthyroidism.

BASELINE ENDOGENOUS THYROID-STIMULATING HORMONE

Indications • Baseline serum TSH is measured to assess thyroid gland function in dogs with suspected hypothyroidism and hyperthyroidism. Theoretically, measurement of TSH in conjunction with serum T_4 concentration, fT_4 concentration, or both should increase accuracy of diagnosing dogs with suspected hypothyroidism. The diagnostic value of serum TSH for identifying occult hyperthyroidism in cats remains to be determined.

Advantages • The test is readily available, the hormone is stable during handling and shipping, and testing may improve accuracy of assessing thyroid gland function when used in conjunction with measurement of other thyroid hormones.

Disadvantages • The test is of minimal diagnostic usefulness by itself: normal TSH concentrations are common in dogs with hypothyroidism, and increased TSH values may occur in dogs with concurrent nonthyroidal illness or with drugs (e.g., phenobarbital). Current TSH assays cannot identify low TSH values.

Analysis • Serum TSH can be measured using immunoradiometric, chemiluminescent immunometric, and enzyme immunometric assays.²⁰ The chemiluminescent immunometric assay has been validated in the cat.⁴³

Normal Values • Dogs, less than 0.03 to 0.6 ng/ml; senior cats, less than 0.03 to 0.15 ng/ml.

Danger Values • None.

Variables That Affect Serum TSH Concentration •

Many of the variables that decrease baseline thyroid hormone concentrations, most notably concurrent illness and drugs, may also increase serum TSH concentrations in euthyroid dogs, potentially causing misdiagnosis of hypothyroidism if the clinician accepts the results out of context (see Table 8-1 and 8-2). Serum TSH concentrations may be normal or increased depending, in part, on the effect of the concurrent illness or drug on fT_4 concentrations and on pituitary function. If pituitary function is suppressed, TSH concentrations will be in the normal range or undetectable. If pituitary response to changes in fT_4 concentration is not affected by the concurrent illness, or drugs, TSH concentrations will increase in response to a decrease in fT_4 . Serum TSH concentrations can easily exceed 1.0 ng/ml in dogs with concurrent nonthyroidal illness.

Causes of Increased Serum TSH Concentration • In dogs, increased serum TSH concentration is caused by primary hypothyroidism, euthyroid sick syndrome, and drugs. Ideally, baseline endogenous TSH should be increased and serum T_4 and fT_4 decreased in dogs with primary hypothyroidism. Unfortunately, serum TSH concentrations measured in hypothyroid dogs overlap with those for euthyroid dogs with concurrent illness, and approximately 20% of hypothyroid dogs have normal TSH concentrations (i.e., <0.6 ng/ml). In most studies, the sensitivity and specificity of the TSH assay have averaged approximately 80%.³⁷ Serum TSH concentration should always be interpreted in conjunction with the serum T_4 or fT_4 concentrations measured in the same blood sample and should never be used as the sole test of thyroid gland function. Finding a low serum T_4 or fT_4 concentration and a high TSH concentration in a blood sample obtained from a dog with appropriate history and physical examination findings supports the diagnosis of primary hypothyroidism, and finding normal serum T_4 , fT_4 , and TSH concentrations rules out hypothyroidism. Any other combination of serum T_4 , fT_4 , and TSH concentrations is difficult to interpret, and reliance on the history, physical examination findings, and serum fT_4 concentration is recommended.

Causes of Nondetectable Serum TSH Concentration • Current TSH assays cannot differentiate between normal and decreased serum TSH concentrations in dogs and cats. The lower limit of the reference range extends to an undetectable value. A nondetectable serum TSH concentration is of limited diagnostic value except in dogs with increased serum T_4 concentration and possibly cats with occult hyperthyroidism. Hyperthyroidism should result in a nondetectable TSH concentration because of negative feedback inhibition of pituitary thyrotroph function.⁴³ Identification of TSH in a blood sample obtained from a dog with an increased serum T_4 concentration or a cat with suspected hyperthyroidism is inconsistent with hyperthyroidism and

supports another reason for the increased serum T_4 concentration.

THYROGLOBULIN AUTOANTIBODIES

Indications • Measurement of thyroglobulin (Tg) autoantibodies is done to identify lymphocytic thyroiditis in dogs with hypothyroidism and possibly as a screening test for lymphocytic thyroiditis in currently euthyroid dogs intended for breeding. Tg autoantibodies occur in conjunction with T_3 and T_4 autoantibodies (see next section on **Thyroid Hormone Autoantibodies**) and are identified in dogs with lymphocytic thyroiditis that are not positive for T_3 and T_4 autoantibodies, implying that Tg autoantibody determination is a better screening test for lymphocytic thyroiditis than T_3 and T_4 autoantibodies. Presence of serum Tg autoantibodies implies thyroid pathology but provides no information on the severity or progressive nature of the inflammatory response or the extent of thyroid gland involvement, nor is this test an indicator of thyroid gland function. Tg autoantibodies should not be used alone in the diagnosis of hypothyroidism. Dogs with confirmed hypothyroidism can be negative and euthyroid dogs can be positive for Tg autoantibodies. Identification of Tg autoantibodies would support hypothyroidism caused by lymphocytic thyroiditis if the dog has clinical signs, physical findings, and thyroid hormone test results consistent with the disorder.

Tg autoantibodies may be used as a prebreeding screen for lymphocytic thyroiditis in breeding dogs. A positive Tg autoantibody test is considered suggestive of lymphocytic thyroiditis. However, the value of serum Tg autoantibodies as a predictor for eventual development of hypothyroidism in dogs that have no clinical signs and normal thyroid function test results remains to be clarified. Currently, a positive Tg autoantibody test is considered suggestive of lymphocytic thyroiditis and supports retesting thyroid gland function in 3 to 6 months.

THYROID HORMONE AUTOANTIBODIES

Indications • Measurement of thyroid hormone autoantibodies is done to explain unusual serum T_4 or T_3 values in dogs with suspected hypothyroidism, to identify lymphocytic thyroiditis in dogs with hypothyroidism, and as a screening test for lymphocytic thyroiditis in currently euthyroid dogs intended for breeding (see previous section on **Thyroglobulin Autoantibodies**). Interpretation of positive test results is similar to the interpretation of positive Tg autoantibody test results. Circulating thyroid hormone antibodies may interfere with RIA techniques used to measure serum T_4 and T_3 concentrations, causing spurious, unreliable numbers.⁴⁰ The type of interference depends on the RIA; falsely low and falsely high results can occur. Fortunately, the prevalence of clinically relevant concentrations of thyroid hormone antibody causing obvious spurious T_4 values accounts for less than 1% of assayed blood samples. Serum fT_4 measured by an equilibrium dialysis technique is not affected by T_4

autoantibodies and should be evaluated in lieu of serum T_4 in dogs suspected of having T_4 autoantibodies. See Appendix I for availability of measurement of circulating antibodies directed against T_3 and T_4 .

3,5,3'-TRIIODOTHYRONINE SUPPRESSION TEST

Indications • A T_3 suppression test is done to confirm hyperthyroidism in the cat with occult disease. The T_3 suppression test evaluates responsiveness of pituitary TSH secretion to suppression by liothyronine sodium. Administration of T_3 to normal cats should suppress pituitary TSH secretion, decreasing the serum T_4 concentration. Subsequent measurement of serum T_4 is accurate because exogenous T_3 cannot be converted to T_4 . Cats with hyperthyroidism experience autonomous secretion of thyroid hormone. Administration of T_3 to hyperthyroid cats should have no suppressive effect.

Advantages • The test is readily available, relatively inexpensive, and easy to interpret.

Disadvantages • It requires 3 days to complete, and accuracy depends on the owner's ability to administer the drug seven times and on the cat swallowing the tablets.

Protocol • Serum is obtained for determination of baseline serum T_4 and T_3 concentration. Owners then administer liothyronine sodium beginning the following morning at a dose of 25 μg three times a day for 2 days. On the morning of day 3, a seventh 25- μg dose is administered. Two to 4 hours later, a second blood sample is obtained for measurement of serum T_4 and T_3 concentrations.

Interpretation • Normal cats have postpill serum T_4 concentrations less than 1.5 $\mu\text{g}/\text{dl}$. Hyperthyroid cats have postpill T_4 concentrations greater than 2.0 $\mu\text{g}/\text{dl}$. Values between 1.5 and 2.0 $\mu\text{g}/\text{dl}$ are nondiagnostic. The percentage of decrease in serum T_4 concentration is not reliable, although suppression greater than 50% below baseline occurs in normal but not hyperthyroid cats.²⁸

Serum T_3 results are used to determine whether the owner successfully administered the thyroid medication to the cat. Serum T_3 concentration should increase in all cats properly tested, regardless of status of thyroid gland function. If serum T_4 concentration fails to decline in a cat in which serum T_3 concentration has not increased, problems with owner compliance are likely and test results are discarded.

THYROID-STIMULATING HORMONE STIMULATION TEST

Indication • The TSH stimulation test evaluates the thyroid gland's responsiveness to exogenous TSH administration. The primary advantage of this test is differentiation of hypothyroidism from the euthyroid sick syndrome in a dog with low baseline serum T_4 and fT_4 concentration.

Advantages • The test is easy to perform and usually accurate in differentiating hypothyroidism from other disorders decreasing serum T_4 concentrations.

Disadvantages • Only recombinant human TSH (rhTSH) is available, and it is expensive. Results are uninterpretable in some dogs.

Protocol • The current TSH stimulation protocol for dogs is 75 μ g of rhTSH per dog administered IV or intramuscularly (IM) and blood for serum T_4 concentration obtained before and 6 hours after rhTSH administration.³ The remaining reconstituted rhTSH can be stored at 4° C for 4 weeks and at -20° C for 8 weeks without loss of biological activity.⁷

Interpretation • Interpretation is based on absolute serum T_4 values. In a euthyroid dog, serum T_4 concentration should be greater than or equal to 2.5 μ g/dl 6 hours after rhTSH administration, and the 6-hour post-rhTSH serum T_4 concentration should be greater than or equal to 1.5 times the baseline serum T_4 concentration. Dogs with primary hypothyroidism typically have a post-rhTSH serum T_4 concentration less than 1.5 μ g/dl (Figure 8-9).²⁵ Post-rhTSH serum T_4 concentrations of 1.5 to 2.5 μ g/dl are nondiagnostic and may occur in early hypothyroidism or may represent thyroid gland suppression because of concurrent illness or drug therapy in an otherwise euthyroid dog. Severe systemic illness can cause post-rhTSH serum T_4 concentrations considered diagnostic for primary hypothyroidism (i.e., <1.5 μ g/dl). Similarly, low baseline serum T_4 concentration with normal post-rhTSH serum T_4 concentration is also difficult to interpret.

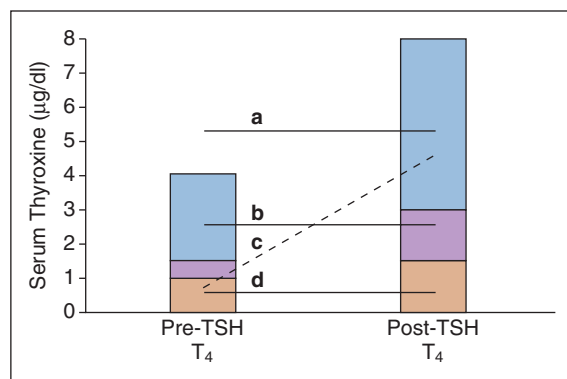


FIGURE 8-9 Interpretation of thyroid-stimulating hormone (TSH) stimulation test in dogs. Post-TSH thyroxine (T_4) values that fall into the normal range usually indicate normal thyroid gland function. Exceptions include high pre-TSH T_4 values with no increase in post-TSH T_4 (line a) and low pre-TSH T_4 values with normal response to TSH (line c). Primary hypothyroidism with anti- T_4 antibodies (lines a and b) and secondary hypothyroidism or the suppressive effects of concurrent disease (line c) should be considered. Low pre-TSH and post-TSH T_4 values (line d) and post-TSH T_4 values in the nondiagnostic range may be indicative of hypothyroidism or the suppressive effects of concurrent disease. (From Nelson RW, Couto CG: *Essentials of small animal internal medicine*, St. Louis, 1992, Mosby-Year Book, p 549.)

Assessment of history, physical findings, clinical pathologic assessment, and sometimes response to trial therapy may be required to ultimately determine thyroid gland function. Pre- and post-rhTSH serum T_4 concentrations above baseline in conjunction with a lack of response of the thyroid gland to TSH (see Figure 8-9) occur occasionally in dogs with hypothyroidism and may reflect circulating thyroid hormone antibodies in a dog with lymphocytic thyroiditis.

PITUITARY ADRENOCORTICOTROPIC HORMONE

Occasionally Indicated • In dogs, pituitary ACTH concentration is determined to differentiate pituitary-dependent from adrenal-dependent spontaneous hyperadrenocorticism and to differentiate primary versus secondary hypoadrenocorticism. Similar indications exist for cats. Measurement of baseline endogenous ACTH concentration is not used to diagnose hyperadrenocorticism because of episodic secretion and overlapping values between normal and pituitary-dependent hyperadrenocorticism (PDH).

Advantage • It is a reliable test to differentiate PDH from adrenocortical tumor (AT).

Disadvantages • ACTH is a fragile hormone, requiring meticulous care in specimen handling (see later). In addition, the test is expensive and has limited availability, and plasma samples must remain frozen until assayed.

Protocol • Although blood for ACTH determination has typically been drawn in the morning, the timing of the blood sample during the day probably does not affect interpretation of results. However, blood for ACTH determination must be drawn prior to performing tests that evaluate the pituitary-adrenocortical axis (i.e., ACTH stimulation test, LDDS). ACTH is a labile protein and adheres to glass. The blood sample must be placed in plastic or silicone-coated EDTA tubes and immediately placed on ice. Blood should be centrifuged immediately and plasma frozen in plastic tubes. This entire procedure should take less than 10 minutes. Samples must be shipped frozen and must stay frozen until assayed for ACTH.

Analysis • ACTH is measured in plasma by RIA or chemiluminescent immunometric assay.³⁴ The assay must be validated for the dog and cat.

Normal Values • Dogs, 10 to 70 pg/ml; cats, 10 to 60 pg/ml. Reference ranges may differ between laboratories. To convert from pg/ml to pmol/L, multiply by 0.220.

Interpretation in dogs and cats with hyperadrenocorticism:

- Less than 10 pg/ml: adrenal-dependent hyperadrenocorticism
- 10 to 45 pg/ml: nondiagnostic
- Greater than 45 pg/ml: pituitary-dependent hyperadrenocorticism

Interpretation in dogs with hypoadrenocorticism:

Less than 10 pg/ml: secondary hypoadrenocorticism

10 to 45 pg/ml: nondiagnostic

Greater than 45 pg/ml: primary hypoadrenocorticism

Danger Values • None.

Artifacts • ACTH concentration may be falsely decreased by storing plasma above freezing, sample thawing during transport to the laboratory, or using glass containers during collection or storage.

Drug Therapy That May Alter ACTH Values • ACTH concentration may be decreased by administration of glucocorticoids (e.g., dexamethasone). It may be increased by insulin.

Causes of Altered Plasma ACTH Concentration •

Alterations in baseline endogenous ACTH concentration result from primary pituitary gland disorders or disorders affecting blood cortisol concentration, with its negative inhibitory effects on ACTH secretion (Box 8-10). Pituitary disorders include PDH, which is associated with increased ACTH secretion (Figure 8-10), and secondary hypoadrenocorticism, which results from loss of function of the pituitary corticotroph cells and decreased ACTH secretion (Figure 8-11). Functional ATs of the zona fasciculata have increased cortisol secretion, which inhibits pituitary ACTH secretion; plasma ACTH typically decreases to undetectable concentrations (see Figure 8-10).³⁴ A similar phenomenon occurs with excessive exogenous glucocorticoid administration (i.e., iatrogenic hyperadrenocorticism). Destruction of the zona fasciculata (e.g., primary hypoadrenocorticism) decreases blood cortisol concentration, thus increasing plasma ACTH concentrations (see Figure 8-11). Similar alterations in plasma ACTH concentration occur in cats.

SERUM CORTISOL

Commonly Indicated • Serum cortisol is measured to assess the pituitary adrenocortical axis in patients with suspected hyperadrenocorticism or hypoadrenocorticism,

BOX 8-10. CAUSES OF ALTERED ENDOGENOUS PLASMA ADRENOCORTICOTROPIC HORMONE (ACTH) CONCENTRATION IN DOGS

Normal to Increased

Pituitary-dependent hyperadrenocorticism
Primary hypoadrenocorticism
Atypical hypoadrenocorticism

Normal to Decreased

Adrenal-dependent hyperadrenocorticism
Iatrogenic hyperadrenocorticism
Spontaneous secondary hypoadrenocorticism
Improper sample collection/storage

and to monitor medical treatment of hyperadrenocorticism. Findings suggestive of hyperadrenocorticism include polydipsia, polyuria, polyphagia, endocrine alopecia, panting, weakness, calcinosis cutis, epidermal and dermal atrophy, conformational changes (e.g., “potbellied appearance”), insulin-resistant diabetes mellitus, hepatomegaly, stress leukogram, increased serum alkaline phosphatase (SAP), hypercholesterolemia, persistent hyposthenuria, proteinuria, and recurring urinary tract infection, especially in middle-aged to older patients. Findings suggestive of hypoadrenocorticism include lethargy, depression, anorexia, vomiting, weakness, weight loss, bradycardia, hypovolemia, hyponatremia, and hyperkalemia, especially in young to middle-aged dogs and cats.

Advantages • The test is readily available, including point-of-care ELISAs, and the hormone is stable. Baseline serum cortisol concentration may help rule out primary hypoadrenocorticism.¹⁹

Disadvantages • Baseline serum cortisol concentration has no diagnostic significance when assessing a dog or cat for hyperadrenocorticism or when assessing response to medical treatment of hyperadrenocorticism; interpretation is only valid after manipulation of the pituitary-adrenocortical axis with ACTH or dexamethasone.

Analysis • Serum cortisol is typically measured by RIA or chemiluminescent immunoassays. Serum should be harvested soon after collection and refrigerated or frozen

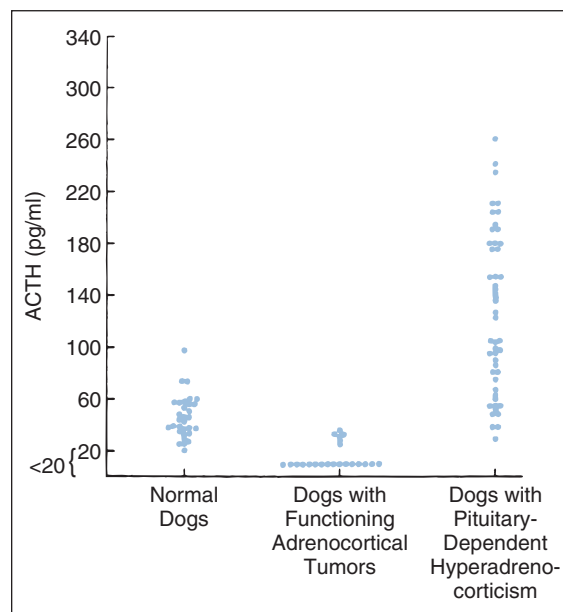


FIGURE 8-10 Endogenous plasma adrenocorticotropic hormone (ACTH) concentrations from clinically normal dogs, dogs with adrenal-dependent hyperadrenocorticism (adrenocortical carcinomas or adenomas), and dogs with pituitary-dependent hyperadrenocorticism. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, Philadelphia, 1987, WB Saunders.)

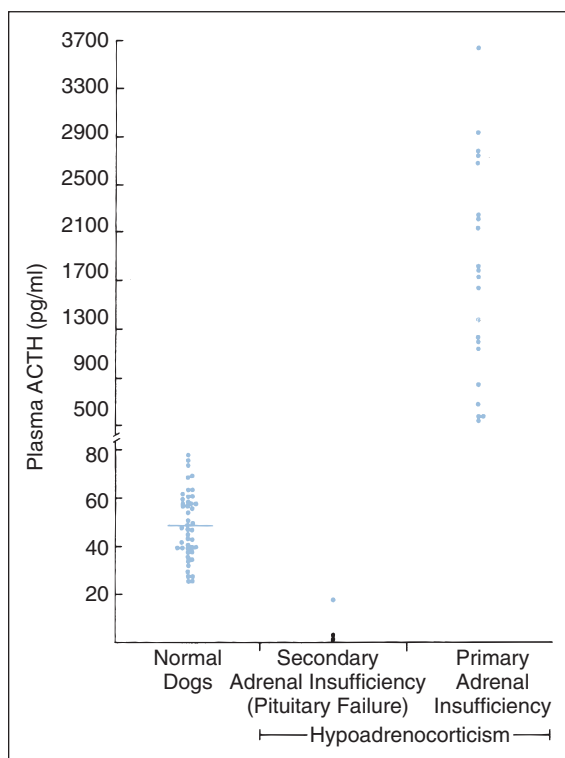


FIGURE 8-11 Endogenous plasma adrenocorticotrophic hormone (ACTH) concentrations in normal dogs, dogs with secondary adrenal failure, and dogs with primary adrenal failure. (From Feldman EC, Peterson ME: Hypoadrenocorticism. *Vet Clin North Am* 14:761, 1984.)

until assayed. If mailed, serum samples should be shipped with cool packs. Repeated freezing and thawing or hemolysis of the sample does not alter cortisol concentrations. Fasting for up to 36 hours does not affect cortisol values, and a clinically detectable diurnal variation does not exist in the dog or cat.

Normal Values • Dogs, 1.0 to 6.0 $\mu\text{g/dl}$; cats, 1.0 to 5.0 $\mu\text{g/dl}$. To convert from $\mu\text{g/dl}$ to nmol/L , multiply by 27.59.

Danger Values • None.

Artifacts Affecting Serum Cortisol Concentration •

Environment (e.g., “stress,” excitement) and chronic disease may increase baseline serum cortisol concentrations. Exogenous glucocorticoid preparations containing hydrocortisone, cortisone, prednisone, prednisolone, and possibly methylprednisolone may cross-react with some cortisol assays, causing spurious increases in measured cortisol values. Dexamethasone does not cross-react with cortisol assays. Serum specimens may be stored overnight at 2° to 8° C. Freezing is preferred for long-term storage.

Drug Therapy That May Alter Serum Cortisol •

Estrogen administration may increase serum cortisol. Chronic androgen or glucocorticoid administration and

BOX 8-11. CAUSES OF ALTERED RESTING PLASMA CORTISOL CONCENTRATIONS IN DOGS AND CATS

INCREASED

Stress, fear, excitement, aggression

Severe or chronic illness

Drugs

Cortisone, hydrocortisone, prednisone, and prednisolone (due to cross-reaction with cortisol assay)

Anticonvulsants

Hyperadrenocorticism

Pituitary-dependent

Adrenal-dependent

DECREASED

Improper storage

Iatrogenic hyperadrenocorticism

Progesterone-secreting adrenal tumor

Hypoadrenocorticism

Primary

Secondary (i.e., pituitary insufficiency)

Atypical (i.e., selective glucocorticoid deficiency)

Drugs

Progestagens

Megestrol acetate

Acute critical illness

progestagens such as megestrol acetate can decrease serum cortisol concentration.

Causes of Hypercortisolemia • The most clinically relevant cause of hypercortisolemia is hyperadrenocorticism (Box 8-11). Additional causes include environmental factors that create “stress” or excitement, chronic illness (e.g., diabetes mellitus, renal failure, hepatic failure, congestive heart failure), and medications that contain glucocorticoid preparations that cross-react with the cortisol assay. For the latter to cause increased cortisol values, medication must have been given within 12 to 24 hours of blood sampling and still be present in blood. Once glucocorticoid in the preparation has been metabolized, serum cortisol concentrations decrease because of the negative inhibitory effects of exogenous glucocorticoid on pituitary ACTH secretion. This phenomenon (i.e., iatrogenic hyperadrenocorticism) creates signs of hyperadrenocorticism, but the pituitary-adrenocortical axis is suppressed, resembling hypoadrenocorticism (Figure 8-12; see *Causes of Hypocortisolemia* later).

Hyperadrenocorticism occurs in dogs and rarely in cats. A tentative diagnosis of hyperadrenocorticism can be established based on history, physical examination, and routine clinical pathologic assessments (e.g., CBC, serum biochemical panel, urinalysis; see description under the Commonly Indicated heading at the beginning of this section). Common abnormalities identified on clinical pathologic assessments include a stress leukogram, increased SAP and ALT activities, hypercholesterolemia,

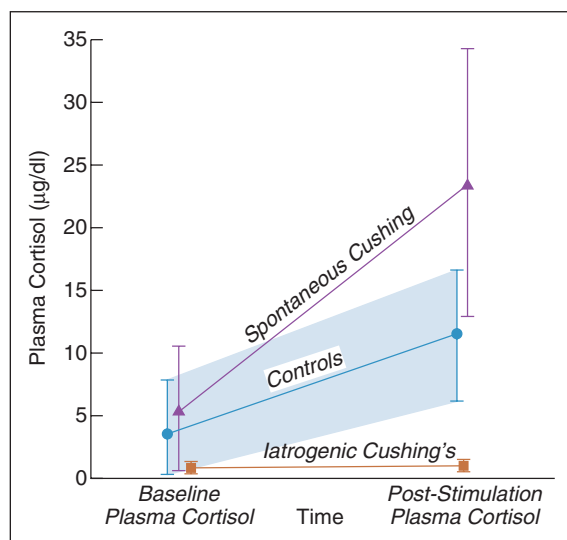


FIGURE 8-12 Mean plasma cortisol concentrations (± 2 SD) determined before and 1 hour after the administration of synthetic adrenocorticotropic hormone (ACTH) in control dogs, dogs with spontaneous hyperadrenocorticism, and dogs with iatrogenic hyperadrenocorticism. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, ed 2, Philadelphia, 1996, WB Saunders.)

and isosthenuria to hyposthenuria, proteinuria, and bacteriuria. In cats with hyperadrenocorticism, hyperglycemia and hypercholesterolemia are the most consistent clinical pathologic findings. Additional abnormalities in both species include increased serum amylase, lipase, and insulin concentrations and decreased baseline serum T_4 , fT_4 , T_3 , and possibly TSH concentrations. Many dogs with hyperadrenocorticism are misdiagnosed with primary hepatic disease because of hepatomegaly, increased hepatic enzymes (especially SAP), increased serum bile acid concentrations, and vacuolar hepatopathy.

Diagnosis of hyperadrenocorticism and differentiation between PDH and AT requires evaluation of serum cortisol concentrations after manipulation of the pituitary-adrenocortical axis with ACTH or dexamethasone (Figure 8-13). Baseline serum cortisol concentration has no diagnostic value for hyperadrenocorticism. Tests to confirm hyperadrenocorticism include the ACTH stimulation test and the LDDS test. Tests to differentiate between PDH and AT include endogenous ACTH concentration, the LDDS test, the high-dose dexamethasone suppression (HDDS) test, and abdominal ultrasonography. Diagnosis of iatrogenic hyperadrenocorticism is based on a history of glucocorticoid administration and ACTH stimulation test results. The reader is referred to appropriate headings that follow for specific information on these tests.

The most commonly used tests to diagnose hyperadrenocorticism in dogs and to differentiate PDH from AT are the ACTH stimulation test, the LDDS test, and abdominal ultrasonography. The urine cortisol:creatinine ratio (UCCR) can be assessed as part of the initial screen for hyperadrenocorticism or, more commonly, is assessed to further rule out hyperadrenocorticism if results of

previously listed tests are normal or inconclusive and the dog has ambiguous clinical signs. The dexamethasone suppression test, UCCR, and abdominal ultrasonography are used to establish the diagnosis of hyperadrenocorticism in cats. In the author's experience, the sensitivity of the ACTH stimulation test has been less than 50% in cats with hyperadrenocorticism.

Causes of Hypocortisolemia • The most clinically relevant cause of hypocortisolemia is primary hypoadrenocorticism (see Box 8-11), caused by destruction of the adrenal zona glomerulosa (mineralocorticoid-producing zone) and zona fasciculata (glucocorticoid-producing zone). Additional causes include iatrogenic hyperadrenocorticism (see previous discussion of **Causes of Hypercortisolemia**), secondary hypoadrenocorticism, progesterone-secreting ATs, megestrol acetate therapy, acute critical illness, and improper sample handling.⁴ Secondary hypoadrenocorticism (i.e., atypical hypoadrenocorticism) results from a selective deficiency of glucocorticoid secretion, which may result from inadequate ACTH secretion as the result of pituitary disease or result from a primary loss of function of the zona fasciculata; the latter is affiliated with an increased plasma ACTH concentration.⁴¹

Primary hypoadrenocorticism typically occurs in young adult dogs and rarely in cats. History, physical examination, and routine clinical pathologic assessments often suggest hypoadrenocorticism. Clinical pathologic abnormalities may include mild, nonregenerative anemia, lack of a stress leukogram in a sick dog or cat, hyperkalemia, hyponatremia, hypochloremia, azotemia, hyperphosphatemia, mild hypercalcemia (i.e., 12 to 14 mg/dl), mild hypoglycemia (i.e., 45 to 60 mg/dl), and metabolic acidosis. Urine specific gravity can range from hypersthenuric to isosthenuric (because of renal sodium wasting and loss of the renal medullary concentration gradient) and so can mimic primary renal disease. Cardiac conduction disturbances may develop with severe hyperkalemia (i.e., >7 mEq/L). If present, these disturbances are readily identified on a lead II rhythm strip of an ECG and may include dampened P wave, prolonged PR interval and QRS complex, spiked T wave, and ventricular arrhythmias.

Hallmark abnormalities identified on clinical pathologic assessments are hyperkalemia, hyponatremia, and hypochloremia.¹ The sodium:potassium ratio reflects changes in these electrolytes. Values are often well below 27:1 in primary hypoadrenocorticism (normal ratios, 27:1 to 40:1). Normal serum electrolyte concentrations, however, do not rule out adrenal insufficiency. Serum electrolyte concentrations may be normal in early disease, in selective hypocortisolism, in hypoadrenal patients that have recently received fluid therapy, and in secondary hypoadrenocorticism. Furthermore, other disorders (especially renal, gastrointestinal, and hepatic) can cause serum electrolyte changes mimicking adrenal insufficiency. The reader should refer to Chapter 6 for other causes of hyponatremia, hypochloremia, and hyperkalemia.

Baseline serum cortisol concentration can be used to rule out hypoadrenocorticism; serum cortisol concentrations greater than 2 µg/dl are inconsistent with the

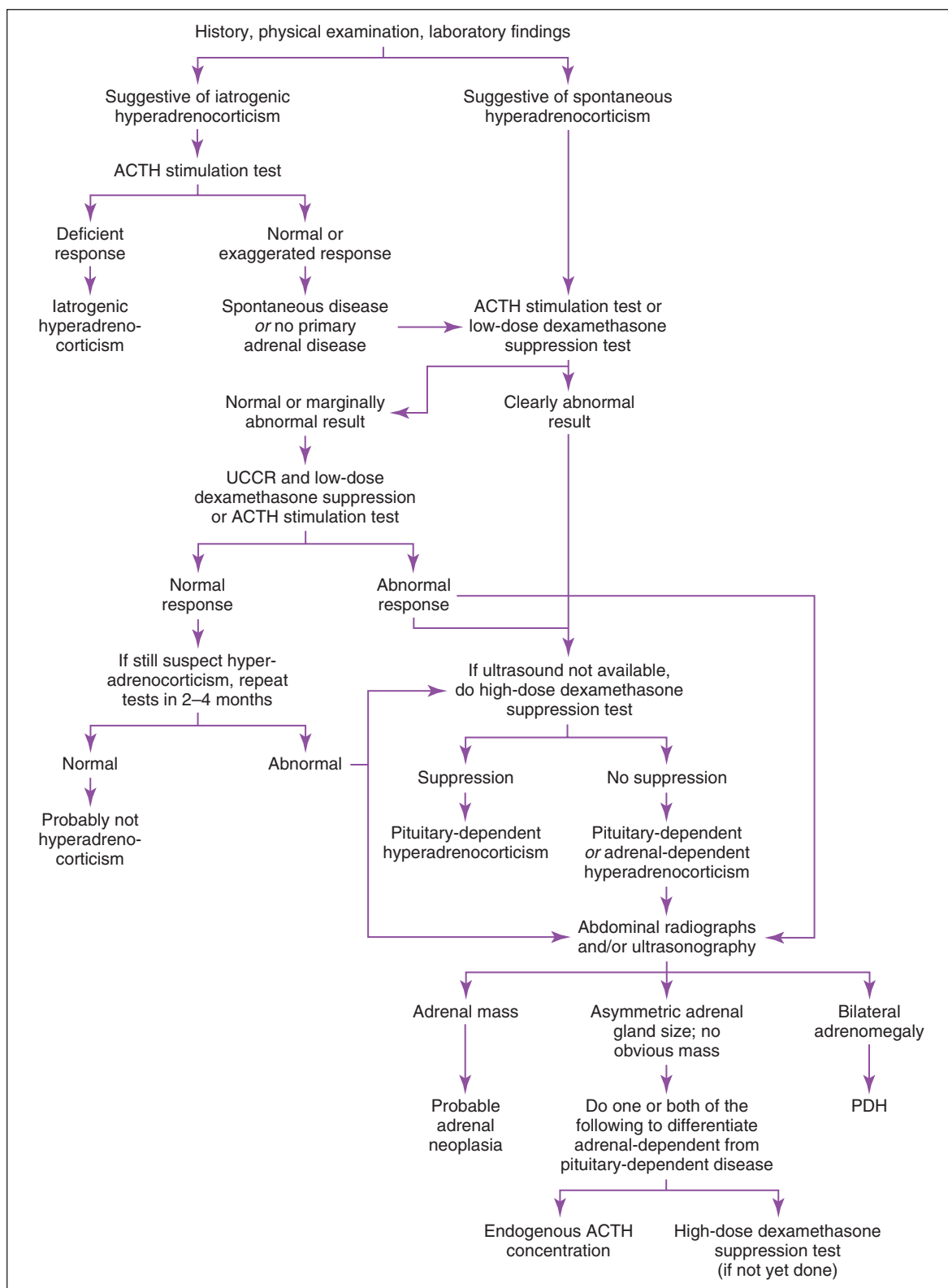


FIGURE 8-13 Evaluation of patients with suspected hyperadrenocorticism based on history (polyuria-polydipsia, polyphagia, panting, weakness), physical examination (truncal alopecia, potbelly, calcinosis cutis, hepatomegaly), or laboratory findings (increased serum alkaline phosphatase [SAP], lymphopenia, eosinopenia, hypercholesterolemia, urinary tract infection). ACTH, Adrenocorticotrophic hormone; PDH, pituitary-dependent hyperadrenocorticism; UCCR, urine cortisol:creatinine ratio.

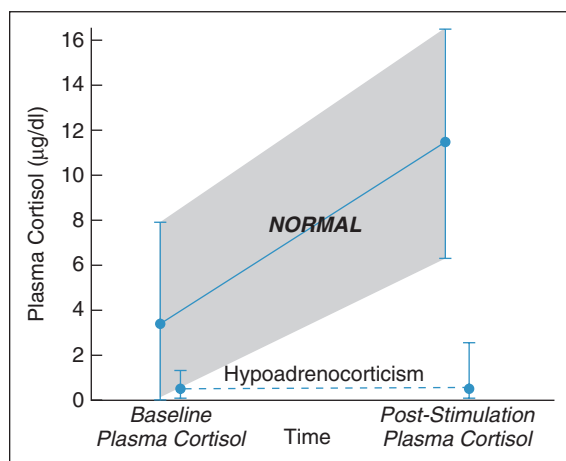


FIGURE 8-14 Plasma cortisol concentrations before and after exogenous adrenocorticotrophic hormone (ACTH) stimulation in normal dogs and in dogs with hypoadrenocorticism. The ranges are means $\pm$ 2 SD. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, ed 2, Philadelphia, 1996, WB Saunders.)

disease.¹⁹ Baseline serum cortisol concentrations less than 2 μ g/dl are identified in many situations (e.g., illness-induced relative adrenal insufficiency²¹) and do not confirm hypoadrenocorticism. Confirmation of hypoadrenocorticism requires an ACTH stimulation test. Animals with adrenal insufficiency have low or low-normal baseline serum cortisol concentration and minimal to no increase in serum cortisol after the administration of ACTH (Figure 8-14). The ACTH stimulation test does not distinguish primary from secondary adrenal insufficiency. Concurrent electrolyte abnormalities imply primary hypoadrenocorticism, but normal electrolyte concentrations do not differentiate early primary from secondary insufficiency.⁴¹ Differentiation requires measurement of baseline endogenous ACTH concentration or plasma aldosterone concentrations during the ACTH stimulation test (see [Plasma Aldosterone](#) later in this chapter).

ADRENOCORTICOTROPIC HORMONE STIMULATION TEST

Commonly Indicated • An ACTH-stimulation test is done to confirm hypoadrenocorticism and iatrogenic hyperadrenocorticism, to screen for spontaneous hyperadrenocorticism, and to monitor mitotane (o,p'-DDD), trilostane, and ketoconazole therapy in dogs with PDH. The ACTH stimulation test does not differentiate between PDH and AT.

Advantages • The test is readily available and easy to interpret.

Disadvantages • The test is expensive; false-positive and false-negative results are common when testing for spontaneous hyperadrenocorticism; results may be affected (i.e., exaggerated) by chronic illness and “stress”;

and the test does not differentiate between primary and secondary hypoadrenocorticism or between PDH and AT.

Protocol • The protocol for the ACTH-stimulation test differs between the dog and cat and with the type of ACTH used. When synthetic ACTH is used, blood for cortisol assay is obtained before and 1 hour after intramuscular (IM) administration of 0.25 mg (250 μ g) synthetic ACTH (irrespective of weight [dogs]) and before, 30 and 60 minutes after IM administration of 0.125 mg (125 μ g) synthetic ACTH (irrespective of weight [cats]). When using synthetic ACTH, a lower dose (5 μ g/kg IV or IM) is also effective in dogs and the unused reconstituted ACTH can be stored frozen at -20° C in plastic syringes for 6 months with no adverse effects on bioactivity of the ACTH. When ACTH gel is used, blood for cortisol assay is obtained before and 2 hours after (dog) and before and 1 and 2 hours after (cat) IM administration of 2.2 IU ACTH gel/kg body weight. The ACTH-stimulation test can be performed any time during the day.

Artifacts and Drug Therapy That May Alter Results • Anything altering serum cortisol concentration can affect ACTH stimulation test results (see [Artifacts That May Alter Serum Cortisol Concentration](#) earlier in this chapter). Anticonvulsant medications may spuriously increase ACTH stimulation test results.

Interpretation of post-ACTH cortisol concentration in dogs:

- Less than 2 μ g/dl: hypoadrenocorticism
- Less than 6 μ g/dl: suggestive of iatrogenic hyperadrenocorticism
- 6 to 18 μ g/dl: normal
- 18 to 24 μ g/dl: suggestive of spontaneous hyperadrenocorticism
- Greater than 24 μ g/dl: strongly suggestive of spontaneous hyperadrenocorticism

Interpretation of post-ACTH cortisol concentration in cats:

- Less than 2 μ g/dl: hypoadrenocorticism
- Less than 5 μ g/dl: suggestive of iatrogenic hyperadrenocorticism
- 5 to 15 μ g/dl: normal
- 15 to 20 μ g/dl: suggestive of spontaneous hyperadrenocorticism
- Greater than 20 μ g/dl: strongly suggestive of spontaneous hyperadrenocorticism
- To convert μ g/dl to nmol/L, multiply by 27.59.

LOW-DOSE DEXAMETHASONE SUPPRESSION TEST

Commonly Indicated • An LDDS test is done to screen for spontaneous hyperadrenocorticism and to differentiate between PDH and AT. This test does not identify iatrogenic hyperadrenocorticism, nor is it used to assess response to mitotane, trilostane, or ketoconazole therapy.

Advantages • The test is readily available, relatively inexpensive, and easy to interpret. It often confirms hyperadrenocorticism and identifies PDH at the same time.

Disadvantages • Results can be affected by acute “stress,” excitement, aggression, fear, and concurrent illness. In addition, other procedures must be avoided until test completion. False-positive and false-negative test results can occur.

Protocol • Ideally, this test should be started between 8:00 and 9:00 AM after the patient has been hospitalized overnight. The patient should rest quietly in the cage except when walked outside or when blood is taken. In the dog, a serum sample for cortisol analysis is obtained immediately before and 4 and 8 hours after IV administration of 0.01 mg dexamethasone/kg body weight. The same dose of dexamethasone is used in the cat, although blood samples are obtained immediately before and 4, 6, and 8 hours after IV dexamethasone administration. Dexamethasone sodium phosphate or dexamethasone in polyethylene glycol can be used.

Analysis and Artifacts • See Serum Cortisol earlier in this chapter.

Artifacts and Drug Therapy That May Alter Results •

Anything altering serum cortisol concentration can affect LDDS test results (see Artifacts That May Alter Serum Cortisol Concentration earlier in this chapter). Results may be affected by stress, fear, excitement, aggressive behavior, concurrent anticonvulsant drugs, concurrent diagnostics, exogenous glucocorticoids, and nonadrenal disease; the more severe the nonadrenal disease, the more likely the chance of obtaining false-positive test results.

Interpretation • Evaluation of the 8-hour post-dexamethasone serum cortisol concentration is used to confirm hyperadrenocorticism (Figure 8-15). Normal dogs have serum cortisol values less than 1.0 µg/dl, whereas dogs with PDH and AT have plasma cortisol concentrations greater than or equal to 1.4 µg/dl at 8 hours. In general, the higher the 8-hour post-dexamethasone serum cortisol concentration is above 1.4 µg/dl, the more supportive the test result is for hyperadrenocorticism. Cortisol concentrations of 1.0 to 1.4 µg/dl are suggestive of but not diagnostic for hyperadrenocorticism; the clinician must consider other information to establish the diagnosis.

If the 8-hour post-dexamethasone cortisol value supports hyperadrenocorticism, the 4-hour post-dexamethasone serum cortisol value may be useful in distinguishing between PDH and AT. Low doses of dexamethasone suppress pituitary ACTH secretion and serum cortisol concentrations in approximately 60% of dogs with PDH during the initial 2 to 6 hours of the test. Suppression does not occur in dogs with AT, nor does it occur in approximately 40% of dogs with PDH. Suppression is defined as (1) a 4-hour post-dexamethasone serum cortisol concentration less than 1.4 µg/dl, (2) a 4-hour post-dexamethasone serum cortisol concentration less than 50% of baseline concentration, and (3) an 8-hour

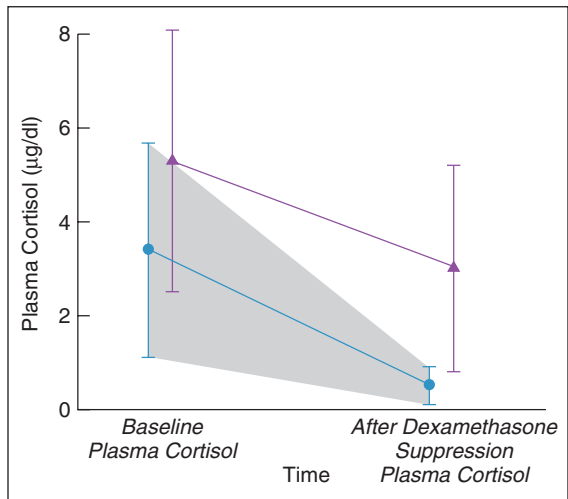


FIGURE 8-15 Mean plasma cortisol concentrations (± 2 SD) determined before and 8 hours after administration of a low dexamethasone dose (0.01 mg/kg) intravenously (IV) in control dogs (•) and in dogs with hyperadrenocorticism (▲). The reader should note the slight overlap of values after dexamethasone. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, Philadelphia, 1987, WB Saunders.)

post-dexamethasone serum cortisol concentration less than 50% of baseline concentration.⁹ Any hyperadrenal dog with serum cortisol concentrations meeting one or more of these three criteria usually has PDH. Failure to meet any of these criteria is consistent with lack of suppression. Lack of suppression is nonspecific, consistent with hyperadrenocorticism but not distinguishing between pituitary and adrenal disease.

This test is difficult to interpret in cats. An occasional normal cat will “escape” the suppressive effects of 0.01 mg dexamethasone/kg given IV and fall outside the normal 8-hour post-dexamethasone reference range (i.e., 8-hour post-dexamethasone serum cortisol > 1.4 µg/dl). A serum cortisol concentration greater than 1.4 µg/dl at 4, 6, and 8 hours strongly suggests hyperadrenocorticism. The test is inconclusive if either the 4- or 6-hour cortisol value is less than 1.4 µg/dl and the 8-hour value is greater than 1.4 µg/dl. These results suggest hyperadrenocorticism but may also occur in normal cats that have escaped the suppressive effects of dexamethasone. The test should be repeated using 0.1 mg dexamethasone/kg (see next section on [High-Dose Dexamethasone Suppression Test](#)). Because of potential for escape in normal cats, the LDDS test should never be the only test used to confirm diagnosis of feline hyperadrenocorticism.

HIGH-DOSE DEXAMETHASONE SUPPRESSION TEST

Infrequently Indicated • An HDDS test is done to distinguish PDH from AT in dogs with confirmed spontaneous hyperadrenocorticism and to confirm

hyperadrenocorticism in cats. The need for this test has declined because of the increasing availability of abdominal ultrasonography to evaluate adrenal glands.

Protocol • Ideally, this test should be started between 8:00 and 9:00 AM after the patient has been hospitalized overnight. The patient should rest quietly in the cage except when walked outside or when blood is obtained. In dogs, a serum sample for cortisol analysis is obtained immediately before and 8 hours after IV administration of 0.1 mg dexamethasone/kg body weight. Obtaining a 4-hour post-dexamethasone blood sample is optional; in the author's experience, the 4-hour HDDS sampling was informative in only 2% of dogs tested with both LDDS and HDDS.⁹ The same dose of dexamethasone is used in cats, although blood samples are obtained immediately before and 4, 6, and 8 hours after IV dexamethasone administration. Dexamethasone sodium phosphate or dexamethasone in polyethylene glycol can be used.

Interpretation • A higher dose of dexamethasone is used in an attempt to suppress pituitary ACTH secretion in dogs with PDH. Suppression is defined as a 4-hour or 8-hour post-dexamethasone serum cortisol concentration less than 1.4 $\mu\text{g/dl}$, and a 4-hour or 8-hour post-dexamethasone serum cortisol concentration less than 50% of baseline concentration. Any dog with hyperadrenocorticism and serum cortisol concentrations meeting one or more of these four criteria most likely has PDH. Approximately 75% of dogs with PDH meet at least one of the four criteria for "suppression" on the HDDS test. Failure to meet any of these criteria is consistent with lack of suppression. Among dogs with hyperadrenocorticism in which suppression is not demonstrated are approximately 25% of dogs with PDH and almost 100% of dogs with ATs (Figure 8-16).

The HDDS test is also used to diagnose feline hyperadrenocorticism. In normal cats, the serum cortisol concentrations should be less than 1.4 $\mu\text{g/dl}$ at 4, 6, and 8 hours. Similar results occur in some cats with PDH. Hyperadrenocorticism should be suspected if the 8-hour serum cortisol concentration is greater than 1.4 $\mu\text{g/dl}$. The likelihood of this disease is increased further if the 4- and 6-hour serum cortisol concentrations are also greater than 1.4 $\mu\text{g/dl}$. Any post-dexamethasone serum cortisol concentration less than 50% of the precortisol value supports PDH once hyperadrenocorticism is confirmed.

URINE CORTISOL: CREATININE RATIO

Indications • The UCCR is an excellent initial screening test for hyperadrenocorticism in dogs. The UCCR is increased in dogs with hyperadrenocorticism, compared with healthy dogs.⁸ Normal UCCR test results can occur in dogs with hyperadrenocorticism but are uncommon. Unfortunately, the specificity of the UCCR is low (20% in one study),³⁸ and this test should not be used as the sole test to diagnose hyperadrenocorticism.

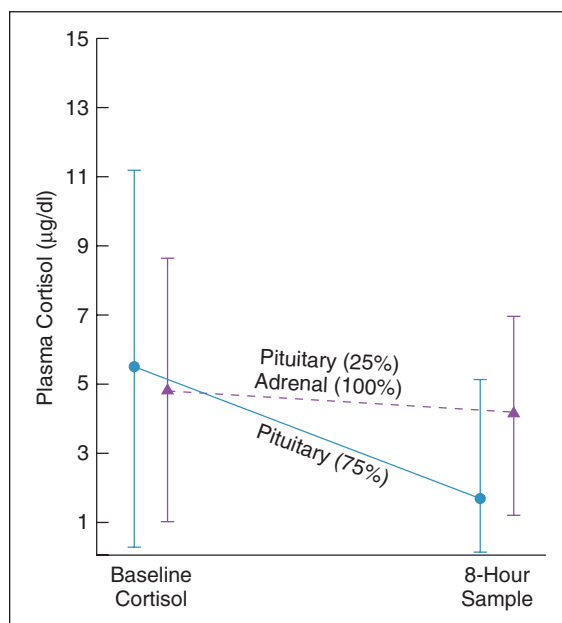


FIGURE 8-16 Patterns of plasma cortisol responses during high-dose (0.1 mg/kg) dexamethasone suppression testing in dogs with pituitary-dependent or adrenal-dependent hyperadrenocorticism. The reader should note that suppression is diagnostic of pituitary dependency. Lack of suppression occurs in all adrenal tumor cases and in 25% of pituitary-dependent cases. (From Feldman EC, Nelson RW: *Canine and feline endocrinology and reproduction*, Philadelphia, 1987, WB Saunders.)

Protocol • Ideally, the UCCR should be determined from free-catch urine samples obtained by the client in the nonstressful home environment. The stress associated with driving the dog to the veterinary hospital and having the dog undergo a physical examination or diagnostic tests before collecting urine can increase test results. Urine collected in the home environment should be refrigerated until the sample can be brought to the clinic for determination of cortisol and creatinine concentrations. The UCCR is determined by dividing urine cortisol concentration (in $\mu\text{mol/L}$) by urine creatinine concentration (in $\mu\text{mol/L}$).

Interpretation • A normal UCCR is inconsistent with hyperadrenocorticism. A UCCR greater than the reference range is consistent with hyperadrenocorticism and supports the performance of additional diagnostics (e.g., LDDS test) to confirm the diagnosis, assuming history, physical examination, and results of routine blood work also support a diagnosis of hyperadrenocorticism. However, an increased UCCR does not, by itself, confirm a diagnosis of hyperadrenocorticism. The UCCR is often increased in dogs with nonadrenal illness and in dogs with clinical signs consistent with hyperadrenocorticism but with a normal pituitary-adrenocortical axis (results that overlap with those obtained from dogs with hyperadrenocorticism). The UCCR is essentially used as a

screening test for normalcy. Additional tests for hyperadrenocorticism are indicated when the UCCR is increased or when the UCCR is normal but the clinical picture strongly suggests hyperadrenocorticism.

Interpretation of the UCCR in cats is similar to that in dogs (i.e., a normal ratio rules out hyperadrenocorticism, whereas an increased ratio is consistent with, but not definitive for, hyperadrenocorticism). UCCR test results must be interpreted based on reference ranges established in cats.

PLASMA ALDOSTERONE

Indications • Plasma aldosterone is measured to (1) identify selective aldosterone deficiency in patients with hyponatremia, hyperkalemia, and normal plasma cortisol responsiveness to ACTH; (2) distinguish primary adrenal disease from secondary adrenal atrophy because of a pituitary or hypothalamic deficiency in dogs with normal serum electrolytes and hypoadrenal ACTH stimulation test results; (3) identify hyperaldosteronism; and (4) assess hormonal function of an adrenal mass. Clinical findings with hyperaldosteronism include lethargy, weakness, hypokalemia, hypernatremia, hypertension, and adrenomegaly.

Analysis • Aldosterone is measured in EDTA or heparinized plasma, or in serum by RIA, or enzyme- or chemiluminescence-based immunoassays. Specimens should be stored frozen. In the author's laboratory, serum and heparinized plasma yield comparable results. Baseline plasma aldosterone concentration is usually diagnostic for primary hyperaldosteronism. Identification of aldosterone deficiency requires interpretation of plasma aldosterone concentration after stimulation with ACTH (see ACTH Stimulation Test earlier in this chapter). Timing of blood sampling is the same as for serum cortisol. For meaningful results, the aldosterone assay must be validated for use in each species to be tested and normal values established. See Appendix I for availability of testing. Handling and shipping are as described for cortisol.

Normal Values • Baseline in dogs: 5 to 345 pg/ml; post-ACTH stimulation: 70 to 760 pg/ml. Baseline in cats: 70 to 140 pg/ml. Reference ranges may differ between laboratories. To convert pg/ml to pmol/L, multiply by 2.77.

Danger Values • None.

Artifacts • Storage at 22° C for greater than or equal to 3 days or at 37° C for 1 day decreases measured plasma aldosterone concentration. High sodium intake may suppress, whereas low sodium intake may increase serum aldosterone concentrations.

Interpretation • Measurement of plasma aldosterone is potentially useful for differentiating primary from secondary hypoadrenocorticism and in the evaluation of dogs with suspected glucocorticoid but not aldosterone deficiency (i.e., atypical hypoadrenocorticism). Hypoaldosteronism is documented by finding low baseline and

post-ACTH plasma aldosterone concentrations. Unfortunately, plasma aldosterone concentrations may be in the lower end of the reference range in dogs with primary hypoadrenocorticism and may be within or below the reference range in dogs with suspected atypical hypoadrenocorticism. Markedly increased baseline plasma aldosterone concentrations suggest primary hyperaldosteronism in dogs with no other explanation for hypokalemia, hypernatremia, and systemic hypertension. Identification of an adrenal mass with abdominal ultrasound further supports the diagnosis.

INSULIN-LIKE GROWTH FACTOR-I

Indication • Measurement of insulin-like growth factor-I (IGF-I) concentration is done as a screening test for acromegaly in diabetic cats with insulin resistance. Most of the growth-promoting effects of growth hormone (GH) are mediated by IGF-I. The liver is a major site of IGF-I synthesis, and much of the circulating IGF-I is believed to be derived from the liver. IGF-I has an approximately 50% homology of structure with proinsulin and insulin, and the IGF-I cell membrane receptor resembles the insulin receptor in its structure. Secretion of IGF-I is under direct control of GH. As such, serum IGF-I concentrations are increased in acromegaly.

Analysis • IGF-I is measured in serum by RIA.

Normal Values • Cats, 5 to 70 nmol/L.

Artifacts • Unknown.

Drug Therapy That May Alter Serum IGF-I • Unknown.

Role as a Diagnostic Test for Feline Acromegaly • Clinical suspicion for acromegaly is based on identification of conformational alterations (e.g., increased body size, large head, prognathia inferior, organomegaly) and a stable or progressive increase in body weight in a cat with insulin-resistant diabetes mellitus. Measurement of serum IGF-I concentration provides further evidence for the diagnosis of acromegaly. Concentrations are usually increased in acromegalic cats, but values may be in the reference range in the early stages of the disease.² Repeat measurements performed 4 to 6 months later will usually demonstrate an increase in serum IGF-I if acromegaly is present. The increase in serum IGF-I typically coincides with development and growth of the pituitary somatotrophic adenoma. Increased serum IGF-I concentrations have been identified in a small number of poorly controlled diabetic cats where poor controlled was not caused by acromegaly. Interpretation of serum IGF-I test results should always take into consideration the status of control of the diabetic state, the presence and severity of insulin resistance, and the index of suspicion for acromegaly based on review of the history, physical examination, and results of routine blood and urine tests and diagnostic imaging. Identifying an increased serum IGF-I concentration in a poorly controlled diabetic cat with insulin resistance and

clinical features suggestive of acromegaly supports the diagnosis and provides justification for computed tomography or magnetic resonance imaging of the pituitary gland.

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Gastrointestinal, Pancreatic, and Hepatic Disorders

9

Michael D. Willard and David C. Twedt

Some gastrointestinal (GI) problems (e.g., vomiting, diarrhea, weight loss, anorexia, icterus, hepatomegaly, abnormal behavior associated with eating, abdominal pain) typically necessitate laboratory testing. On the other hand, dysphagia, regurgitation, ptyalism, halitosis, constipation, mucoid stools, hematochezia, and melena are usually best approached initially by other means (e.g., physical examination, radiology, ultrasonography, endoscopy, laparotomy, and/or biopsy).

DIFFERENTIATION OF EXPECTORATION, REGURGITATION, AND VOMITING

Whenever fluid, mucus, foam, food, or blood is expelled from the mouth, one must determine whether vomiting, regurgitation, gagging, or expectoration is occurring. The history sometimes allows differentiation.

Expectoration

Expectoration is the coughing up of material from the lungs or major airways. The material typically is frothy mucus or red blood; bile and food are absent. The characteristic sequence of coughing followed by oral expulsion must be determined from the history or observation. Regurgitation and vomiting typically occur without simultaneous coughing, although regurgitation is often accompanied by tracheitis and aspiration pneumonia. However, a patient that is expectorating may “gag” so hard because of pharyngeal/laryngeal irritation that it eventually vomits.

Regurgitation

Regurgitation is due to oral, pharyngeal, or esophageal dysfunction and is typically characterized as a relatively passive expulsion of esophageal contents. Gagging is the expulsion of oral or pharyngeal material and may be associated with disorders causing dysphagia (i.e., difficult swallowing) or regurgitation. The relatively minor

abdominal contractions associated with gagging are typically different than the vigorous abdominal contractions that classically occur with vomiting. Regurgitation may follow seconds to hours after eating or drinking. Patients may regurgitate white foam (i.e., salivary secretions that have been swallowed) and/or food. Regurgitated food material is undigested and sometimes has a tubular form conforming to the shape of the esophageal lumen. Most clients cannot reliably distinguish undigested from digested food. Regurgitated material that has remained in the esophagus for long time periods can appear “partially digested” because it is macerated, odoriferous, and mixed with saliva. If blood is present, it is usually undigested (i.e., bright red), whereas blood originating from the stomach is usually partially digested by gastric acid and has a “coffee grounds” appearance readily distinguishing it from the undigested form (unless the patient vomits before the blood can be partially digested).

It is sometimes difficult to differentiate vomiting from regurgitation via history, and in some patients the processes are concurrent. Vomiting may cause secondary esophagitis and subsequent regurgitation, or a patient with long-standing esophageal disease may develop another concurrent disorder causing vomiting. It is therefore important to clarify the chronologic order of specific signs. Finally, some patients with signs “classic” for regurgitation are vomiting instead. To aid in differentiation, one may attempt to observe the act of expulsion by feeding the patient, although this is very unreliable (“watched” regurgitating patients often do not regurgitate). Watching the patient eat occasionally helps if there is obvious pharyngeal dysphagia that suggests oropharyngeal disease. Some patients with pharyngeal dysphagia also have concurrent esophageal dysfunction. Contrast radiographs and/or fluoroscopy of the pharynx and esophagus can usually differentiate vomiting from regurgitation.

Regurgitation is usually best evaluated by history, physical examination, plain and contrast radiographs, and/or esophagoscopy (Figure 9-1). Contrast radiographs should use barium instead of iodide contrast agents unless esophageal rupture is suspected (e.g., finding air or fluid in the mediastinum on plain radiographs). The

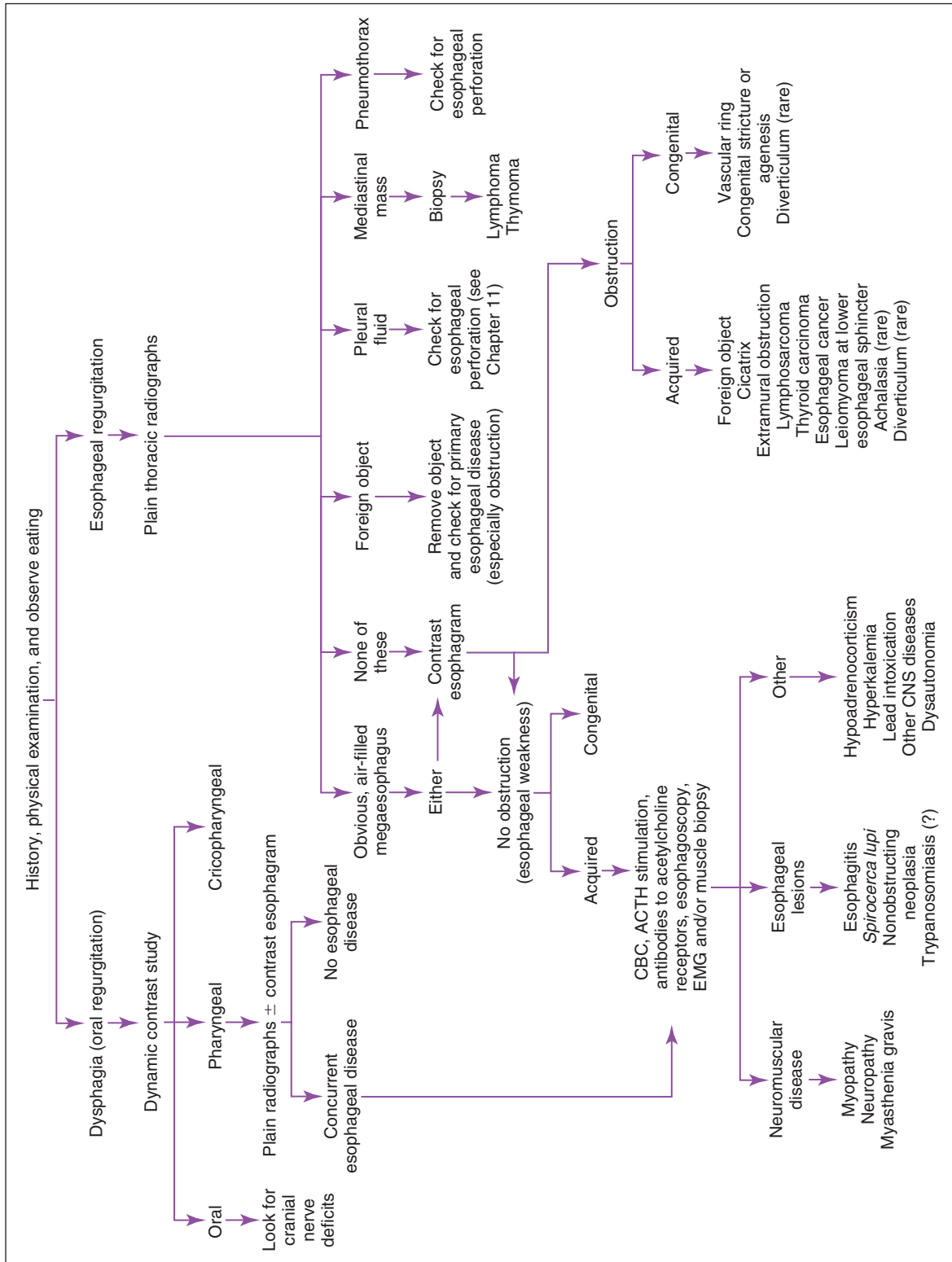


FIGURE 9-1 Diagnostic approach to chronic regurgitation in dogs and cats. ACTH, Adrenocorticotrophic hormone; CBC, complete blood count; CNS, central nervous system; EMG, electromyogram.

main purpose of a contrast esophagram is to distinguish esophageal motility abnormalities from anatomic lesions (e.g., obstruction, mass, inflammation, fistula). Some drugs (e.g., xylazine, ketamine) can cause esophageal hypomotility, making the radiographs potentially misleading. Esophagoscopy is insensitive for diagnosing esophageal muscular weakness but sensitive for finding anatomic lesions, differentiating intramural from extramural obstruction, identifying esophagitis, and removing foreign objects. Patients with acquired esophageal weakness should be evaluated for myopathies, neuropathies, and myasthenia gravis (generalized or localized to the esophagus). Occasionally, hypoadrenocorticism, hyperkalemia, lead poisoning, *Spirocerca lupi*, and selected central nervous system (CNS) disorders (e.g., distemper, hydrocephalus) may be responsible. Generalized or localized myopathies and neuropathies have several causes (e.g., trauma, dermatomyositis, thymoma, botulism, tick paralysis, systemic lupus erythematosus, nutritional factors, toxoplasmosis, trypanosomiasis). Dysautonomia occurs in dogs and cats, causing generalized dysfunction of the autonomic nervous system producing esophageal hypomotility. It is important to detect underlying disorders so that one may treat the cause rather than just the symptoms. It is also wise to evaluate patients with unexpected esophageal foreign objects (e.g., a relatively small bolus of food) for partial obstructions (e.g., subclinical vascular ring anomaly, stricture).

Vomiting

Vomiting is a reflex act originating in the CNS that can be stimulated by various conditions. One must consider primary GI disease and non-GI disorders (e.g., metabolic, inflammatory, and toxic conditions) as causes of vomiting. Many vomiting patients have non-GI problems.

Vomiting is classically characterized by prodromal nausea (i.e., salivation, licking of lips) followed by retching or forceful abdominal contractions. Vomiting may occur any time after eating or drinking (seconds to hours). A patient may vomit food, water, fresh blood, or mucus that is indistinguishable from regurgitated material. Bile, partially digested blood (i.e., “coffee grounds”), or expelled material with a pH of 5 or less strongly suggests vomiting as opposed to regurgitation. Vomited duodenal contents may have a pH greater than or equal to 7 and are usually positive for bile. A urine dipstick with a pH indicator is useful in making pH determinations. A patient that has “dry heaves” is typically vomiting as opposed to regurgitating.

Vomiting patients are best divided into those with acute (<2 weeks) versus those with chronic (>2 weeks) vomiting. The most common categories of causes for each are listed in [Boxes 9-1 and 9-2](#). Patients with acute vomiting often spontaneously resolve if they are supported by fluid therapy. A thorough history and physical examination are indicated first. Laboratory evaluation and/or imaging should be considered if the disease is severe or a serious disease (e.g., obstruction) is suspected. If vomiting persists, is progressive, or is attended by other clinical signs (e.g., polyuria-polydipsia [pu-pd], weight loss, icterus, painful abdomen, ascites, weakness, hematemesis), additional testing is indicated ([Figure 9-2](#)).

BOX 9-1. MAJOR CAUSES OF ACUTE VOMITING IN DOGS AND CATS

Motion Sickness

Acute Gastritis-Enteritis (various viral or bacterial agents or toxins)

Parvoviral enteritis (dogs and cats)

Hemorrhagic gastroenteritis

Parasites

Gastrointestinal (GI) Obstruction

Foreign body (obstructing or linear)

Intussusception

Diet

Overeating

Poor-quality or spoiled food

Food to which patient is allergic or intolerant

Acute Pancreatitis

Iatrogenic (Drugs)

Amoxicillin plus clavulanic acid

Chemotherapeutics (e.g., cisplatin, cyclophosphamide, doxorubicin)

Chloramphenicol

Digitalis

Erythromycin

Narcotics

Nitrofurantoin

Tetracyclines (including doxycycline)

Theophylline

Xylazine

Intoxication

Ethylene glycol

Mushrooms

Organophosphates

Pesticides (including herbicides, fungicides, etc.)

Diet and Parasites

Diet and parasites commonly cause acute and chronic vomiting; hence, dietary change (to a bland or hypoallergenic diet), fecal examination, and broad-spectrum anthelmintic therapy (e.g., fenbendazole, pyrantel) are reasonable initial choices in patients not suspected of having a clinically important disease. Continued vomiting is an indication for laboratory tests or imaging.

Obstruction

Gastric or intestinal obstruction does not usually require clinicopathologic testing for diagnosis. A complete blood count (CBC) may suggest sepsis, disseminated intravascular coagulation (DIC), or severe blood loss. Renal function, electrolyte, and acid-base evaluations are recommended before anesthesia. One cannot reliably predict changes in these parameters even when the site of obstruction is known. Persistent and profuse loss of gastric contents from any cause may produce

BOX 9-2. MAJOR CAUSES OF CHRONIC VOMITING IN DOGS AND CATS**Gastrointestinal Obstruction**

Foreign objects (*common*)
 Intussusception
 Neoplasia (gastric or intestinal)
 Pyloric stenosis (*infrequent*)
 Gastric antral mucosal hyperplasia
 Inflammatory infiltrates of the stomach or intestines (e.g., pythiosis, eosinophilic masses)
 Chronic partial gastric volvulus (*uncommon*)
 Hypomotility of stomach/intestines (physiologic obstruction) (*uncommon*)
 Congenital structural abnormalities (*rare*)

Abdominal Inflammation

Pancreatitis (*common*)
 Chronic enteritis (dietary-responsive or antibiotic-responsive) (*common*)
 Gastrointestinal ulceration/erosion
 Peritonitis (sterile or septic)
 Inflammatory bowel disease
 Chronic gastritis (*infrequent*)
 Pharyngitis (caused by upper respiratory virus in cats) (*rare*)
 Parasites (e.g., *Physaloptera*) (*regionally important*)

Systemic (extra-alimentary tract diseases) (*common*)

Hepatic disease/insufficiency (*common*)
 Hypoadrenocorticism (*uncommon but important*)
 Diabetic ketoacidosis (*common*)
 Uremia (*common*)
 Hypercalcemia (*important*)
 Cholecystitis
 Pyometra (*common*)
 Feline hyperthyroidism (*common*)
 Central nervous system (CNS) disease (e.g., "limbic epilepsy", tumor, encephalitis, or increased intracranial pressure) (*rare*)
 Psychotic or behavioral changes (*rare*)

hypokalemic, hypochloremic metabolic alkalosis with aciduria. However, most patients with gastric vomiting are not alkalotic. Insignificant acid-base changes or metabolic acidosis due to dehydration are probably more common. Intestinal obstruction may cause acidosis due to loss of pancreatic bicarbonate, although some patients have a normal blood pH or a metabolic alkalosis if the obstruction is high in the duodenum.

Abdominal radiographs and ultrasound are the best initial tests. In otherwise occult cases, contrast radiographs may be necessary, in which case barium is preferred over iodide compounds unless intestinal rupture is suspected. Barium leakage into the abdomen causes peritonitis and requires vigorous abdominal lavage at the time of surgery (see Chapter 10).

Extra-alimentary Tract Disease

A serum chemistry profile should be obtained to help rule out hepatic disease (alanine aminotransferase [ALT], serum alkaline phosphatase [SAP], blood urea nitrogen [BUN], and albumin), hypoadrenocorticism (sodium and potassium), hypercalcemia (calcium and albumin), uremia (creatinine, BUN, and urinalysis), and diabetic ketoacidosis (glucose and urinalysis). Very young (i.e., <12 to 14 weeks of age) and very small (i.e., <3 kg) patients should undergo blood glucose monitoring to detect secondary hypoglycemia. More precise testing is occasionally required to diagnose these disorders (e.g., serum bile acids for hepatic insufficiency, adrenocorticotrophic hormone [ACTH] stimulation test for hypoadrenocorticism). Other tests to consider are serum gastrin for gastrinoma, and serum thyroxine for feline hyperthyroidism.

Pancreatitis

Acute pancreatitis occurs commonly. Predisposing causes in dogs include hyperlipidemia, fatty meals, or obesity. Pancreatitis can occur in any dog, but middle-aged obese female dogs, schnauzers, and Yorkshire terriers seem to be predisposed. Vomiting may or may not be associated with eating, abdominal pain, fasting hyperlipidemia, bloody diarrhea, and, rarely, diffuse subcutaneous fat necrosis. On radiographic examination, a mass or indistinctness due to localized peritonitis may be visible in the cranial right abdominal quadrant. CBC, serum amylase, and serum lipase activities are insensitive and nonspecific; patients with pancreatitis can have almost any result on these tests. Increased ALT and SAP concentrations (as the result of the proximity of the pancreas to the liver and obstruction of the biliary duct) are common but insensitive and nonspecific. Mild to moderate hypocalcemia sometimes occurs. Abdominal ultrasonography can be very specific for canine pancreatitis, but its sensitivity depends upon operator skill and timing (i.e., ultrasound findings can change dramatically in a matter of hours). If a pancreatic mass is discovered during surgery, it must be biopsied; chronic pancreatitis can be grossly indistinguishable from pancreatic neoplasia. The canine immunoreactive pancreatic lipase (spec cPL) (IDEXX, Westbrook, ME) test is the most sensitive test for pancreatitis, but its specificity for clinically important disease is still being determined. Chronic pancreatitis may be presumptively diagnosed if one finds exocrine pancreatic insufficiency in a breed that is not affected by pancreatic acinar cell atrophy.

Pancreatitis is an important but difficult-to-diagnose disease in cats. Chronic pancreatitis in older cats sometimes occurs in conjunction with cholangiohepatitis and/or inflammatory bowel disease (IBD) (often referred to as a *triaditis syndrome* involving all three organs). Vomiting is not as prominent in feline pancreatitis as it is in canine pancreatitis. Feline trypsin-like immunoreactivity (fTLI) concentrations are increased in some patients. Abdominal ultrasonography is specific, but the sensitivity is uncertain. A pancreatic biopsy may be required for a definitive diagnosis. The feline immunoreactive pancreatic lipase (spec fPL) test appears to be useful in diagnosing pancreatitis. Feline pancreatitis occasionally is due to

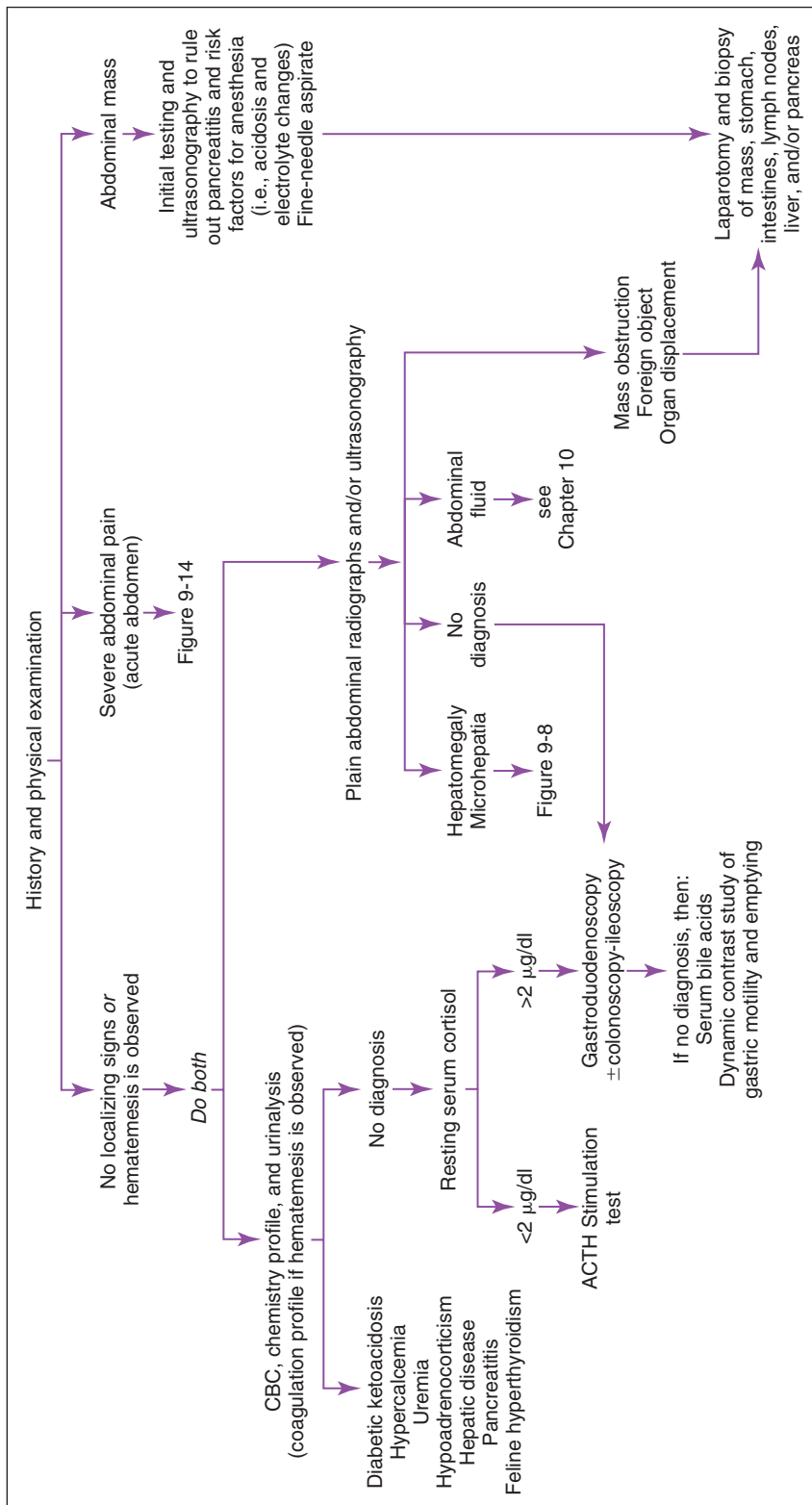


FIGURE 9-2 Diagnostic approach to chronic vomiting in a dog or cat that has been unresponsive to dietary change and anthelmintic therapy. ACTH, Adrenocorticotropic hormone; CBC, complete blood count.

toxoplasmosis or to feline infectious peritonitis (FIP) (see Chapter 15).

Gastritis, Enteritis, and Colitis

Chronic enteritis, colitis, or gastritis can cause various degrees of vomiting and may require mucosal biopsy for diagnosis. Abdominal ultrasound is potentially specific and may delineate infiltrative or inflammatory intestinal patterns, but it is insensitive. If gastritis or enteritis is suspected or if the other major causes of chronic vomiting have been ruled out, gastric and intestinal mucosal biopsies via endoscopy or laparotomy may be helpful. Inflammatory bowel disease is an important cause of feline chronic vomiting. Duodenitis is also a significant cause of vomiting without diarrhea in dogs; therefore both gastric and intestinal biopsies should be performed. Finally, because 10% to 20% of patients with colitis vomit, it is sometimes helpful to perform endoscopy on the upper and lower intestinal tracts in patients (especially cats) with chronic vomiting. It is critical that good-quality mucosal tissue samples be taken and handled properly to avoid artifacts, which can render them nondiagnostic.

Hematemesis

Hematemesis is the vomiting of blood; it suggests GI ulceration/erosion, coagulopathy, or ingestion of blood. The vomitus may contain bright-red blood or digested blood that resembles coffee grounds. Administration of nonsteroidal anti-inflammatory drugs (especially concurrently with corticosteroids) is a major reason for canine ulceration. Hepatic failure, mast cell tumor, shock, submaximal exertion, and dexamethasone administration must also be considered. After these have been ruled out, endoscopy is indicated and allows diagnosis of ulceration (especially because of a foreign object, inflammatory disease, or neoplasia). Depending upon the particulars of the case, one may perform endoscopy or treat symptomatically.

Abdominal Inflammation

Septic or nonseptic peritonitis (or inflammation of any abdominal organ) may cause vomiting. Abdominocentesis or abdominal lavage (see Chapter 10) may be needed, especially if physical examination or abdominal imaging suggests abdominal fluid. Occult cases may require laparoscopy or exploratory surgery for diagnosis.

AMYLASE

Rare Indications • Used to diagnose pancreatitis, this test is no longer recommended.

Disadvantages • The test has poor sensitivity and specificity.

Drug Therapy That May Cause Hyperamylasemia • Some drugs occasionally cause pancreatitis (Box 9-3) and may cause hyperamylasemia. Corticosteroids sometimes increase serum amylase concentrations.

Causes of Hypoamylasemia • Insignificant.

BOX 9-3. DRUGS THAT MAY CAUSE ACUTE PANCREATITIS

Asparaginase
Azathioprine
Clomipramine
Furosemide
N-methylglucamine
Metronidazole
Potassium bromide (*unproven association*)
Salicylazosulfapyridine (Azulfidine)
Sulfonamides
Tetracycline
Thiazide diuretics

NOTE: These drugs do not reliably cause pancreatitis, and a history of administration of one of these drugs plus signs of pancreatitis cannot be assumed to be cause and effect. A patient with acute pancreatitis that is receiving one of these drugs, however, should undergo drug withdrawal, if possible.

Causes of Hyperamylasemia • Decreased glomerular filtration (i.e., azotemia) and pancreatitis are causes of hyperamylasemia. Patients with pancreatitis may have normal to markedly increased values. Intestinal disease, ruptured intestines, and hepatic disease can increase serum amylase.

Causes of Increased Fluid Amylase • When abdominal fluid amylase is greater than serum amylase concentrations, pancreatic disease is possible. Bowel rupture is also possible.

LIPASE

Rare Indications • Used to diagnose pancreatitis, the test is not recommended.

Disadvantages • The test has poor sensitivity and specificity. Duodenal foreign objects, chronic gastritis, and abdominal carcinomas sometimes cause increased serum lipase activity.

Drug Therapy That May Cause Hyperlipasemia • Drugs causing pancreatitis and hyperlipasemia are the same as for amylase (see Box 9-3) plus heparin. Corticosteroids (dexamethasone) may increase serum lipase activity without histologic evidence of acute pancreatitis.

Causes of Hypolipasemia • Not significant.

Causes of Hyperlipasemia • Causes of hyperlipasemia are similar to the causes of hyperamylasemia. Renal dysfunction increases serum lipase. Not all patients with acute pancreatitis have increased serum lipase, and the increase in serum lipase activity is not proportional to the severity of the pancreatitis. Extremely increased lipase values are sometimes caused by pancreatic carcinomas.

CANINE IMMUNOREACTIVE PANCREATIC LIPASE (SPEC CPL)

Common Indications • Measurement of spec cPL is indicated in patients with vomiting, abdominal pain, nonseptic inflammatory abdominal exudate, icterus, or a prior history of pancreatitis.

Advantages • The spec cPL is the most sensitive test for pancreatitis. It only requires a serum sample and can be run in house when using SNAP technology (IDEXX, Westbrook, ME).

Disadvantages • Specificity for clinically important pancreatic lesions (as opposed to pancreatic lesions that are not causing clinical disease) is currently unknown.

Analysis • The spec cPL is measured in serum by enzyme-linked immunosorbent assay (ELISA).

Normal Values • Less than 200 µg/L is normal.

Artifacts • Uncertain.

Causes of Decreased Values • Exocrine pancreatic insufficiency (EPI) or isolated pancreatic lipase deficiency may decrease spec cPL. More overlap exists between normal dogs and dogs with EPI than exists for serum trypsin-like immunoreactivity (TLI) concentration. TLI is the test of choice for EPI. Because the spec cPL test is so sensitive, a negative result strongly suggests that acute pancreatitis is not present, and the clinician should look elsewhere for the cause of the vomiting.

Causes of Increased Values • Values greater than 400 µg/L are consistent with and suggestive of pancreatic inflammation or necrosis. It is currently unclear whether all microscopic pancreatic lesions (i.e., inflammation, necrosis) that are associated with a high spec cPL are clinically important.

FELINE IMMUNOREACTIVE PANCREATIC LIPASE (SPEC fPL)

Common Indications • Indications for measurement of spec fPL are cats suspected of having pancreatitis and those with vague clinical signs (e.g., unexplained anorexia, abdominal discomfort, unexplained weight loss) that remain undiagnosed despite testing.

Advantages • The spec fPL is the most sensitive test for feline pancreatitis.

Disadvantages • The spec fPL has uncertain specificity for clinically important pancreatic disease.

Analysis • The spec fPL is measured in serum by ELISA.

Normal Values • Less than 3.5 µg/L.

Artifacts • None known.

Drug Therapy That May Alter spec fPL • None known, but any drug that can cause pancreatitis may increase the spec fPL.

Causes of Decreased Values • Uncertain, but EPI might be a cause.

Causes of Increased Values • Values greater than 5.4 µg/L are consistent with and suggestive of pancreatitis.

GASTRIN

Occasional Indications • Measurement of gastrin is indicated in cases of chronic vomiting, diarrhea, weight loss, suspected gastrinoma, or gastric or duodenal ulceration of unknown cause. This test is usually not requested until more common diseases have been ruled out.

Advantages • The test detects otherwise occult gastrinomas.

Disadvantages • The sample requires careful handling (i.e., rapid harvesting of serum and freezing if analysis is delayed).

Analysis • Gastrin is measured in serum by radioimmunoassay (RIA).

Normal Values • Depends on laboratory (the assay must be validated for the species). To convert pg/ml to ng/L, multiply pg/ml $\times$ 1.0 = ng/L.

Artifacts • Gastrin may be falsely decreased by hormone degradation as the result of sample storage for several days at temperatures above freezing.

Drug Therapy That May Increase Gastrin • Antacids, including histamine₂ (H₂) receptor antagonist drugs and proton pump inhibitors, may increase gastrin concentration.

Causes of Hypogastrinemia • Not significant.

Causes of Hypergastrinemia • Atrophic gastritis (uncommon), antral G-cell hyperplasia (rare), short bowel syndrome, hyperparathyroidism, gastric ulcers, gastric outlet obstruction, renal failure, and gastrinoma may cause hypergastrinemia; the last four are probably the most common. If gastrinoma is suspected in a patient that has a normal or equivocal serum gastrin concentration, secretin or calcium stimulation tests may be performed. A rise in the serum gastrin concentration after giving either of these drugs suggests a gastrinoma.

ACUTE DIARRHEA

Patients with diarrhea are best classified into those with acute (<2 to 3 weeks) versus those with chronic (>2 to 3 weeks) diarrhea. Acute diarrhea (Box 9-4) is usually self-limiting, although some conditions may be severe and cause mortality (e.g., acute hemorrhagic

BOX 9-4. MAJOR CATEGORIES OF CAUSES OF ACUTE DIARRHEA IN DOGS AND CATS**Intestinal Parasites (common)**

Hookworms

Roundworms

Whipworms (primarily dogs)

Coccidia

Giardia (sometimes difficult to diagnose)*Strongyloides**Trichostrongylus axei* (primarily cats)**Diet (common)**

Poor-quality food/food poisoning

Sudden dietary change (especially young animals)

Food intolerance/allergy

Acute Viral or Bacterial Enteritis

Parvovirus (canine and feline) (common)

Coronavirus (canine and feline)

Clostridium perfringens (common)

Campylobacteriosis (rare)

Salmonellosis (rare)

Escherichia coli (suspected, but not proven)**Intussusception****Intoxication**

Garbage

Food poisoning

Heavy metal

Organophosphate

Hemorrhagic Gastroenteritis**TABLE 9-1. DIFFERENTIATION OF CHRONIC SMALL INTESTINAL DIARRHEA FROM CHRONIC LARGE INTESTINAL DIARRHEA**

	SMALL INTESTINAL DIARRHEA	LARGE INTESTINAL DIARRHEA
Weight loss (very important criterion)	Expected	Uncommon except with severe disease (e.g., histoplasmosis, pythiosis, or cancer)
Polyphagia	Often present	Uncommon
Vomiting	May occur	Occurs in 10%–20% of patients
Volume of feces	May be normal or larger than normal	May be normal or smaller than normal
Frequency of defecation	Normal to slightly increased	Normal to markedly increased, may have many small defecations per bowel movement
Slate-gray feces (steatorrhea)	Rare	No
Hematochezia	No	Sometimes
Melena	Rare	No
Mucoid stools	Rare (unless ileum is diseased)	Often present
Tenesmus/dyschezia	Rare	Sometimes

gastroenteritis, parvoviral disease, hookworms, intoxication). History should explore the possibility of recent dietary change and exposure to infectious agents. Diet, bacteria, viruses, and parasites are the major identifiable causes of acute diarrhea in dogs and cats. Because intestinal parasites may contribute to any diarrheic state, fecal examinations (direct and flotation) are typically warranted in diarrheic patients. Giardiasis may require special diagnostic techniques (see [Fecal *Giardia* Detection](#) later in this chapter). The need for diagnostics depends upon (1) the severity of the problem (i.e., more severely ill patients require more diagnostics), and (2) the likelihood that the patient has an infectious agent that has potential to be nosocomial or zoonotic.

Feeding bland or elimination diets may be diagnostic and therapeutic. Depressed, weak, and dehydrated patients should undergo electrolyte and acid-base evaluations to aid in selecting fluid replacement therapy. All patients less than 12 to 14 weeks of age and those that are emaciated or weighing less than 3 kg should undergo blood glucose monitoring to detect secondary hypoglycemia. CBC is indicated in most febrile or severely depressed patients so that sepsis or transmural inflammation can be detected. Fecal cultures, ELISA, and/or polymerase chain reaction (PCR) for *Salmonella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica*, verotoxin-positive *Escherichia coli*, *Clostridium perfringens*, or *Clostridium difficile* may be

performed, but establishing a cause-and-effect relationship between the organism and disease can be difficult.

Not all patients with canine parvoviral diarrhea are severely ill, diarrheic, febrile, or have identifiable leukopenia. Leukopenia may only persist 24 to 36 hours and be missed if a CBC is not performed during that period. Other diseases causing severe sepsis (i.e., perforating linear foreign body with peritonitis or overwhelming salmonellosis) can cause leukopenia indistinguishable from that of canine parvoviral enteritis. Routinely used vaccination schedules do not necessarily guarantee protection against canine parvovirus. In-house ELISA tests for parvovirus performed on feces appear to be specific for parvoviral antigen, but testing may be negative if done too early or too late. Fecal shedding of viral particles may not occur for 1 to 3 days after signs begin and decreases rapidly with time. The test result should be strongly positive within 3 days of the onset of clinical signs and remain positive for several days. A recent vaccination may result in a weakly positive fecal ELISA.

CHRONIC DIARRHEA

Chronic diarrhea should first be defined as either small intestinal or large intestinal in origin ([Table 9-1](#)). Occasionally, large and small intestines are concurrently

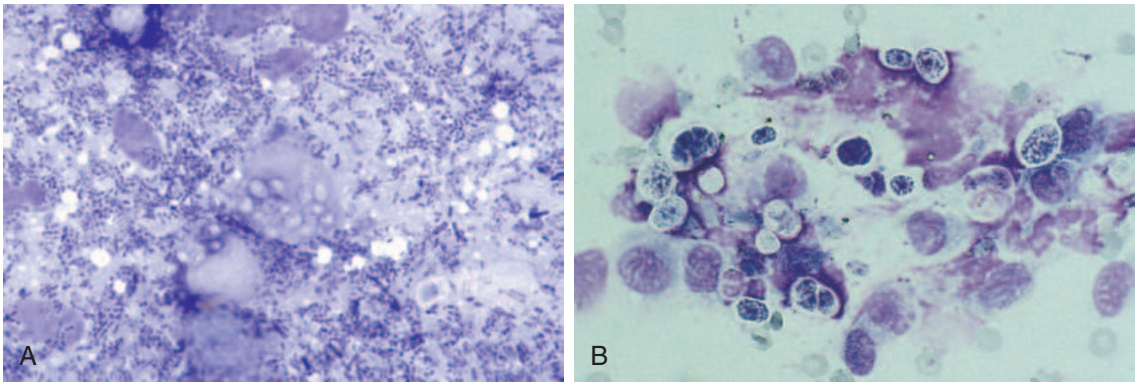


FIGURE 9-3 **A**, A canine rectal scraping showing a macrophage with numerous engulfed yeasts. These are *Histoplasma capsulatum*. **B**, A canine rectal scraping that shows inflammatory cells and spherical organisms with a clear halo. The organisms are *Prototheca*. (Courtesy of Dr. Rick Cowell.)

involved. Patients with chronic diarrhea in which clinical disease is not severe are often treated with therapeutic trials before aggressive diagnostics are instituted. The specifics of the therapeutic trials are influenced by whether the patient has large or small bowel disease. Patients should usually have at least three fecal examinations at 48-hour intervals. If these tests are negative, it is still acceptable (depending upon the risk of parasites in the geographic location) to treat empirically for *Giardia* infection and whipworms before aggressive diagnostics are begun. Giardiasis may be particularly difficult to diagnose (see [Fecal Giardia Detection](#) later in this chapter). Adverse food reactions (i.e., allergy, intolerance, fiber deficiency) commonly cause chronic diarrhea. Dietary intolerances are a reaction to a particular substance in the diet, whereas true food allergies are immunologic reactions to specific antigens. Dietary food trials are indicated in suspected cases. There are antibiotic-responsive intestinal diseases that are also treated empirically; however, the specific therapy varies with whether the patient has large or small bowel disease (see next section). Failing to respond to empirical anthelmintic, dietary, and antibacterial therapy indicates the need for further diagnostics.

Large Intestinal Disease

Large intestinal disease has different parasites (i.e., *Trichuris vulpis*, *Tritrichomonas fetus*), dietary problems (i.e., fiber-responsive diarrhea), and bacterial problems (i.e., so-called *clostridial colitis* that responds best to tylosin or amoxicillin) than small bowel disease. Once parasitic, dietary, and “*clostridial colitis*” are eliminated by diagnostics and therapeutic trials, additional diagnostic steps, such as rectal mucosal scrapings (not swabs) with cytologic examination ([Figure 9-3](#)) might be appropriate. Persistent large intestinal disease that fails to respond to these therapeutic trials or that is associated with hypoalbuminemia or obvious weight loss is usually an indication for abdominal ultrasound followed by fine-needle aspiration and/or colonoscopy-ileoscopy plus biopsy. Rigid colonoscopy of the descending colon is adequate for diagnosis in most cases. Flexible endoscopy allows access to the descending, transverse, and ascending colon;

ileocolic valve; cecum; and ileum. If flexible endoscopy is unavailable, abdominal ultrasonography may reveal lesions in areas not accessible with rigid endoscopy.

Small Intestinal Disease

Small intestinal disease has different parasites (e.g., *Giardia*), dietary problems (e.g., lymphangiectasia), and bacterial problems (i.e., so-called *antibiotic-responsive enteropathy* [ARE] or *dysbiosis* that may respond to a variety of antibacterials) than large bowel disease. Chronic and severe small intestinal diarrhea necessitates differentiation of maldigestion, protein-losing enteropathy (PLE), and malabsorptive disease without protein loss ([Figure 9-4](#)). Weight loss and diarrhea are usually present, but some patients only have weight loss.

Maldigestion

Maldigestion due to bile acid insufficiency caused by biliary obstruction is rare. Intestinal lactase deficiency is uncommon, but a lactose-free diet may be tried in selected patients (especially cats). EPI is the principal cause of maldigestion but is rare in cats. Differentiation of EPI from malabsorptive intestinal disease is important. EPI is often overlooked in afflicted dogs or may erroneously be diagnosed in patients without the malady. Clinical trials using pancreatic enzyme preparations are very insensitive and nonspecific. Powdered enzyme is often superior to tablet formulations, and some enzyme preparations are clearly superior to others. Some dogs with EPI also require a low-fat diet, antacid therapy (rare), or treatment for concurrent ARE (common) before the enzyme replacement therapy becomes effective, even when appropriate enzymes are administered. Up to 15% of dogs with EPI never respond to therapy. Too often, failure of empirical enzyme replacement therapy leads to unnecessary tests (i.e., biopsy) because EPI was incorrectly eliminated. No consistent hematologic or serum chemistry profile changes are seen. Undigested fats can often be found in the feces; however, this is inconsistent. The fat absorption test yields many false results. The TLI assay is the standard test for EPI. It is important to note that the TLI tests are species specific. Measurement of fecal proteolytic activity

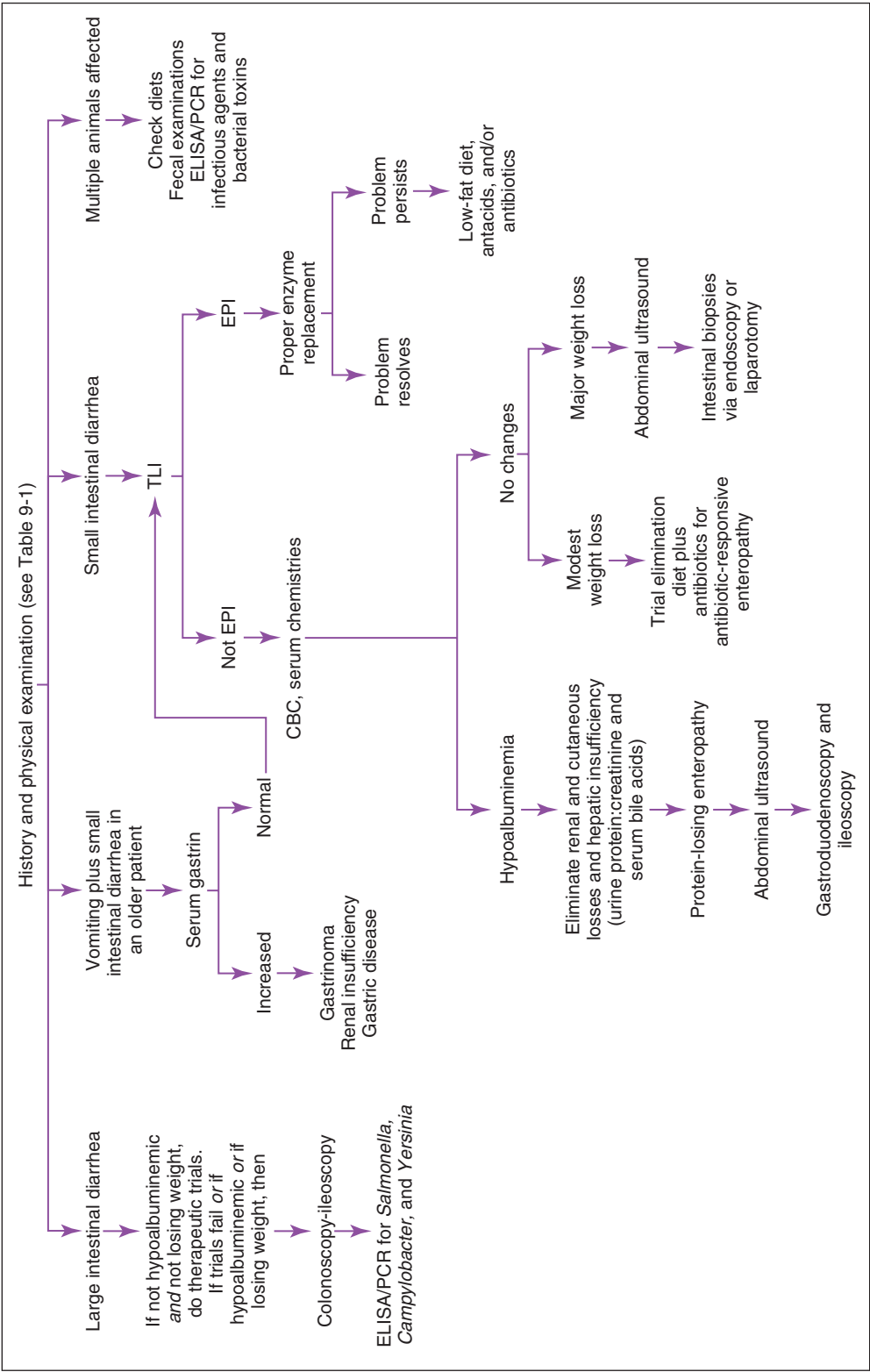


FIGURE 9-4 Diagnostic approach to chronic diarrhea in dogs and cats in which multiple fecal examination results are negative and empirical anthelmintic, antiprotozoal, and dietary therapy do not resolve the diarrhea. CBC, Complete blood count; ELISA, enzyme-linked immunosorbent assay; EPI, exocrine pancreatic insufficiency; PCR, polymerase chain reaction; TLI, trypsin-like immunoreactivity.

is often accurate for diagnosing EPI; however, it is more cumbersome and has limited availability.

Malabsorptive Disease Without Protein Loss

Once maldigestion has been eliminated, malabsorption becomes the most likely diagnosis in diarrheic animals with weight loss. One must then decide whether to perform diagnostic therapeutic trials or diagnostic tests. Patients that are emaciated, have serum albumin less than 2.1 g/dl, or are progressing rapidly should usually next undergo abdominal ultrasonography and intestinal biopsies (preferably via endoscopy unless ultrasonography shows lesions that cannot be diagnosed endoscopically or that can be aspirated with ultrasound guidance). Patients that are not critically ill may first receive carefully designed therapeutic trials. Therapeutic trials may be chosen more rationally with the aid of minimal laboratory data (e.g., biochemical profile, fecal examinations). The two major therapeutic trials are (1) food trials for dietary-responsive disease (i.e., elimination diets) and (2) antibacterial trials for ARE or dysbiosis.

ARE (previously called "small intestinal bacterial overgrowth" or SIBO; now sometimes called dysbiosis) may exist by itself or coexist with another GI malady. No consistent CBC or serum chemistry profile changes are seen in this syndrome. Fecal culture is not informative, and ultrasound and intestinal biopsy are seldom diagnostic. A barium contrast study very rarely identifies a segmental lesion or partial obstruction responsible for secondary ARE. Quantitated culture of duodenal or proximal jejunal fluid for aerobes and anaerobes is difficult to interpret, because clinically normal dogs may have as many as or more bacteria than clinically affected dogs. Serum cobalamin and folate concentrations are insensitive and nonspecific for ARE. Dogs with ARE usually respond within 3 to 4 weeks to appropriate antibacterial therapy (e.g., tetracycline, tylosin, metronidazole $\pm$ enrofloxacin), usually combined with a high-quality elimination diet unless irreversible mucosal changes or primary underlying intestinal disease are present.

Dietary intolerance is relatively common, and elimination diets (e.g., fish and potato, turkey and potato, tofu and beans, hydrolyzed) are reasonable trials. At least 3 and preferably 4 weeks should be allotted for such a dietary trial, during which time absolutely nothing else should be fed (including flavored treats or medications).

If dietary, antibiotic, and repeated anthelmintic and antiprotozoal therapies are ineffective, ultrasonography followed by small intestinal biopsy is probably necessary. Ultrasonography is done to look for lesions that can be aspirated (thus avoiding the need for anesthesia and biopsy) and to ensure that endoscopy can reach the lesion. In most patients, the stomach, duodenum, ileum, and colon may be endoscopically sampled. Duodenal cytology is helpful in some disorders (e.g., eosinophilic enteritis, purulent enteritis, giardiasis, lymphoma). If laparotomy is performed, multiple representative full-thickness specimens (e.g., stomach, duodenum, jejunum, ileum, mesenteric lymph node) are indicated, because lesions can be spotty, even in severely affected patients. If endoscopy is performed, multiple high-quality tissue

samples (e.g., ≥ 6 to 8) from each site are obtained. It is critical that the endoscopist be accomplished and trained in obtaining high-quality tissue samples. Many endoscopically obtained tissue samples are nondiagnostic because of the operator's lack of training.

Protein-Losing Enteropathy

PLE is uncommon in cats but seen with some regularity in dogs. PLEs are classically described as causing panhypoproteinemia. However dogs with diseases causing hyperglobulinemia (e.g., chronic skin disease, rickettsial disease, heartworm disease) and some breeds (e.g., basenji dogs) may have only hypoalbuminemia because the serum globulin concentration is initially increased, and even though much of this fraction is lost into the intestines, the amount remaining in the blood keeps concentrations in the normal range. If red blood cells (RBCs) are also being lost, iron deficiency anemia may occur (see Chapter 3).

PLE may be the result of various GI diseases (e.g., hookworms, chronic intussusception, fungal infections, ulcers and erosions), but lymphangiectasia, alimentary lymphosarcoma, ARE, and IBD seemingly are the most common causes in adult dogs. Intestinal lymphangiectasia produces some of the lowest serum albumin concentrations that occur in alimentary disease (e.g., <1.0 g/dl). Hypocholesterolemia is common, and peripheral lymphocyte counts occasionally are decreased. If hepatic insufficiency and loss from the kidneys and skin have been eliminated in a severely hypoalbuminemic patient, PLE becomes the major differential diagnosis by process of elimination. If PLE is suspected in a patient that has another potential explanation for its hypoalbuminemia (e.g., renal protein loss, hepatic insufficiency, severe exudative skin disease), then measuring fecal α_1 -protease inhibitor concentrations may clarify whether or not excessive GI protein loss is occurring. α_1 -protease is relatively stable and resistant to GI degradation; consequently, it can be measured in the feces. Intestinal biopsy is usually the definitive test for determining the cause of PLE. Full-thickness biopsy may risk dehiscence if the serum albumin level is less than 1.5 g/dl; serosal patch graft techniques decrease the risk of dehiscence. Gastroduodenoscopy-ileoscopy plus biopsy is relatively safe and often diagnostic. Occasionally the intestinal lesion is inaccessible via endoscopy. Although not recommended, dietary trial with an ultra-low-fat diet may be substituted for biopsy in patients suspected of having lymphangiectasia. Therapeutic trials with steroids without a definitive diagnosis can be potentially dangerous (especially in dogs) and are not recommended.

FECAL CHARACTER

Muroid feces should be approached as a large intestinal or a distal small intestinal problem. In dogs and cats with large bowel disease but no weight loss or hypoalbuminemia, multiple fecal examinations, digital rectal examination, and therapeutic trials (i.e., dietary, antibacterial and/or anthelmintic) are often the best initial steps. If these are unsuccessful, then colonoscopy-ileoscopy plus biopsy generally becomes the most useful

diagnostic tool. Hematochezia should also be considered as a large bowel problem. Melena signifies swallowed blood from any source, coagulopathy, or gastric and upper intestinal bleeding. Therefore before performing an exploratory laparotomy, one should consider all the possible causes of ingesting blood (e.g., coughing up blood from the respiratory tract, posterior nasal bleeding). Ingestion of bismuth subsalicylate or liver can cause feces to appear melanic. Diet and changes in intestinal bacterial flora influence fecal color but do not generally signify disease.

FECAL ENZYME-LINKED IMMUNOSORBENT ASSAY FOR PARVOVIRUS

Occasional Indications • A fecal ELISA for parvovirus is indicated in dogs suspected of having parvoviral enteritis (especially those not displaying classic signs), or in acute neutropenia of unknown cause. The test can be used in cats to diagnose panleukopenia caused by canine parvovirus 2a and 2b.

Advantages • The test is quick and available, and has good sensitivity and specificity if done at the appropriate time (e.g., approximately 1 to 3 days after onset of clinical signs).

Disadvantages • Dogs with parvoviral enteritis can have negative reactions, especially very early or very late in the course of the disease.

Analysis • Fresh feces are used according to kit instructions (see Chapter 15). The instructions must be carefully followed or false results might be obtained.

Normal Values • Dogs should be negative for fecal parvoviral antigen.

Interpretation • A positive result supports canine parvoviral enteritis. Not all dogs affected with parvoviral enteritis have diarrhea and fever; some show only anorexia, vomiting, or fever. Theoretically, if coproantibody binds all of the antigen in the feces, a false-negative result may occur. If the test is performed too early in the disease, it may yield negative results. With such dogs, one should repeat the test in 36 to 48 hours. Shedding of viral particles decreases after the first week of disease, and a test performed too late in the disease might yield negative results. Modified-live virus vaccination results in transient fecal shedding and can give a weak positive fecal ELISA test result (5 to 15 days after vaccination).

FECAL ANALYSIS FOR CLOSTRIDIUM PERFRINGENS

Occasional Indications • Fecal analysis for *C. perfringens* is occasionally indicated in dogs with acute, nosocomial diarrhea or chronic large bowel diarrhea of unknown cause.

Disadvantages • The test has uncertain sensitivity and specificity for *C. perfringens*-associated disease. Presence of toxin does not mean the patient has disease due to that toxin. Conversely, absence of toxin does not guarantee the patient is not diseased because of the bacteria producing the toxin. Animals may be toxin-positive and not have diarrhea, and dogs with toxin-negative diarrhea may respond to antibiotics. Old fecal samples might yield false-positive results.

Analysis • Fresh or frozen feces are used according to the instructions on the test kit. Reverse passive latex agglutination (RPLA) (i.e., PET-RPLA Toxin detection kit; Oxoid Limited, Cambridge, UK) and ELISA (i.e., *C. perfringens* Enterotoxin Test; TechLab, Blacksburg, VA) methods are available for *C. perfringens* enterotoxin.

Interpretation • Results from ELISA methodology appear to correlate better with disease than do results from RPLA methodology. Finding *C. perfringens* enterotoxin in feces plus clinical signs consistent with clostridial diarrhea has been considered diagnostic. However, enterotoxin cannot be found in all patients responding to tylosin or amoxicillin therapy. In suspected cases with a negative toxin assay, one may repeat the test again at the onset of recurrence of clinical signs or perform a therapeutic trial with amoxicillin or tylosin.

Fecal spore counts do not correlate well with *C. perfringens* enterotoxin production or with the presence of diarrhea. Examining fecal smears (see [Fecal Microscopic Cytology](#) later in this chapter) to look for the presence of spores is not an acceptable screening procedure.

FECAL ANALYSIS FOR CLOSTRIDIUM DIFFICILE

Occasional Indications • The importance of *C. difficile* in small animal medicine is unknown. The test seems most appropriate for suspected nosocomial or antibiotic-associated diarrheas.

Advantages • The ELISA for *C. difficile* antigen is very sensitive and has excellent negative predictive value. The ELISA for *C. difficile* toxin is thought to be specific, but of uncertain sensitivity.

Disadvantages • Interpretation is not simply based upon positive/negative test results. Presence of toxin does not mean the patient has disease due to that toxin. Conversely, absence of toxin does not guarantee the patient is not diseased because of *C. difficile*. Animals may be toxin-positive and not have diarrhea, and dogs with toxin-negative diarrhea may respond to appropriate antibacterials.

Analysis • ELISA methodology is available to look for *C. difficile* toxin A (ImmunoCard Toxin A; Meridian Diagnostics, Cincinnati, OH).

Drug Therapy That May Alter Analysis • Unknown.

Interpretation • The best approach is to first check for the presence of bacteria using the ELISA for the bacterial antigen. If the test is negative, then it is very unlikely that *C. difficile* is present. If the ELISA for the bacterial antigen is positive, then one should test for the toxin using an ELISA that detects both A and B toxin. Finding *C. difficile* toxin A in feces of diarrheic patients seems suggestive of a cause-and-effect relationship. However, the importance of *C. difficile* in canine and feline medicine and any risk it may have for people is currently not understood.

FECAL CULTURE

Rare Indications • Fecal culture is rarely indicated for dogs and cats with diarrhea (especially large bowel) that appears to be nosocomial, is potentially infectious (e.g., fever, leukocytosis, neutrophilic fecal cytology), or is suspected of being due to a specific infectious agent (i.e., *Salmonella* spp., *C. jejuni*, verotoxin-positive *E. coli*, and *Y. enterocolitica*). Nonculture techniques looking at DNA are probably more useful but are generally limited to research laboratories.

Disadvantages • The clinician must specify which pathogen(s) to culture for and must provide the laboratory with fresh feces or feces submitted in appropriate transport media. Testing requires a microbiology laboratory familiar with the specific enrichment and isolation techniques for each pathogen. Using culture swabs is not adequate for isolation of most enteric pathogens. Finally, growing a “pathogen” does not mean that it is responsible for clinical signs.

Analysis • Fresh feces must be promptly submitted to the laboratory, and the laboratory must know the specific pathogen(s) sought. To submit old feces or feces that have not been collected or handled properly or to request a “general culture for pathogens” is generally a waste of time and money. It requires laboratories that are properly equipped to culture for enteric pathogens. Culture for *C. perfringens* is not helpful diagnostically.

Interpretation • Any clinically normal animal may have any of the pathogens listed earlier cultured from its feces, although *Y. enterocolitica* is particularly uncommon in the United States. Interpretation of the fecal culture must consider the history, physical examination, laboratory data, and sometimes numbers of organisms (i.e., number of bacterial colony-forming units per gram of feces) found.

FECAL FAT

Rare Indications • Fecal fat testing may be used to detect malabsorption or maldigestion in animals with diarrhea or unexplained weight loss.

Advantages (Semiquantitative Analysis) • Fecal fat has minimal expense, availability, and reasonable accuracy as a screening test.

Disadvantages (Semiquantitative Analysis) • The test occasionally produces misleading results.

Analysis • The clinician performs a semiquantitative analysis for undigested fats by mixing a drop of fresh feces with a drop of Sudan III, heating the slide to a boil, and examining the smear microscopically. The clinician performs analysis for digested fats by mixing one drop of fresh feces, one drop of 36% acetic acid, and one drop of Sudan III. This is put on a microscope slide, heated to boiling, and examined while still warm. In both cases, identifying orange droplets is a positive finding. It is important that the patient has been eating a moderate- to high-fat diet. Feeding low-fat diets to malabsorptive dogs may cause the test result to be negative.

Normal Values • Semiquantitative: few or no undigested and digested fat globules per high-power field (hpf).

Artifacts • The semiquantitative analysis may have unexplained false-negative and false-positive reactions. Administration of barium sulfate, bismuth, psyllium fiber, mineral oil, or castor oil or feeding a low-fat diet may also confuse semiquantitative analysis.

Causes of Increased Fecal Fat • Finding several orange globules/hpf, if repeatable on several examinations, is principally caused by malabsorption or maldigestion. It is a reasonable screening test and helps distinguish EPI (positive for undigested fats) from malabsorption (positive for digested fats). Despite occasional false-positive reactions, strongly positive results for undigested fecal fat in a dog with signs consistent with maldigestion are an indication for TLI. Fecal fat may not be detectable in some dogs with EPI.

FECAL PROTEOLYTIC ACTIVITY

This test was used to detect maldigestion in animals with chronic diarrhea or weight loss of unknown cause. Theoretically it may diagnose EPI in rare patients that have EPI secondary to obstruction of the pancreatic duct or ducts. However, the radiograph film digestion test is useless and should never be used. The most reliable procedure for measuring fecal proteolytic activity is difficult to perform and requires special handling of the feces; it is described in prior editions. The TLI test is the test of choice for EPI.

FECAL ALPHA-1 PROTEASE INHIBITOR ACTIVITY

Infrequent Indications • Hypoalbuminemia of uncertain cause or suspected PLE in a patient with concurrent hepatic insufficiency or protein loss from the kidneys or skin are indications for testing fecal alpha-1 protease inhibitor activity.

Advantages • The test can define the GI tract as the source of protein loss. Alpha-1 protease inhibitor is a plasma protein. If it leaks into the intestinal lumen, it

resists GI degradation and hence can be measured in the feces.

Disadvantages • The test has limited availability. The magnitude of alpha-1 protease inhibitor in the feces is variable and may not reflect the severity of the disease.

Analysis • Three 1-g fecal samples from three different bowel movements are submitted in tubes provided by the laboratory. It is critically important that three samples (preferably from different days or at least different bowel movements) be submitted, that the feces be collected promptly after defecation, and that the feces not be collected by digitally removing them from the rectum. Samples must be frozen while one awaits shipping and must be shipped on a cold pack. Currently the only laboratory offering this test is GI Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4474.

Normal Values • 0.23 to 5.67 $\mu\text{g/g}$ feces. The clinician must look at individual values and the mean of all three values.

Causes of Abnormalities • Abnormally high values in the feces indicate loss of serum proteins into the alimentary tract and might indicate that PLE is the cause of hypoalbuminemia. Interpretation of the magnitude of the loss is as per the laboratory.

FECAL MICROSCOPIC CYTOLOGY

Rare Indications • Fecal microscopic cytology is rarely indicated in cases of large or small intestinal diarrhea.

Advantages • The test is widely available and easy to perform.

Disadvantages • The test has poor sensitivity and variable specificity for different agents.

Analysis • Thin, air-dried, fresh fecal smears are stained with new methylene blue (NMB) or Wright stain and examined using high-power and oil immersion microscopy. Rectal and colonic mucosal scrapings obtained with a curette are also a means of examining mucosal cells.

Normal Values • A mixed population of rod and cocci bacteria, few bacterial spores or yeast, occasional epithelial cells and amorphous debris.

Artifacts • Old fecal samples may alter results (white blood cells [WBCs] do not remain identifiable in feces for long time periods, and the bacterial population changes and bacterial spores may increase). Fecal debris may resemble degenerate WBCs.

Drug Therapy That May Alter Fecal Microscopic Cytology • Administration of barium and psyllium fiber may make interpretation difficult, and antibiotics change bacterial flora composition.

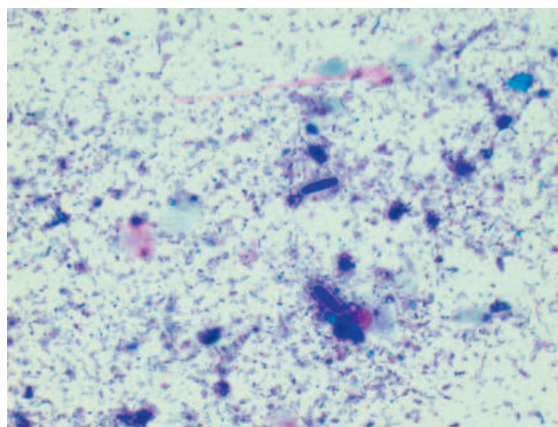


FIGURE 9-5 Cytology of a fecal smear showing large yeast bodies (i.e., *Cyniclomyces guttulatus*).

Interpretation • Fecal WBCs (specifically neutrophils) can be observed with bacterial (e.g., salmonellosis, campylobacteriosis) and inflammatory mucosal disease. Transmural colitides occasionally have increased fecal WBCs. Fecal WBCs can be an indication to biopsy colonic mucosa in patients with chronic colitis. Eosinophils may sometimes be seen with allergic or parasitic colitis. Increased numbers of yeast or a uniform population of bacteria may help identify the cause of diarrhea in a patient, but the mere presence of an organism (e.g., *Cyniclomyces guttulatus* [Figure 9-5]) does not ensure that it is causing disease.

FECAL OCCULT BLOOD

Rare Indications • A fecal occult blood test may be used to detect GI bleeding that is not grossly apparent.

Disadvantages • See Artifacts.

Analysis • Fresh feces are smeared on a test pad. The patient must have been on a meat-free diet for at least 3 days before the feces are obtained. Sensitivity varies markedly between different assays.

Normal Values • See Artifacts.

Artifacts • Fecal occult blood results may be falsely decreased by sampling unmixed feces (blood may not be distributed homogeneously throughout the feces) and vitamin C supplementation. Results may be falsely increased by diets containing fresh meats (i.e., hemoglobin) or fresh uncooked vegetables (i.e., peroxidases), which cause a positive reaction.

Causes of Fecal Occult Blood • Bleeding into the GI tract at any level and as the result of any cause may result in fecal occult blood. GI blood loss of

volumes of 2 ml blood/30 kg body weight will give positive results.

FAT ABSORPTION TEST

Rare Indications • A fat absorption test may be used to detect and distinguish maldigestion from malabsorption in chronic small intestinal diarrhea or unexplained weight loss. The test has many false-negative and false-positive results and is not recommended.

Analysis • The test is described in prior editions.

TRYPSIN-LIKE IMMUNOREACTIVITY

Common Indications • TLI testing is indicated in patients with chronic small bowel diarrhea or unexplained weight loss.

Advantages • TLI has high sensitivity and specificity for EPI. The test only needs one serum sample that does not require special or cumbersome handling procedures.

Disadvantages • The test is species specific. Currently the only laboratory offering the fTLI test (for cats) is the GI Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4474.

Analysis • TLI is performed on serum using ELISA.

Normal Values • Dogs, 5 to 35 µg/L; cats, 12 to 82 µg/L.

Danger Values • None.

Artifacts • Theoretically, EPI caused by an obstructed pancreatic duct instead of acinar cell atrophy would yield a normal or even increased serum TLI value.

Drug Therapy That May Alter TLI • Drugs causing acute pancreatitis (see Box 9-3) might increase serum TLI. Oral pancreatic enzyme supplementation does not affect serum TLI concentrations.

Causes of Decreased TLI • TLI is the test of choice for EPI. A serum TLI concentration less than 2.5 µg/L (dog) or 8 µg/L (cat) is generally considered diagnostic for EPI. Subclinical canine EPI may be suspected by finding intermediate values (>2.5 µg/L and <5.0 µg/L). In such cases repeated testing should be performed. Some dogs will later develop EPI, whereas others will not.

Causes of Increased TLI • Values greater than 50 µg/L in dogs and greater than 100 µg/L in cats may occur with pancreatitis (the spec PL is better than TLI for this purpose in dogs and cats), renal failure, prerenal azotemia (may increase two times), and malnutrition. An increased fTLI test is potentially consistent with pancreatitis. In dogs, TLI seems to increase early in pancreatitis but then quickly returns to reference ranges.

SERUM COBALAMIN AND SERUM FOLATE

Occasional Indications • Chronic small bowel diarrhea, unexplained weight loss, or uncertain but suspected small intestinal disease are occasional indications for serum cobalamin and folate testing. These tests are more important in the cat than in the dog.

Advantages • Only one serum sample is needed to measure both values.

Disadvantages • The test has poor sensitivity and specificity for ARE and uncertain sensitivity and specificity for other intestinal diseases or EPI. This test should be an adjunct to other tests in patients with possible intestinal disease. The test is specific for cobalamin deficiency.

Analysis • Cobalamin and folate are measured in serum by bioassay or immunoassay. “No boil” methods are unreliable in dogs. Serum should be transported in a covered tube.

Normal Values • Depend on the laboratory. Normal ranges vary widely between laboratories. The particular laboratory must validate the assay for dogs and cats.

Danger Values • None.

Artifacts • Cobalamin concentration may be falsely decreased by sample degradation caused by exposure of serum to sunlight.

Drug Therapy That May Alter Serum Cobalamin Concentrations • Dietary content or vitamin supplementation of cobalamin and folate can affect serum concentrations. Drugs that affect intestinal bacterial concentrations (i.e., antibacterials) may alter values.

Causes of Decreased Serum Cobalamin Concentrations • The major reasons for decreased serum cobalamin concentrations in dogs and cats are ileal disease or resection (rare), EPI, intestinal mucosal disease, and ARE (“dysbiosis”). In cats, hepatic disease and hyperthyroidism might cause hypocobalaminemia. The major differentiation to be made is among EPI and intestinal disease; therefore decreased serum cobalamin is an indication to measure serum TLI. Not all dogs with EPI, mucosal disease, or ARE have decreased serum cobalamin. Cats with EPI, severe small intestinal disease (e.g., lymphoma, IBD), and some hepatic diseases (e.g., idiopathic hepatic lipidosis) can have very low cobalamin concentrations. Finding a significantly decreased serum cobalamin concentration can be an indication of small intestinal disease in animals that were previously not suspected to have such disease.

Causes of Increased Serum Cobalamin Concentrations • Cobalamin concentration may be increased by cobalamin supplementation.

Causes of Decreased Serum Folate • Severe mucosal disease of the proximal small intestine decreases serum folate. Not all patients with such disease have decreased folate levels.

Causes of Increased Serum Folate • ARE, EPI, and dietary supplementation are probably the major causes. Many patients with these diseases do not have increased folate levels. The combination of low cobalamin plus increased folate is consistent with ARE, but is insensitive and nonspecific.

C-REACTIVE PROTEIN (CRP)

Occasional Indications • CRP has been used in one formula for determining a Canine IBD Activity Index (CIBDAI).

Advantages • The test is very sensitive for detecting inflammation.

Disadvantages • The test will detect inflammation almost anywhere in the body; it is nonspecific as far as type or location or cause.

Analysis • CRP is measured on a sample of refrigerated or frozen fasting serum.

Normal Values • Less than 7.6 mg/L.

Artifacts • None known.

Drug Therapy That May Alter C-Reactive Protein • Unknown.

Interpretation • Any inflammation almost anywhere in the body can increase the CRP. The greatest utility in measuring CRP is to see what change occurs in a given patient after therapy (i.e., whether therapy is associated with an increase or decrease in CRP). Changes in the CRP can reveal resolving or worsening inflammation, even if all values are normal.

HYDROGEN BREATH TEST

Rare Indications • The hydrogen breath test is rarely indicated in cases of chronic small bowel diarrhea or unexplained weight loss. The test detects hydrogen production as a by-product of bacterial fermentation of carbohydrates. Increase in hydrogen production indicates ARE or carbohydrate malabsorption. It can only be done in clinics/laboratories with specialized equipment and experience. The clinician should contact the laboratory for specifics.

Interpretation • Carbohydrate malabsorption and ARE may increase expired hydrogen. The only source of hydrogen is bacterial fermentation of carbohydrates. The sensitivity and specificity of this test for ARE in dogs are unknown.

FECAL SMEAR (WET MOUNT) FOR PARASITES

Common Indications • A fecal smear is used to screen for parasites and parasitic ova; it is indicated in any patient with diarrhea, melena, hematochezia, fecal mucus, weight loss, or vomiting.

Advantages • The test is widely available, easy to perform, and inexpensive.

Disadvantages • A fecal smear requires fresh feces, and is insensitive compared with concentration and molecular techniques.

Analysis • A thin smear is made of very fresh (<5 minutes old) feces, mixed with a drop of saline solution or water and coverslipped to prevent dehydration. It should be examined immediately. If protozoa are visible and better cytologic detail is desired, a drop of Lugol's iodine or Dobell and O'Connor's iodine may be placed at the corner of the coverslip.

NOTE: Iodine kills protozoa, thus stopping motility.

Normal Values • No parasites or ova.

Artifacts • Cooling of the slide or dehydration inhibits the motility of protozoa.

Drug Therapy That May Alter Results • Orally administered compounds containing kaolin, pectin, barium sulfate, bismuth, and other intestinally active compounds (e.g., cathartics, enemas) may make it difficult to find and identify parasites, ova, and cysts.

Parasites, Bacteria, and Ova That May Be Identified • *Giardia* spp. (Figure 9-6A), *Tritrichomonas* spp. (see Figure 9-6A), *Entamoeba histolytica*, *Balantidium coli*, *Strongyloides stercoralis* (Figure 9-6B), and *Aelurostrongylus abstrusus* may be detected. Any ova may be found, but this test may be useful for detecting *Spirocerca lupi* and *Trichuris vulpis* ova. With oil immersion, small motile bacterial spirochetes in conjunction with fecal WBCs suggest *Campylobacter* spp. as a possible cause.

FECAL FLOTATION

Common Indications • Indications for a fecal flotation test are as for a fecal smear.

Advantages • The test has reasonable sensitivity, high specificity, availability, and low cost.

Analysis • Feces are well mixed with either a saturated sugar solution or a zinc sulfate solution (prepared by mixing 331 g ZnSO₄ • 7 H₂O in 1 L water to attain a specific gravity of 1.18 to 1.20 [as determined with a hydrometer]). This is the best fecal flotation technique for

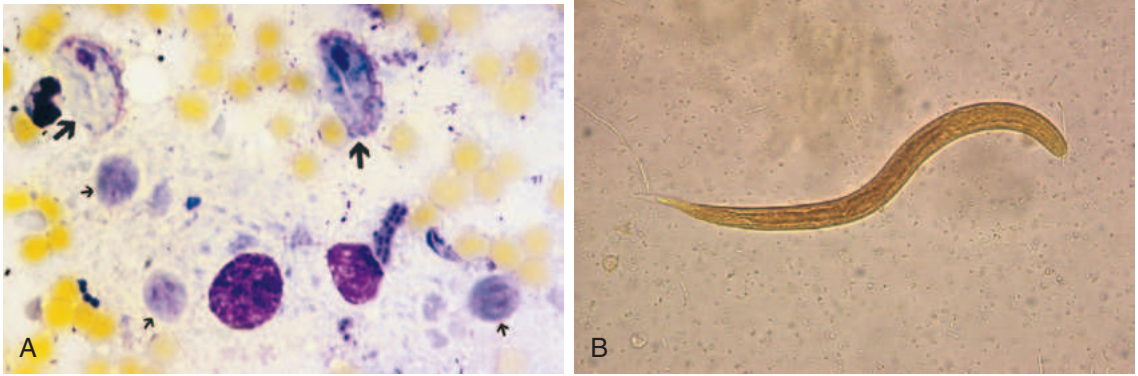


FIGURE 9-6 **A**, Comparison of *Giardia* trophozoites (small arrows) and *Tritrichomonas* trophozoites (large arrows) in a smear that has been stained to enhance internal structures. Note that the *Tritrichomonas* trophozoites are larger and have one large undulating membrane. **B**, A fecal smear stained with iodine showing larvae from *Strongyloides stercoralis*. The larvae of other strongylids (e.g., hookworms) appears identical, so it is important to use only fresh feces when looking for *S. stercoralis*. (A and B courtesy of Dr. Tom Crain, Texas A&M University.)

Giardia spp. because it does not distort the cysts. Ova and cysts are allowed to rise to the surface and are retrieved with a coverslip. Samples for *Giardia* detection should be examined within 15 minutes to avoid distortion and lysis of cysts. Centrifugation of the sample increases the sensitivity of the procedure. Samples that will be sent to an outside laboratory for analysis may be refrigerated (not frozen) for 1 to 2 days or preserved by mixing 1 part feces with 3 parts sodium acetate–acetic acid–formalin (prepared by mixing 1.5 g sodium acetate + 2 ml glacial acetic acid + 4 ml 40% formaldehyde solution + 92.5 ml water).

Normal Values • No ova or oocysts present.

Artifacts • Diarrhea may decrease ova concentration within a sample.

Parasite Ova and Cysts That May Be Identified • *Ancylostoma* spp., *Toxocara* spp., *Toxascaris leonina*, *Trichuris vulpis*, *Spirocerca lupi*, *Physaloptera rara* (using dichromate solution), *Capillaria aerophila*, *Capillaria plica*, *Oncicola canis*, *Diectophyme renale*, *Isospora* spp., *Giardia* spp., *Toxoplasma gondii*, *Cryptosporidium* spp., *Paragonimus kellicotti*, and some tapeworms may be detected.

FECAL SEDIMENTATION

Rare Indications • Indications for fecal sedimentation testing are the same as for fecal smear and flotation, especially if flukes are being considered. If feces contain excessive fat, then formalin and ethyl acetate is probably better than water for sedimentation.

Disadvantages • The test requires more time than a direct fecal smear or fecal flotation.

Analysis • Feces are mixed with the sedimentation solution (e.g., water or saline), strained once or twice to remove large debris, and allowed to settle for 30 minutes

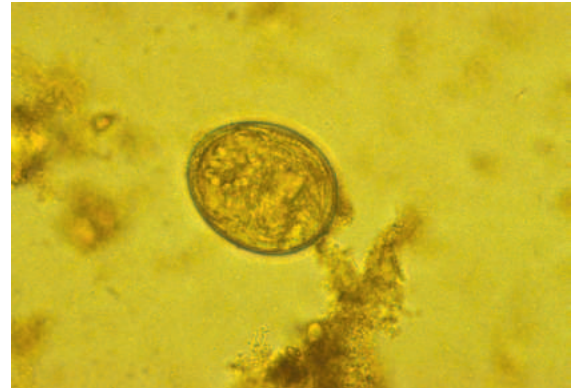


FIGURE 9-7 Ova of *Heterobilharzia americana* in a fecal sedimentation. (Courtesy of Dr Tom Craig, Texas A&M University.)

to 2 hours. The sediment is then examined microscopically. When formalin and ethyl acetate are used, the strained feces are centrifuged, the pellet is resuspended in 9 ml of 5% formalin solution, 3 ml ethyl acetate is added, and the mixture is shaken vigorously. This is recentrifuged, the debris at the formalin and ethyl acetate interface is discarded, and the sediment is then examined.

Normal Values • No ova.

Artifacts • Same as discussed in the previous section on Fecal Flotation.

Parasite Ova That May Be Identified • Fecal sedimentation may detect all the ova that may be found by fecal flotation, plus *Alaria canis*, *Nanophyetus salmincola*, and *Heterobilharzia americana* (Figure 9-7).

FECAL *GIARDIA* DETECTION

Common Indications • Fecal testing for *Giardia* is indicated in patients with chronic diarrhea, unexplained weight loss, intermittent bilious vomiting, or when *Giardia* is suspected clinically and multiple zinc sulfate flotations using centrifugation are negative. Techniques include duodenal aspiration and cytology, fecal ELISA antigen test (e.g., ProSpecT Microplate ELISA Assay for *Giardia*; Alexon, Lenexa, KS), and immunofluorescence assay (IFA) (e.g., MeriFluor *Cryptosporidium/Giardia*; Meridian Diagnostics, Cincinnati, OH) performed on feces.

Advantages • These tests are more sensitive than direct smear or fecal flotation for diagnosing *Giardia*.

Disadvantages • Duodenal aspirate requires surgery or endoscopy.

Analysis • Fresh samples should be used for analysis with fecal ELISA and fecal IFA. The IFA is more sensitive than the ELISA. Duodenal fluid aspirates require fresh direct wet mount observation of motile trophozoites.

Normal Values • No trophozoites or fecal antigen present.

FECAL *TRITRICHOMONAS* DETECTION

Occasional Indications • Fecal testing for *Tritrichomonas* is indicated for chronic large bowel diarrhea in cats, especially exotic breeds such as Somalis, ocicats, and Bengals.

Advantages • Culture is more sensitive (approximately 1000 organisms/50 mg of feces) than direct fecal examination, while PCR is the most sensitive test (approximately 10 organisms per 200 mg feces).

Disadvantages • Different techniques have different sensitivities that the clinician must be aware of. Feces should be fresh, not refrigerated. Old feces can give false-negative results for direct examination and culture. Direct fecal examination is relatively insensitive (<15%).

Analysis • Fresh fecal samples can be examined microscopically (see Fecal Smear earlier in this chapter) (see Figure 9-6A). Feces (very fresh, approximately 0.05 g) can be cultured using commercially available pouches designed for culturing *Trichomonas foetus* from cattle (i.e., In Pouch TF; Biomed Diagnostics, White City, Oregon). This test is best done in the clinic without sending off the feces or the pouch. The inoculated pouch is incubated upright in the dark at either 37° C or room temperature for 2 or 12 days, respectively. It should be examined microscopically at least every 48 hours.

Finally, feces may be preserved (approximately 200 mg in 3 to 5 ml of 70% isopropyl alcohol; be sure to avoid including litter) and mailed to a laboratory for PCR analysis.

Normal Values • Negative.

FECAL *CRYPTOSPORIDIUM* DETECTION

Rare Indications • Fecal testing for *Cryptosporidium* may be indicated in patients with chronic diarrhea. Cats (especially with feline immunodeficiency virus [FIV] infection) may be more likely to have cryptosporidiosis than dogs, but the prevalence of this disorder and its clinical significance in dogs and cats is currently unknown.

Disadvantages • Oocysts are small and may be difficult to find.

Analysis • Fresh fecal samples should be sent to a referral laboratory experienced in finding *Cryptosporidium*. Special fecal flotation techniques, direct fecal smears stained with an acid-fast stain, or ELISA methodology (e.g., ProSpecT *Cryptosporidium* Microplate Assay; Alexon, Lenexa, KS) can be used. The ELISA methodology appears to be the most sensitive.

Normal Values • Negative.

FECAL *HETEROBILHARZIA* DETECTION

Occasional Indications • Fecal PCR testing for *Heterobilharzia* is primarily used in dogs with intestinal or hepatic disease or unexplained hypercalcemia from areas where *Heterobilharzia* is endemic.

Advantages • The test is more sensitive than fecal sedimentation; it can detect 1 to 2 ova/g of feces.

Disadvantages • There is limited availability of testing. The test is currently available at GI Laboratory, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-4474.

Analysis • PCR is performed on feces.

Artifacts • None known.

Drug Therapy That May Alter Test Results • None known.

Interpretation • A positive result in an animal with clinical signs consistent with heterobilharziasis is an indication to treat.

HEPATIC ABNORMALITIES

Perhaps the most difficult aspect of dealing with hepatic disease is distinguishing primary hepatic disease (i.e., the liver is or will be the cause of the patient's illness) from secondary hepatic disease (i.e., the liver disease is caused by the patient's nonhepatic illness). Primary hepatic disease may be heralded by relatively suggestive signs (e.g., hepatomegaly, microhepatia, icterus, ascites, hepatic encephalopathy) or associated with nonspecific signs (e.g., depression, weight loss, anorexia, vomiting). The latter are common presenting complaints of many diseases, which is why serum biochemistry profiling is indicated in patients with chronic signs or evidence of systemic disease. There are no signs or laboratory abnormalities consistently found in patients with primary hepatic disease. When screening for hepatic disease, one should request at least a CBC, serum ALT, SAP, total bilirubin, albumin, cholesterol, BUN, glucose, urinalysis, and abdominal imaging. Hepatic function tests (i.e., serum bile acids and/or blood ammonia) and ultrasound are often very helpful. Hepatic cytology/biopsy is usually necessary for definitive diagnosis except in patients with portovascular anomalies. Abnormalities in hepatic-specific enzymes may result from primary hepatic disease or hepatic involvement secondary to nonhepatic disease (e.g., glucocorticoid hepatopathy, septicemia, IBD, pancreatitis). After identifying abnormalities in ALT, aspartate aminotransferase (AST), SAP, or gamma-glutamyl transpeptidase (GGT), one should investigate first for a secondary hepatic disease because these are the most common causes of increased values. In such cases the liver usually has reactive but reversible degenerative changes. Laboratory tests and ultrasound should be used for two main purposes: (1) to identify the presence of hepatic disease and (2) to help determine if hepatic biopsy is indicated.

Microhepatia: Small Liver

A small liver suggests atrophy (i.e., congenital portosystemic shunt [PSS], hepatic arteriovenous [AV] fistula), fibrosis and cirrhosis, or diffuse massive hepatic necrosis (Figure 9-8). Hepatic atrophy tends to be characterized by sharp borders as opposed to the rounded or blunted hepatic margins typically associated with fibrosis and cirrhosis. Some patients with primary hepatic fibrosis severe enough to cause portal hypertension also have sharp hepatic margins, however. Many patients with marked hepatic atrophy due to congenital PSS are relatively young (<3 to 4 years) and have had signs of hepatic disease since (or before) weaning, whereas most patients with cirrhosis are middle-aged or older and clearly have late onset of clinical signs. Hepatic AV fistula is an uncommon cause of microhepatia, but it is usually diagnosed in dogs less than 2 years of age. However, some dogs with congenital PSS are first diagnosed when they are more than 10 years old. Likewise, some dogs with acquired PSS due to cirrhosis are diagnosed when less than 6 months old.

Hepatic atrophy typically causes abnormalities in hepatic function tests (e.g., serum bile acids, blood ammonia) but may yield normal or abnormal ALT, SAP, BUN, and serum albumin. A single normal or abnormal hepatic function test result does not mean that other hepatic function tests will have similar results. Pre- and postprandial serum bile acid concentrations are generally

sensitive function tests. (NOTE: Cholestatic diseases also increase bile acids; therefore, bile acids are not a "pure" test of hepatic function.) However, if hepatic disease is strongly suspected and the serum bile acid concentrations are not as high as anticipated, one should not hesitate to perform other tests to characterize the liver. If hepatic atrophy is likely, abdominal ultrasonography, advanced imaging (e.g., contrast portography, computed tomography, magnetic resonance imaging), hepatic biopsy, or a combination of these might be considered.

Small livers with clearly rounded or blunt hepatic margins are often fibrotic/cirrhotic. Significant increases in serum ALT and SAP are often present, but some dogs with marked hepatic cirrhosis have normal hepatic enzymes. Serum albumin and BUN are more variable. If cirrhosis appears likely, a biopsy is often indicated. An obviously nodular or "cobblestone" appearance is very suggestive of cirrhosis; however, significant fibrosis can be present without major gross changes, and some noncirrhotic diseases (e.g., hepatic collapse with nodular regeneration, nodular hyperplasia) may grossly resemble cirrhosis. Acquired multiple shunts visible at laparoscopy or laparotomy are usually due to cirrhosis but can be secondary to congenital hepatic AV fistula, veno-occlusive disease, portal vein obstruction, or infiltrative disease.

Hepatomegaly: Enlarged Liver

Focal or asymmetric hepatic enlargement generally necessitates further laboratory investigation, imaging, and possibly biopsy. Neoplasia is a prominent but not invariable cause of focal hepatomegaly. The magnitude of the enlargement is not prognostic.

Generalized hepatomegaly necessitates careful clinicopathologic evaluation. Hepatomegaly may be caused by primary or secondary hepatic disease. Diagnosis may be confirmed with a history of exposure to certain toxins (Boxes 9-5 and 9-6) or diagnosis of a systemic disease (e.g., hyperadrenocorticism) known to affect the liver. *Changes in ALT, SAP, hepatic function tests, and hepatic size, although suggestive of hepatic disease, are not diagnostic of specific entities.* This is true even in breeds with predispositions to specific hepatic diseases (e.g., Doberman pinschers, Bedlington terriers). Changes in the SAP or ALT may also be caused by primary nonhepatic disease (e.g., hyperadrenocorticism, IBD, diabetes mellitus, heart failure). A definitive diagnosis usually requires hepatic biopsy. The clinician should first seek to rule out nonhepatic causes of secondary hepatic dysfunction. Hepatic biopsy should be considered in patients with obviously significant hepatic disease, those that do not have obvious nonhepatic causes (e.g., hyperadrenocorticism, right heart failure), and those that have persistent (>1 month) changes in serum ALT consistent with chronic or progressive hepatic disease or abnormal hepatic function tests (see Figure 9-8). It is not always possible to accurately distinguish primary hepatic from secondary hepatic disease before biopsy; therefore whenever hepatic biopsy is performed via laparotomy or laparoscopy, the rest of the abdomen should be explored and other organs sampled if they might be involved. Fine-needle aspirates with cytology are sometimes useful in detecting diffuse hepatic infiltrative disease and hepatic lipidosis (Figure

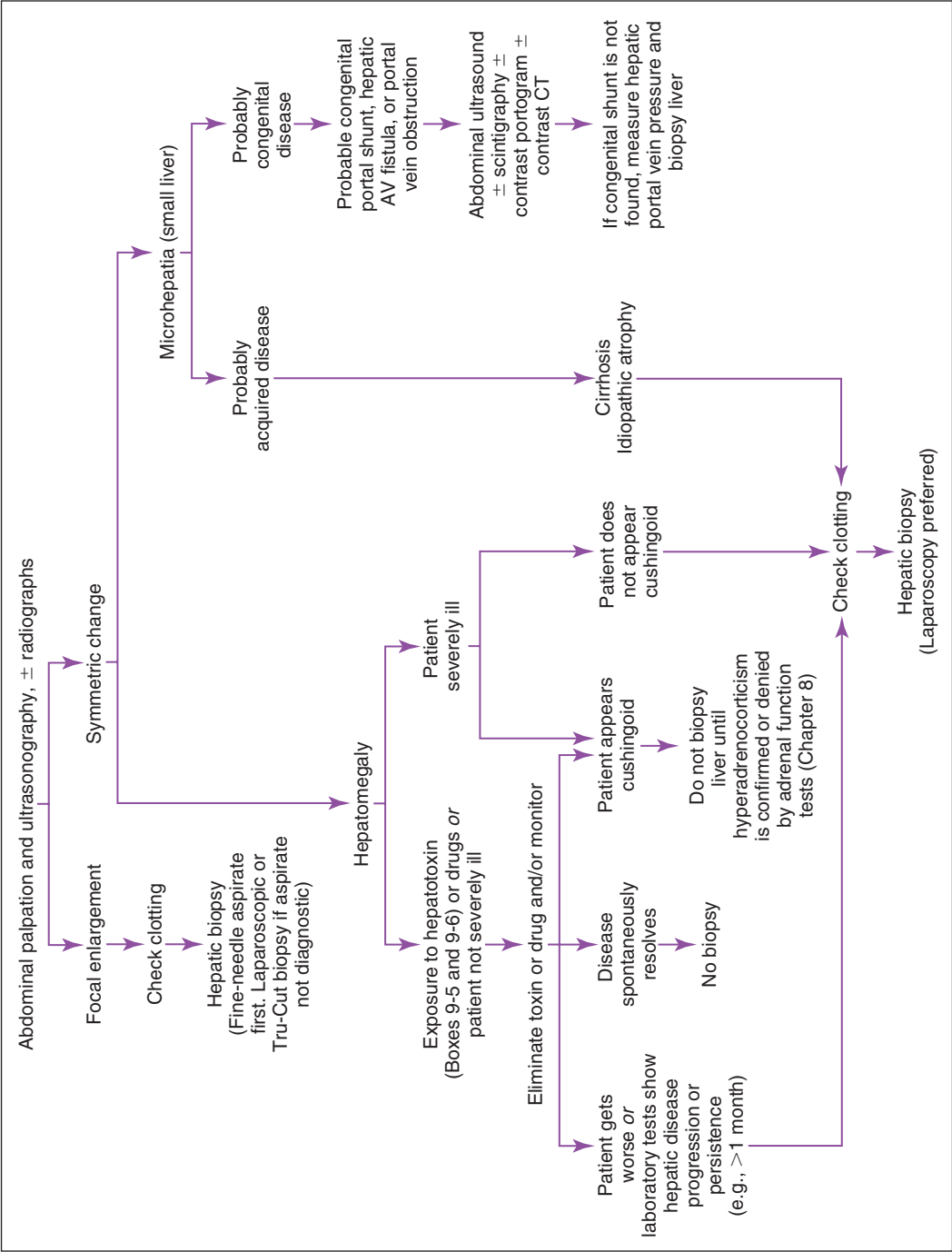


FIGURE 9-8 Diagnostic approach to altered hepatic shape or size in dogs and cats. AV, Arteriovenous; CT, computed tomography.

BOX 9-5. DRUGS THAT HAVE BEEN DOCUMENTED OR SUSPECTED TO CAUSE INCREASED ALANINE AMINOTRANSFERASE (ALT) LEVELS DUE TO HEPATIC DISEASE

Acetaminophen (especially cats) (*important*)
 Amiodarone
 L-Asparaginase
 Azathioprine
 Barbiturates (*important*)
 Carprofen (*important*)
 Clindamycin
 Doxycycline
 Diazepam
 Erythromycin estolate
 Glucocorticoids (dogs only) (*important*)
 Griseofulvin
 Ibuprofen
 Itraconazole
 Ketoconazole
 6-Mercaptopurine
 Methimazole
 Methotrexate
 Nitrofurantoin
 Phenobarbital (*important*)
 Primidone (*important*)
 Salicylates
 Salicylazosulfapyridine
 Sulfonamides
 Tetracycline
 Trimethoprim-sulfa drug (*important*)

NOTE: These drugs do not reliably cause hepatic disease. In a patient with an increased ALT that is receiving one of these drugs, the medication probably should be stopped, if possible, and the ALT rechecked 2 to 4 weeks later. Those drugs that most reliably increase ALT are marked (*important*). The other drugs are less consistent but may still cause severe hepatic disease. Almost any drug could cause an increased ALT in a particular patient.

BOX 9-6. DRUGS THAT HAVE BEEN DOCUMENTED OR SUSPECTED TO CAUSE CHOLESTASIS OR HEPATIC ENZYME INDUCTION RESULTING IN INCREASED SERUM ALKALINE PHOSPHATASE (SAP) LEVELS

Anabolic steroids/androgens
 Asparaginase
 Azathioprine
 Barbiturates (*important*)
 Cephalosporins
 Cyclophosphamide
 Dapsone
 Erythromycin estolate
 Estrogens
 Glucocorticoids (dogs only) (*important*)
 Gold salts
 Griseofulvin
 Ibuprofen
 6-Mercaptopurine
 Methimazole
 Methotrexate
 Nitrofurantoin
 Phenobarbital (*important*)
 Phenothiazines
 Primidone (*important*)
 Progesterone
 Salicylates
 Testosterone
 Tetracyclines
 Thiabendazole
 Trimethoprim-sulfa drug
 Vitamin A

NOTE: Those drugs that most reliably increase SAP are marked (*important*). The other drugs are less consistent.

9-9); however, fine-needle aspirates (even when guided by ultrasound) often miss infiltrative processes. A negative cytologic finding from a fine-needle aspirate never excludes infiltrative hepatic disease. Hepatic biopsy may be done with ultrasound guidance, laparoscopy, or laparotomy. Laparoscopy allows for much superior hepatic biopsies compared with ultrasound-guided core needle biopsies and is not as invasive as surgery.

Hepatic Encephalopathy

Abnormal behavior, sometimes associated with eating, may be caused by hepatic encephalopathy; however, hypoglycemia, primary CNS disease, and epilepsy must also be considered. Whenever possible, glucose should be measured on blood obtained during an episode. Evaluation of hepatic function is indicated in patients with behavioral changes (which may be obvious or subtle),

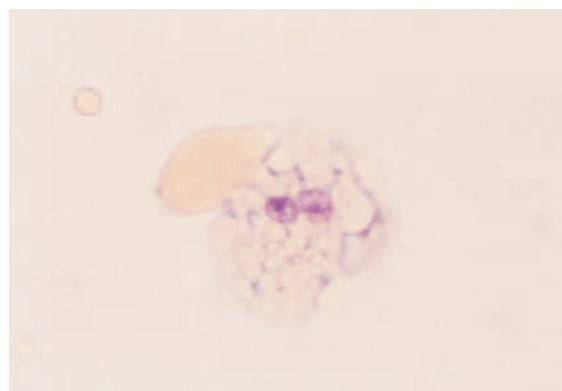


FIGURE 9-9 Feline fatty liver. This binucleated hepatocyte was stained with both a Sudan stain and new methylene blue to document the abundant fat in intracellular vacuoles and free outside the hepatocyte.

transient blindness, seizures, coma, or vague CNS abnormalities. Congenital PSS and severe acquired hepatic disease (e.g., cirrhosis) may cause encephalopathy. Hepatic function testing is mandatory because these diseases may not significantly change serum ALT, SAP, albumin, BUN, glucose, or bilirubin concentrations. Resting blood ammonia concentrations are very specific for hepatic dysfunction, but they are not as sensitive as serum bile acids. A patient in an episode of hepatic encephalopathy may have increased or normal resting blood ammonia concentrations. Ammonia tolerance testing (ATT) and pre- and postprandial serum bile acid concentrations appear to be the most sensitive and specific tests for hepatic dysfunction that causes hepatic encephalopathy. A very rare congenital urea cycle enzyme deficiency may cause hepatic encephalopathy and hyperammonemia without affecting enzymes or bile acids, in which case analysis of urea cycle enzymes in hepatic samples is necessary for diagnosis.

Icterus

Icterus is detected at physical examination or when serum or plasma is inspected at the laboratory. Hyperbilirubinemia always denotes hepatobiliary or hematopoietic disease (Figure 9-10). Hepatic and hematopoietic diseases are not always associated with icterus, and disease in either system may be secondary to other disorders. The presence or absence of icterus is not diagnostic or prognostic. Sepsis, pancreatitis, and IBD sometimes cause secondary hepatobiliary dysfunction that may include icterus.

TOTAL SERUM BILIRUBIN

Common Indications • Measurement of total serum bilirubin is indicated in cases of icterus (on either physical examination or inspection of nonhemolyzed serum or plasma), bilirubinuria (any amount in a cat or significant amounts in a dog), or suspected hepatic disease that is not apparent on other tests. The sclera has detectable

icterus when the serum bilirubin is greater than 3 to

4 mg/dl, and the plasma is icteric when the serum bilirubin is greater than 1.5 to 2 mg/dl.

NOTE: Icterus is absent in many animals (especially dogs) with hepatic disease. Serum bilirubin is not a sensitive test for hepatic disease.

Measurement of direct (conjugated) and indirect (unconjugated) bilirubin fractions is not useful because hemolytic, hepatic, and biliary tract diseases have unpredictable variation in the amount of each fraction.

Analysis • Total bilirubin is measured in serum or heparinized plasma by spectrophotometric and dry reagent methods. The latter require dilutions if the bilirubin is greater than 7.5 mg/dl. Bilirubin is stable at 4° C for 7

days if not exposed to bright light. Measurement of urine bilirubin is discussed in Chapter 7.

Normal Values • Dogs, less than 1.0 mg/dl; cats, less than 1.0 mg/dl (depends upon the laboratory).

Danger Values • Dogs, uncertain, but values greater than 20 mg/dl cause concern (i.e., kernicterus); cats, unknown.

Artifacts • Exposure to bright sunlight or fluorescent lighting can decrease bilirubin by 50% per hour (see Chapter 1).

Drug Therapy That May Alter Serum Bilirubin • Decreased bilirubin may be caused by drugs that cause hepatic enzyme induction (e.g., phenobarbital). Increased bilirubin may be the result of drugs causing hemolytic anemia or acute hepatic necrosis or dysfunction (see Box 9-5).

Causes of Hyperbilirubinemia • Hemolytic disease and hepatobiliary disease are the two main causes (see Figure 9-10). A CBC should be determined in every icteric patient to help rule out hemolytic disease. RBC numbers must decrease rapidly and significantly (i.e., packed cell volume [PCV] usually <20%) to cause clinical icterus. Very regenerative anemias may suggest that icterus is due to immune-mediated hemolytic anemia (IMHA). Reticulocytosis, hemoglobinemia, hemoglobinuria, erythrocytic autoagglutination, spherocytosis, positive Coombs test results, splenomegaly, or hepatomegaly are often present. Chapter 3 includes further discussion of IMHA and other regenerative anemias (e.g., Heinz body, zinc intoxication, *Babesia*, *Cytauxzoonosis*, hemotrophic *Mycoplasma*). Bilirubinuria theoretically should be absent in hemolytic disease but is typically present in IMHA because canine kidneys conjugate bilirubin. The clinician must not be misled by increases in ALT, because severe, acute hemolytic anemia may cause increased ALT (ostensibly caused by acute hepatic hypoxia).

Severe hepatic disease (especially acute necrosis) is sometimes accompanied by DIC and subsequent hemolytic anemia. These cases may be difficult to distinguish from IMHA. However, anemia caused by DIC is usually not as regenerative as in IMHA; in addition, the presence of RBC fragments, thrombocytopenia, increased fibrin degradation products (FDP), decreased antithrombin III, prolonged clotting time, and abnormal hepatic function tests usually allow differentiation, as do vomiting, abdominal pain, and encephalopathy when present.

Dogs and cats often have relatively severe hepatic disease before icterus is observed; however, the magnitude of the total serum bilirubin is not prognostic or diagnostic. Secondary hepatic disease (reactive disease or so-called *bystander phenomenon* as the result of septicemia, toxemia, or inflammation) may cause icterus. Certain bacterial endotoxins and acute phase inflammatory mediators are thought to alter normal bilirubin metabolism and cause increases in total bilirubin concentrations.

Most feline hepatic diseases eventually cause icterus; the most common conditions include hepatic lipidosis, cholangitis and cholangiohepatitis, hepatic lymphoma,

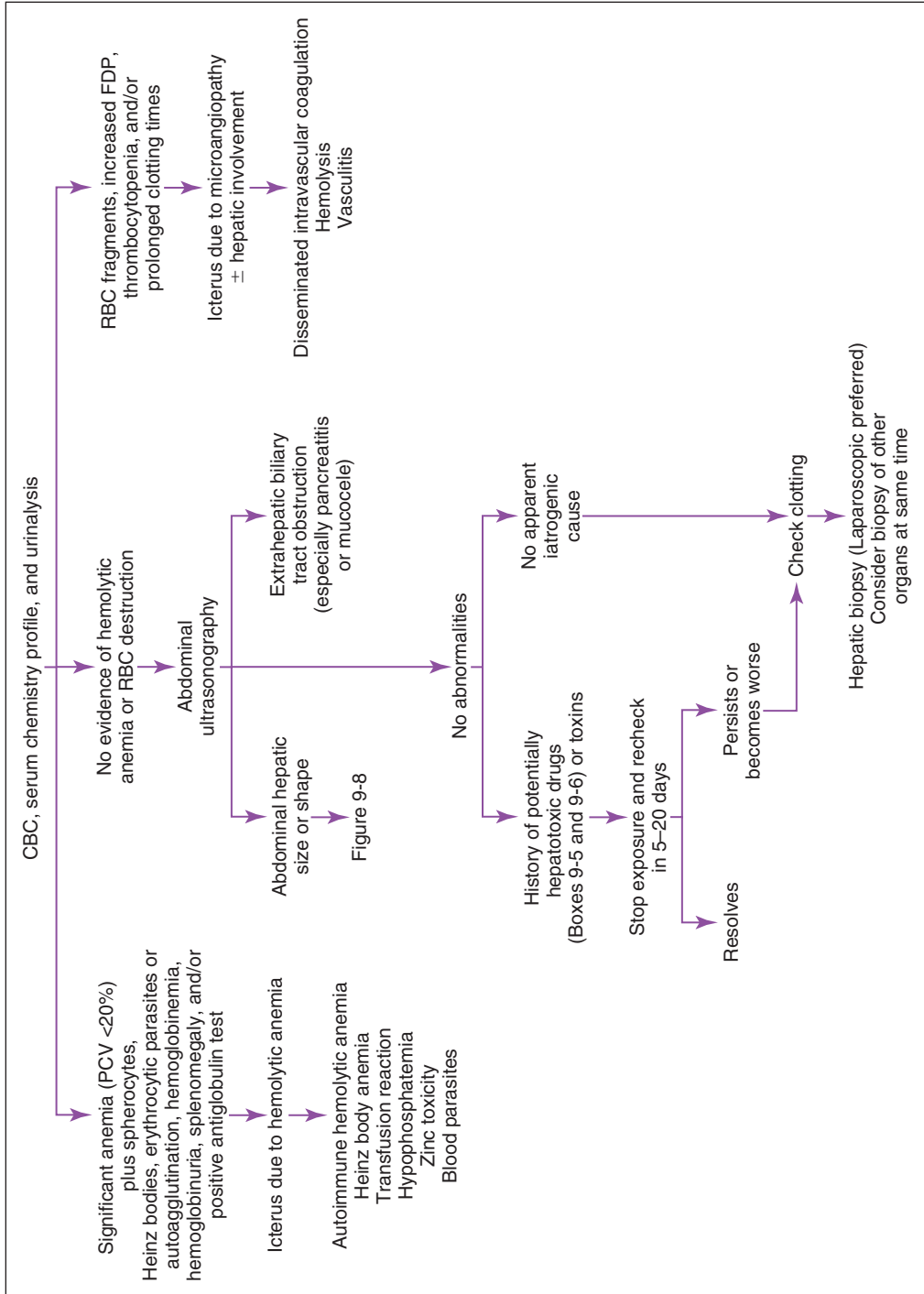


FIGURE 9-10 Diagnostic approach to hyperbilirubinemia in dogs and cats. CBC, Complete blood count; FDP, fibrin degradation products; PCV, packed cell volume; RBC, red blood cell.

and FIP. Icterus in cats is an indication for a CBC and serum biochemistry panel. Icterus in cats that is not caused by hemolysis usually indicates a hepatic biopsy, because most of these cats have primary hepatic disease. Biopsy is necessary to differentiate causes and institute specific treatment.

Common causes of nonhemolytic icterus in dogs include pancreatitis obstructing the bile duct, gallbladder mucoceles, cholecystitis, chronic hepatitis, hepatic lymphoma, acute hepatic necrosis, hepatic cirrhosis, and intrahepatic cholestasis. Icterus in dogs is an indication for a CBC and serum biochemistry panel (to include at least ALT, SAP, BUN, cholesterol, and albumin). Ultrasonography is indicated to help determine if primary hepatic disease or biliary tract obstruction exists. If primary hepatic disease is diagnosed, hepatic biopsy is usually indicated. If pancreatitis is present, surgery is not indicated unless a persistent bile duct obstruction necessitates a biliary drainage procedure (e.g., cholecystoduodenostomy or biliary tract stenting—both are very rarely needed) or a pancreatic abscess is symptomatic despite percutaneous drainage. If coexisting extrahepatic disease is found, it should be investigated.

ALANINE AMINOTRANSFERASE

ALT was formerly known as *serum glutamic-pyruvic transaminase* (SGPT).

Common Indications • Systemic disease, including weight loss, hepatomegaly, vomiting, diarrhea, icterus, ascites, depression, and anorexia, is an indication for measurement of ALT; also, ALT is used as a screening procedure for hepatic disease in patients with undiagnosed illness. Most patients with known chronic hepatitis should undergo periodic ALT determinations to monitor the problem.

Advantages • The serum ALT is specific for the liver.

Disadvantages • The test has lack of sensitivity (i.e., patients with significant hepatic disease such as cirrhosis or hepatic neoplasia may have normal ALT) and cannot distinguish among different hepatic diseases or determine if there is secondary nonhepatic disease involvement.

Analysis • ALT is measured in serum (heparinized plasma in selected assays) by spectrophotometric and dry reagent methods. ALT is stable in separated serum for approximately 1 (at 22° C) to 7 (at 4° C) days.

Normal Values • Serum enzyme activity may vary markedly among laboratories, depending on the technique and the units used.

Danger Values • Despite correlation between ALT and active hepatic damage, no correlation exists between ALT and hepatic function; hence, no danger values exist.

Artifacts • See Chapter 1.

Drug Therapy That May Alter Serum ALT • Any drug causing hepatocellular damage (i.e., drug-induced

hepatopathy) may cause increased ALT. The list of all

drugs suspected to cause increased ALT is extensive and includes many that are safe in the majority of patients. A list of selected drugs documented to cause increased ALT in human beings, dogs, and cats is given in Box 9-5. Administration of one of these drugs does not automatically explain an increased ALT, however.

NOTE: A patient can have an idiosyncratic reaction to almost any drug, causing an increased ALT.

Causes of Decreased ALT • Not significant.

Causes of Increased ALT • Increase in ALT is principally caused by hepatocellular damage from any cause (Box 9-7). RBCs and striated muscle cells contain small amounts of ALT, and damage to these may cause relatively minor increases (i.e., less than two to three times normal) in serum ALT, as may exercise. Dogs with muscular dystrophy may have major increases in ALT, but should also have increases in AST and creatine kinase (CK) values.

Hepatocytes contain substantial amounts of ALT in the cytosol, and major increases in serum ALT (i.e., three or more times normal) indicate hepatocellular leakage of the enzyme but do not always signify primary or irreversible hepatic disease. Hepatic disease may have normal to significantly increased serum ALT activity. The magnitude

BOX 9-7. SELECTED CAUSES OF INCREASED SERUM ALANINE AMINOTRANSFERASE LEVELS (>3 TIMES NORMAL)

DOGS	CATS
Hepatobiliary Disease	Hepatobiliary Disease
Cholangitis	Cholangitis
Cholangiohepatitis	Cholangiohepatitis
Cirrhosis	Feline infectious peritonitis (FIP)
Copper storage disease	Hepatic lymphoma
Hepatic malignancy	Cirrhosis
Chronic hepatitis	Hepatic toxin
Hepatic toxin	Trauma
Trauma	Pancreatitis
Pancreatitis	Hyperthyroidism
Other Disorders	Other Disorders
Anoxia because of anemia/shock	Anoxia because of anemia/shock
Iatrogenic (see Box 9-5)	Iatrogenic (see Box 9-5)

NOTE: Almost any disease affecting the liver can cause increased ALT levels. The disorders listed are those that may be more likely to cause a significant increase. However, any of these diseases can exist with minor or no increase in ALT values.

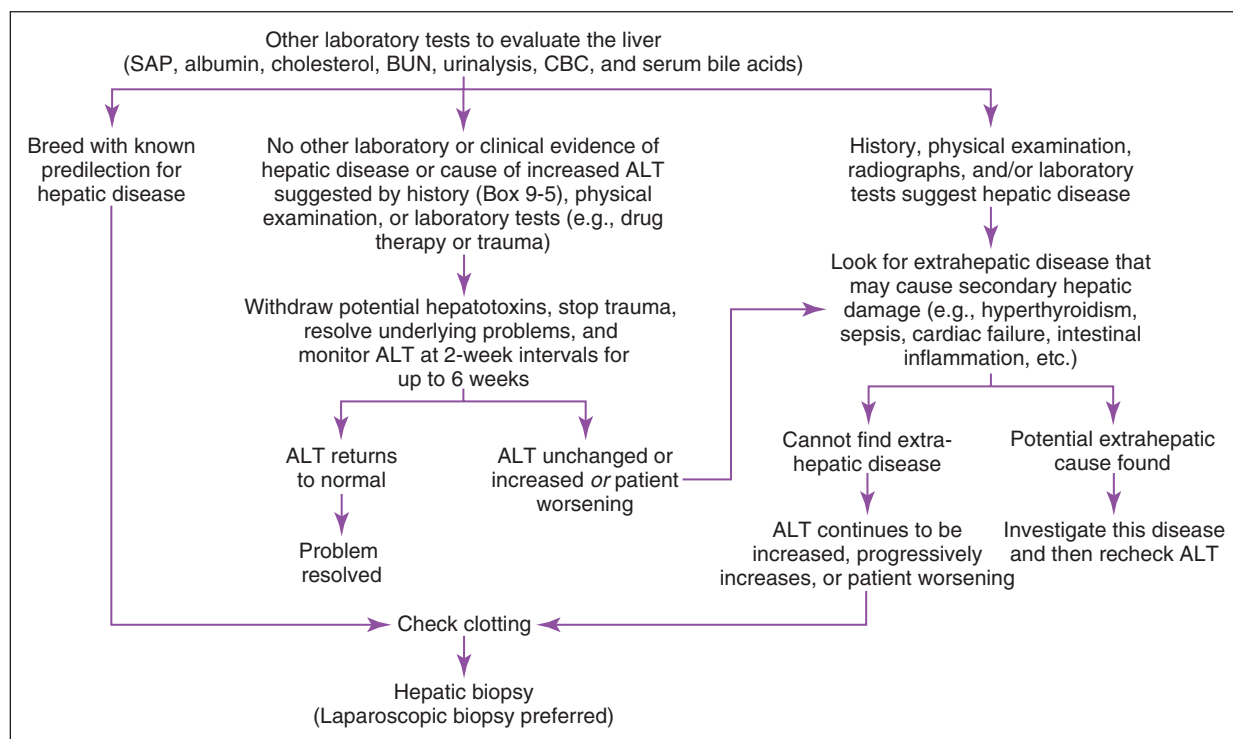


FIGURE 9-11 Diagnostic approach to alanine aminotransferase (ALT) greater than three times normal in dogs and cats. BUN, Blood urea nitrogen; CBC, complete blood count; SAP, serum alkaline phosphatase.

of the increase in ALT does not correlate with the seriousness of the hepatic disease and is not a prognostic indicator unless a specific disease is being considered. The serum ALT half-life is approximately 1 to 2 days or less, and serum ALT is expected to decrease over 1 to 2 weeks once active hepatic damage ceases. It is thought that ALT remains elevated during hepatic regeneration.

After increased serum ALT is identified, many factors must be considered (Figure 9-11). If no other evidence of disease is found, the increased ALT indicates the need for periodic monitoring because it may be the first detectable sign of significant hepatic disease. If other abnormalities consistent with hepatic disease are found, the approach is like that in any other patient with hepatic disease. Common causes of serum ALT more than three times normal include hepatic anoxia, poor hepatic perfusion, spontaneous and surgical trauma (e.g., hit by a car, surgery), chronic hepatitis, cirrhosis, cholangitis and cholangiohepatitis, acute biliary obstruction, hepatic necrosis as the result of any cause, acute pancreatitis, hepatic neoplasia, sepsis, and certain drugs. Sepsis, especially septicemia and toxemia, may secondarily damage hepatocytes. Abdominal inflammation may do the same. The pancreas is close to the liver, and inflammation in the pancreas may cause mechanical damage to the liver. In Doberman pinschers, Bedlington terriers, dalmatians, West Highland white terriers, and Labrador retrievers, a persistently increased serum ALT suggests chronic hepati-

tis that might be associated with increased hepatic copper concentrations.

ASPARTATE AMINOTRANSFERASE

AST was formerly known as *serum glutamic-oxaloacetic transaminase* (SGOT).

Occasional Indications • Indications for AST are the same as for ALT.

Disadvantages • Serum AST is not as specific for the liver as ALT.

Analysis • Measurement of AST is the same as for ALT.

Drug Therapy That May Alter AST • Decreased AST may be caused by metronidazole therapy. Hepatotoxic drugs may cause increased AST (see Box 9-5).

Causes of Decreased AST • None.

Causes of Increased AST • Like ALT, AST is present in significant quantities in hepatocytes. Although ALT is present in the cytosol, AST is present in the mitochondria. Increased serum ALT reflects cell membrane damage and leakage; significant AST increases tend to reflect more serious hepatocyte damage because the mitochondria are

not damaged as readily as is the cell membrane. AST is, however, present in significant quantities in many other tissues, including muscle and RBCs; therefore increased AST is not as specific for hepatic injury as is increased ALT. Exercise and intramuscular (IM) injections may increase serum AST. The most common causes of increased AST include hepatic disease, muscle disease (inflammation or necrosis), or hemolysis (spontaneous or artifactual). Increased AST is an indication to measure serum ALT to determine whether the increased AST is from the liver (significant increases in both ALT and AST suggest that AST increases are of hepatic origin). One may also measure the hematocrit and observe the color of the plasma and serum on a centrifuged blood sample to check for hemolysis.

SERUM ALKALINE PHOSPHATASE

Common Indications • Systemic disease, including weight loss, hepatomegaly, vomiting, diarrhea, ascites, icterus, depression, or anorexia, is an indication for measurement of SAP; also, SAP is used as a screen for hepatic disease and hyperadrenocorticism.

Advantages • SAP is useful in evaluating the liver for cholestatic disease.

Disadvantages • Measurement of SAP is affected by corticosteroids (endogenous and exogenous), bone lesions, and osteoblastic activity in young growing dogs.

Analysis • SAP is measured in serum or heparinized plasma by spectrophotometric methods. Different techniques (i.e., heat stability, phenylalanine, electrophoresis) have been used to distinguish SAP of bone origin from SAP of hepatic origin. The diagnostic usefulness of this distinction is doubtful because dogs with various types of hepatic disease often have endogenous steroids released due to the stress of illness.

Normal Values • May vary markedly from laboratory to laboratory. Immature dogs characteristically have SAP (bone origin) activities up to twice those of sexually mature dogs.

Danger Values • There is no correlation with hepatic function; therefore no danger values exist.

Artifacts • See Chapter 1.

Drug Therapy That May Increase SAP • Any drug that causes hepatic enzyme induction or cholestasis (see Box 9-6) may increase SAP. Glucocorticoids, primidone, and barbiturates typically increase SAP in dogs, but other drugs are less consistent. Although glucocorticoids can cause marked SAP increases in dogs, cats are almost never affected.

Causes of Decreased SAP • Not significant.

Causes of Increased SAP • SAP of bone origin is commonly increased (SAP less than three times normal) in

BOX 9-8. CAUSES OF INCREASED SERUM ALKALINE PHOSPHATASE LEVELS

DOGS	CATS
Biliary Tract Abnormalities	Biliary Tract Abnormalities
Pancreatitis	Same as for dogs
Bile duct neoplasia	
Cholelithiasis	
Cholecystitis	
Gall bladder mucocele	
Ruptured gallbladder	
Hepatic Parenchymal Disease	Hepatic Parenchymal Disease
Cholangiohepatitis	Cholangiohepatitis
Chronic hepatitis	Hepatic lipidosis
Copper storage disease	Hepatic lymphoma
Cirrhosis/fibrosis	Feline infectious peritonitis (FIP)
Hepatic neoplasia	
Lymphoma	
Hemangiosarcoma	
Hepatocellular carcinoma	
Metastatic carcinoma	
Toxic hepatitis	
Aflatoxin	
Mushroom	
Sago palm	
Drug induced	
Other Disorders	Other Disorders
Diabetes mellitus	Diabetes mellitus
Endogenous steroids (from physiologic stress or pathologic hyperadrenocorticism)	Hyperthyroidism
Chronic passive congestion from right heart failure	
Diaphragmatic hernia	
Septicemia	
Ehrlichiosis*	
Young dog with bone growth	
Osteomyelitis*	
Iatrogenic (see Box 9-6)	Iatrogenic (see Box 9-6)*

NOTE: Almost any disease affecting the liver can cause increased SAP levels. The disorders listed are those that may be more likely to cause a significant increase. However, any of these can exist with minor or no increase in SAP values.

*Rarely of importance.

dogs less than 6 to 8 months old. Bone disease (e.g., osteosarcoma, osteomyelitis) may increase SAP (usually a minor increase).

Increased SAP is interpreted differently in dogs and cats (Box 9-8). Cats have less hepatocellular SAP, which is readily excreted by their kidneys. Therefore any increase in feline SAP is considered important. Not all cats with hepatic disease have increased SAP. The major causes of increased SAP in cats are hepatic lipidosis,

cholangitis and cholangiohepatitis, hyperthyroidism, and diabetes mellitus. SAP increases are generally more specific than GGT in cats with hepatic lipidosis (cats with lipidosis classically have very high SAP with little to no increase in GGT; however, this finding is not consistent enough to allow a diagnosis). Hyperadrenocorticism (spontaneous and iatrogenic) very rarely increases SAP in cats. Increased SAP in a cat is an indication for serum thyroid hormone determination, urinalysis, blood glucose, and serum ALT measurement, $\pm$ hepatic function testing (e.g., serum bile acids). If hepatic disease is the apparent cause of the increased SAP, one must determine if hepatic biopsy is indicated (see the discussion under Serum Bile Acids later in this chapter).

The major causes of SAP values more than three times normal in dogs are hepatobiliary disease, endogenous steroids (i.e., hyperadrenocorticism or stress), and therapy with glucocorticoids or anticonvulsants. Hepatic disease with increased SAP usually has a cholestatic component; however, this does not imply icterus or gross obstruction of the biliary tract. Intrahepatic cholestasis caused by diffuse or focal compression of bile canaliculi may occur in various hepatopathies, even those secondary to septicemia, toxemia, and chronic stress-induced vacuolar (i.e., hydropic change) hepatopathy. Acute hepatocellular necrosis can transiently increase SAP. Extrahepatic biliary tract obstruction and enzyme induction caused by endogenous or exogenous glucocorticoids or drug administration may increase SAP more than 10 times normal. As with ALT, the magnitude of the increase in SAP does not correlate with prognosis.

In dogs, it is important first to rule out young age, drug therapy, and hyperadrenocorticism to avoid performing unnecessary diagnostics (Figure 9-12). Hyperadrenocorticism can easily be confused with primary hepatic disease because it typically causes hepatomegaly, pu-pd, increased ALT, and sometimes increased serum bile acids. If a patient has signs of hepatic failure (i.e., icterus, hepatic encephalopathy, hypoglycemia, weight loss, vomiting, hypoalbuminemia, ascites, microhepatia), one generally assumes that the increased SAP is due to primary hepatic disease. If it is not obvious that the patient probably has primary hepatic disease and the patient has signs that might be consistent with hyperadrenocorticism (e.g., pu-pd, cutaneous changes, potbelly), then adrenal gland function testing may be appropriate. If the SAP is increased but the ALT and serum bilirubin concentrations are normal, clinically important hepatic disease is unlikely. If the ALT is substantially increased (and especially if it is higher than the SAP), then primary hepatic disease is much more likely. If a hepatic biopsy specimen is obtained from a patient with hyperadrenocorticism or other systemic disease that is causing "stress," vacuolar hepatopathy is found.

GAMMA-GLUTAMYL TRANSPEPTIDASE

Rare Indications • GGT is rarely indicated; the indications are similar to those for SAP. SAP appears to be more sensitive for hepatobiliary disease in dogs; however, in

cats, GGT has slightly greater sensitivity and perhaps greater specificity for hepatic disease (except hepatic lipidosis). Therefore it is more frequently indicated in cats than in dogs. GGT is less influenced than SAP by secondary hepatic disease conditions or enzyme-inducing drugs. The use of SAP and GGT together has a higher predictive value of hepatic disease.

Analysis • GGT is measured in serum, urine, and body fluids by spectrophotometric methods. GGT is stable in serum at 4° C for at least 3 days and at 20° C for up to 1 year.

Normal Values and Danger Values • Same as for SAP.

Artifacts • See Chapter 1.

Drug Therapy That May Affect GGT • The same drugs that may affect SAP may affect GGT.

Causes of Decreased GGT • Not significant.

Causes of Increased GGT • Causes are similar to those of increased SAP and tend to parallel the magnitude of the rise in SAP, but bone lesions are not recognized to increase GGT. It is induced by glucocorticoid therapy and certain drugs, as is SAP. In cats, GGT may increase more than SAP, except in hepatic lipidosis (where classically the SAP is usually quite high, but GGT values show only a mild [or no] increase). GGT does not tend to increase after acute hepatic necrosis, as does SAP. Increased GGT should be pursued as for increased SAP (see Figure 9-12). Increased GGT may suggest pancreatitis obstructing the bile duct, as for SAP.

Causes of Increased Urine GGT • Increased 24-hour urinary excretion of GGT can be caused by various nephrotoxins (e.g., gentamicin).

LACTATE DEHYDROGENASE

Rare Indications • This test is not recommended.

Disadvantages • Lactate dehydrogenase (LDH) has lack of specificity.

Causes of Increased LDH • LDH is found in many body tissues. Inexplicable increases of small to great magnitude are not uncommon.

SERUM BILE ACIDS

Frequent Indications • Measurement of serum bile acids (SBAs) is indicated in patients with suspected occult hepatic disease, chronic weight loss, abnormal CNS signs, hepatomegaly, and microhepatia. SBAs are used routinely as a screening test for hepatic dysfunction (e.g., PSS).

Advantages • SBAs are easy to measure, and few extrahepatic factors affect it.

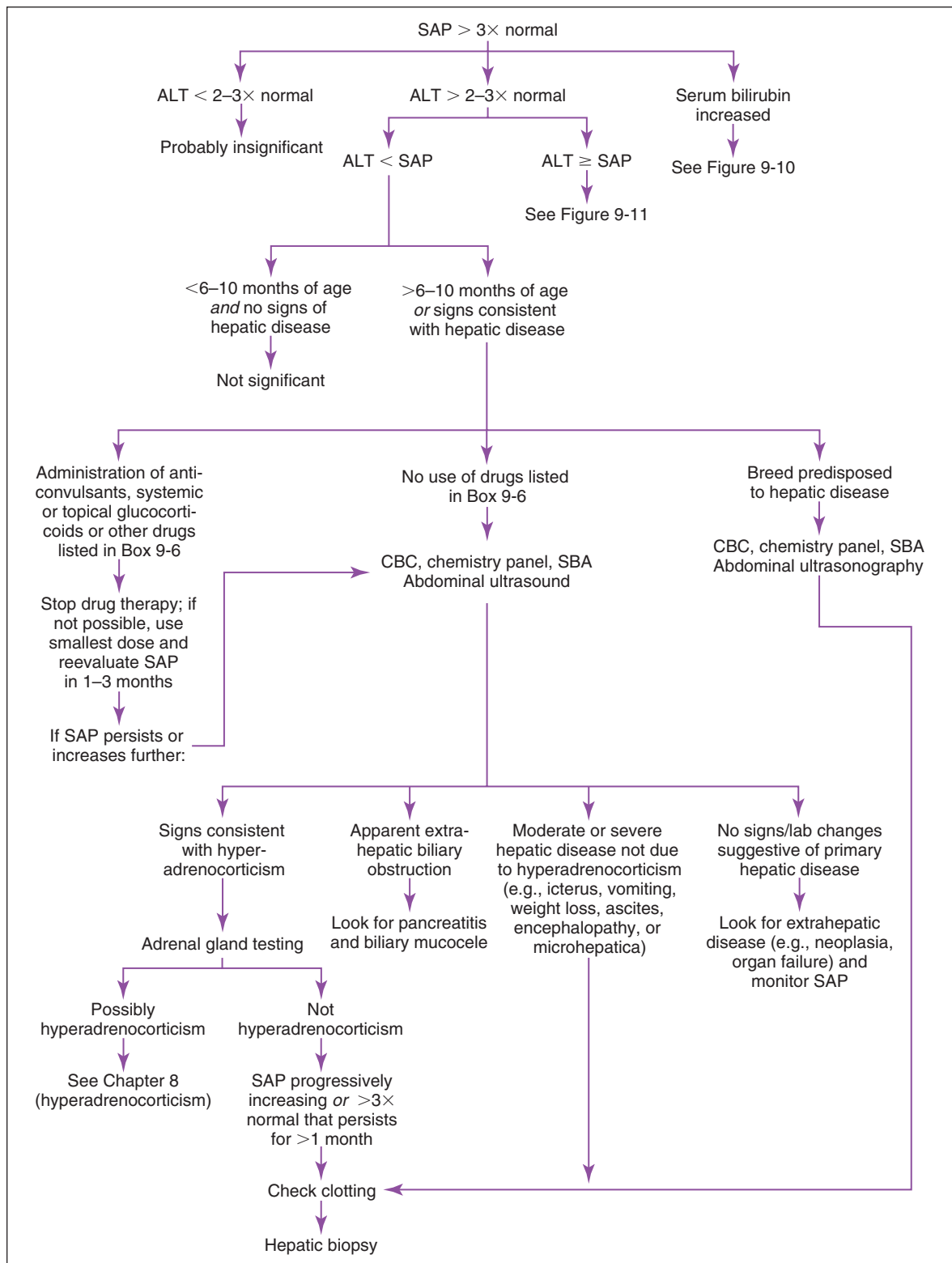


FIGURE 9-12 Diagnostic approach to increased serum alkaline phosphatase (SAP) in dogs. ALT, Alanine aminotransferase; CBC, complete blood count; SBA, serum bile acids.

Disadvantages • Measurement of SBAs does not reliably distinguish among different hepatobiliary diseases. Values may spontaneously change enough from day to day that it can be difficult to use SBA concentrations to determine if a change in hepatic function has occurred.

Analysis • SBAs are measured in serum by either a direct enzymatic method that quantifies total serum 3- α -hydroxylated bile acids or an RIA procedure that measures specific bile acids. It is important that a validated assay for dogs and cats be used because some methods are not accurate. Values for enzymatic and RIA procedures cannot be compared.

Maximum information is obtained by determining a 12-hour fasting preprandial and 2-hour postprandial SBA concentration. Dogs and cats should be fed canned food containing moderate fat content, causing the gallbladder to contract. Preprandial and postprandial SBA concentrations together improve the sensitivity of the test, making it more sensitive than resting blood ammonia concentration. Ceruletide has been administered after taking the fasting sample in an attempt to ensure gallbladder contraction and have more consistent results. It has not demonstrated increased sensitivity and specificity when looking for PSS, but it might be more sensitive when looking for hepatic disease associated with respiratory tract abnormalities.

Normal Values • Because of different techniques and assays ($\mu\text{mol/L}$ or $\mu\text{g/ml}$), normal values must be established for each laboratory.

Danger Values • None.

Artifacts • Very increased serum dehydrogenase activities may require modification of the spectrophotometric technique. Severe lipemia (i.e., chylomicronemia) and hemolysis may falsely decrease SBA measurements, and hypertriglyceridemia may falsely increase SBA concentrations when spectrophotometric techniques are used, but they do not affect RIA. This test is not useful in icteric patients.

Drug Therapy and Other Factors That May Alter SBA Concentration

• Cholestyramine lowers serum concentrations by binding to bile acids in the intestinal lumen, preventing their reabsorption. Ursodeoxycholic acid (a synthetic bile acid) therapy may increase total SBA concentrations. Resection of the ileum (the principal site of bile acid reabsorption), severe ileal disease, or cholecystectomy may also cause SBAs to inaccurately reflect hepatic function. Prolonged anorexia (>1 to 2 days) may cause fasting SBA concentrations to be less than would be found if the patient were eating normally. Intestinal hypomotility may cause the 2-hour postprandial sample to be a less sensitive indicator of hepatic disease because of failure to deliver the bile acids to the ileum in a timely fashion. Hepatic insufficiency does not decrease SBA concentrations.

Causes of Decreased SBA Concentration • Delayed gastric emptying, rapid intestinal transit, malabsorption disorders, and ileal resection may cause subnormal

values. With ARE, total measurable moieties may or may not decrease, but it is expected that unconjugated serum bile acid concentrations may increase.

Causes of Increased SBA Concentration • SBA concentrations are increased because of hepatocellular disease, cholestatic disease, hepatic microvascular dysplasia, or PSS. When both fasting and 2-hour postprandial SBA concentrations are determined, the sensitivity of these tests becomes greater than with other hepatic function tests except perhaps the ammonia tolerance test. Because of the ease of performing and the wide availability of the test, it has replaced other clinical hepatic function tests. SBAs offer no additional information in icteric patients with hepatic or extrahepatic biliary tract disease. In nonicteric patients suspected of having hepatic disease, SBAs are a good screening test to support further diagnostic evaluations. Not all patients with primary hepatic disease have increased SBA concentrations. The relative increase in SBAs as well as the percent increase from preprandial to postprandial values is not prognostic or diagnostic for a specific disease. Reported fasting SBAs that are greater than $20 \mu\text{mol/L}$ or postprandial values greater than $25 \mu\text{mol/L}$ suggest primary hepatic disease, microvascular dysplasia, or PSS and dictate further hepatic evaluation and possibly hepatic biopsy. If only fasted SBA values are determined and found to be normal, postprandial measurements are indicated. Most animals with chronic hepatitis, marked hepatic necrosis, cholestasis, and hepatic neoplasia have abnormal values. SBAs are usually not markedly altered by secondary hepatic disease or with glucocorticoid or anticonvulsant therapy. Some animals without histologic evidence of primary hepatic disease will have markedly increased SBAs. It has been suggested that these animals might have undiagnosed hepatic microvascular dysplasia, but that is uncertain.

Increased SBAs are possibly the most sensitive biochemical indicator of congenital PSS, but the values will not distinguish between PSS and microvascular dysplasia. Rare dogs with PSS can have normal resting and postprandial SBA concentrations.

URINARY BILE ACIDS

Occasional Indications • Measurement of urinary bile acids (UBAs) may be indicated when samples for fasting and postprandial SBA determinations are difficult to obtain.

Advantages • The client can bring a urine sample into the clinic instead of bringing the patient. Because the urine bile acids:creatinine ratio represents an average SBA value over time, the urine bile acids:creatinine ratio is probably not substantially affected by transient variation in SBA concentrations and appears to be as or more specific than a random single measurement of SBAs. Bilirubin and glucose in the urine are unlikely to interfere with test results. Treatment with ursodeoxycholic acid is not known to interfere with test results.

Disadvantages • The test may not be as sensitive as SBAs.

Analysis • UBAs are measured in urine (approximately 5 ml) using enzymatic methodology; the UBA concentration is standardized for urine flow and concentration by measuring concurrent urine creatinine concentration and calculating the ratio (μmol of urine bile acids/mg of urine creatinine).

Normal Values • Dog, less than 7.3 $\mu\text{mol/g}$; cat, less than 4.4 $\mu\text{mol/g}$.

Artifacts • Hematuria and hemoglobinuria may interfere.

Drug Therapy That May Alter UBA Concentrations • None known.

Causes of Low UBAs • Not significant.

Causes of High UBAs • UBA concentrations are increased by the same factors as for SBA.

BLOOD AMMONIA AND AMMONIA TOLERANCE TESTING

Frequent Indications • Indications for blood ammonia measurement are the same as for SBAs.

Advantages • Blood ammonia test has good sensitivity and specificity.

Disadvantages • There are specific procedural requirements for submitting the samples, and there is a likelihood of vomiting or CNS signs with ATT.

Analysis • Ammonia is measured in blood, serum, plasma (heparinized is recommended), CSF, or urine by enzymatic, selective electrode, dry reagent, and resin absorption methods. There does not appear to be any advantage of arterial over venous blood. Blood must be drawn into an ice-chilled tube, which is stoppered tightly after filling, immediately put back on ice, and promptly taken to the in-house laboratory. Unless the test is well established at the clinic, a control sample should be taken at the same time using the same technique. The test must be performed within 20 minutes, or the plasma must be frozen at -20°C , which stabilizes the ammonia concentration for at least 2 days. If an ATT is to be performed, samples for ammonia determination should be taken before and 30 or 45 minutes after administration of 100 mg $\text{NH}_4\text{Cl/kg}$ of body weight. The NH_4Cl may be administered orally (20 to 50 ml of a 5% aqueous solution), as a dry powder in gelatin capsules, or rectally (recommended) as a 5% solution.

Warning: Administration of NH_4Cl to patients with increased resting blood ammonia concentrations may cause encephalopathy. The clinician should not perform ATT if the patient is showing obvious encephalopathic signs or is hyperammonemic. Lack of obvious encephalopathic signs does not guarantee that blood

ammonia levels are normal. The ATT is not recommended in cats.

Normal Values • Resting blood ammonia: dogs, 45 to 120 $\mu\text{g/dl}$; cats, 30 to 100 $\mu\text{g/dl}$. ATT, ammonia at 30 minutes: dogs, minimal change from normal values.

Danger Values • Dogs, greater than 1000 $\mu\text{g/dl}$ (hepatic encephalopathy may be imminent, although poor correlation exists between clinical signs of encephalopathy and blood ammonia concentrations); cats, unknown.

Artifacts • Blood ammonia concentration may be falsely increased by allowing the blood to stand, and by strenuous exercise (see Chapter 1).

Drug Therapy That May Alter Ammonia • Decreased blood ammonia may be the result of intestinal antibacterial drugs, lactulose, *Lactobacillus acidophilus* cultures, enemas, and diphenhydramine. Increased blood ammonia may be the result of valproic acid, asparaginase, narcotics, diuretics causing hypokalemia or alkalosis, hyperalimentation, ammonium salts, and high-protein meals (including blood from spontaneous GI bleeding).

Causes of Hyperammonemia • Urea cycle disorders (extremely rare) and hepatic insufficiency (especially congenital or acquired PSS) can cause hyperammonemia. Resting blood ammonia concentrations are probably less sensitive than SBAs in detecting hepatic dysfunction, whereas the ATT is possibly as sensitive as preprandial and postprandial SBAs in detecting PSS. A significantly increased fasting blood ammonia concentration is specific for hepatic insufficiency and renders an ATT unnecessary. Clinical signs are not well correlated with blood ammonia concentrations. An abnormal ATT result or resting blood ammonia concentration in a patient with hepatic disease is generally an indication for imaging (e.g., ultrasound, scintigraphy) $\pm$ hepatic biopsy. Rarely, blood ammonia is increased because of urinary tract obstruction, especially if complicated by infection with urease-producing bacteria. Some young dogs (notably Scottish deerhounds in Great Britain) have elevated resting blood ammonia values that spontaneously return to normal as the dog ages. Therefore caution must be used when diagnosing congenital PSS in at least some breeds solely by evaluating the resting blood ammonia concentration.

PROTEIN C

Occasional Indications • Measurement of protein C may help distinguish dogs with congenital PSS from those with hepatic microvascular dysplasia. Protein C concentrations are low in dogs suffering from aflatoxin poisoning.

Advantages • Sensitivity of protein C is less than SBAs for PSS, but the specificity is approximately the same.

Disadvantages • There is limited availability of the test. Protein C testing is currently available at Cornell University Diagnostic Laboratory.

Analysis • Protein C is measured by chromogenic assay performed on citrated plasma (serum gives falsely high values). Samples must be shipped refrigerated or frozen.

Normal Values • 75% to 135%.

Artifacts • Hemolysis and lipemia may increase values.

Drug Therapy That May Alter Protein C • Unknown.

Causes of Low Protein C • Protein C is decreased by altered hepatic function and coagulation abnormalities. Protein C less than 70% is suggestive of PSS as opposed to microvascular dysplasia.

Causes of High Protein C • Protein C greater than 70% is suggestive of microvascular dysplasia as opposed to PSS. A protein C value that increased to 70% or greater after corrective surgery for PSS suggests that portal blood is being directed through the liver and not through the shunt.

HEPATIC MINERAL ANALYSIS

Occasional Indications • Hepatic mineral analysis may be used to distinguish between patients in which copper is the cause of the hepatic disease versus those in which it is the effect of the hepatic disease.

Advantages • Hepatic mineral analysis is more specific and definitive than special stains for copper.

Disadvantages • Hepatic mineral analysis requires 50 mg of liver tissue for analysis.

Analysis • Measurement of hepatic minerals is done by atomic absorption spectroscopy or atomic emission spectroscopy.

Normal Values • Less than 400 µg/g dry weight of liver.

Artifacts • Measurement of hepatic minerals may be affected by an inadequate tissue sample or a contaminated sample.

Drug Therapy That May Alter Mineral Analysis • Unknown.

Interpretation • Secondary hepatic copper retention from cholestasis may range from 400 to 1000 µg/g. Hereditary copper-associated hepatopathies can range from 750 µg/g and higher (often in the several thousands).

WEIGHT LOSS OR ANOREXIA OF UNKNOWN CAUSE

BOX 9-9. MAJOR CAUSES OF WEIGHT LOSS IN DOGS AND CATS

Lack of Calories (inadequate food or poor-quality food)

Inability or Refusal to Eat

Dysphagia

Oral lesion

Anorexia for any reason

Regurgitation

Pharyngeal or esophageal disease

Vomiting (see Box 9-2)

Maldigestion (EPI)

Intestinal Malabsorption (does not always cause diarrhea)

Malassimilation (organ failure)

Hepatic failure

Cardiac failure

Diabetes mellitus

Uremia

Cancer cachexia syndrome

Hypoadrenocorticism

Excessive Use or Loss of Calories

Hyperthyroidism

Excessive demand for calories because of environment or exertion

Lactation

Muscle Wasting

Myopathy

Neuropathy

Weight loss has many causes (Box 9-9). Concurrent problems with fewer potential causes (e.g., regurgitation, vomiting, diarrhea, icterus) should be considered first. If a patient had a reasonable appetite when weight loss began, major differential diagnoses are small intestinal disease, maldigestion, hepatic disease, increased use of calories (e.g., hyperthyroidism, lactation), increased loss of calories (e.g., diabetes mellitus) or caloric-deficient diet. If no other identifiable problems (other than weight loss or anorexia) can be pursued, a systematic search is indicated (Figure 9-13). One should first preclude as many causes as possible with the history and physical examination (i.e., lack of food, calorie-deficient food, inability to eat, regurgitation, vomiting and diarrhea). Next, extensive clinicopathologic screening is indicated. Imaging is considered an extension of the physical examination, and abdominal ultrasound plus thoracic radiographs are appropriate. Thoracic radiographs may be very revealing, even if a patient does not have coughing or abnormal lung sounds. Abdominal ultrasonography is particularly desirable and often more useful than radiographs, assuming the operator is accomplished. If laboratory or radiographic abnormalities are not present or are unconvincing, one may repeat the tests at 1- to 3-week intervals, depending on the clinical condition of the patient, or immediately proceed to function tests, biopsies, or both. Certain

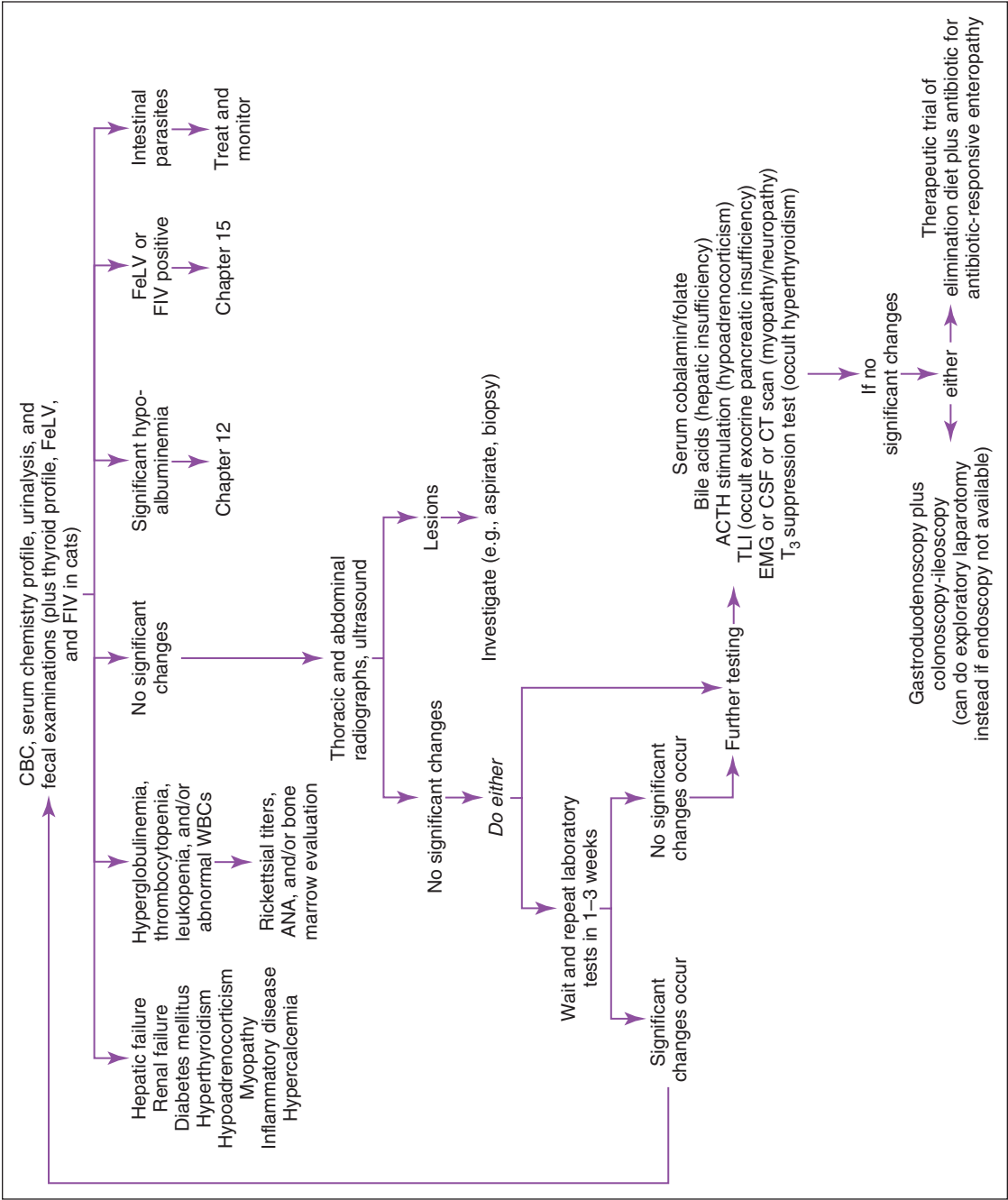


FIGURE 9-13 Diagnostic approach to chronic weight loss in dogs and cats when no other abnormalities are found on history or physical examination and the animal is not ingesting adequate calories (see Box 9-9). ACTH, Adrenocorticotropic hormone; ANA, antinuclear antibodies; CBC, complete blood count; CSF, cerebrospinal fluid; CT, computed tomography; EMG, electromyogram; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; T₃, triiodothyronine; TLI, trypsin-like immunoreactivity; WBC, white blood cell.

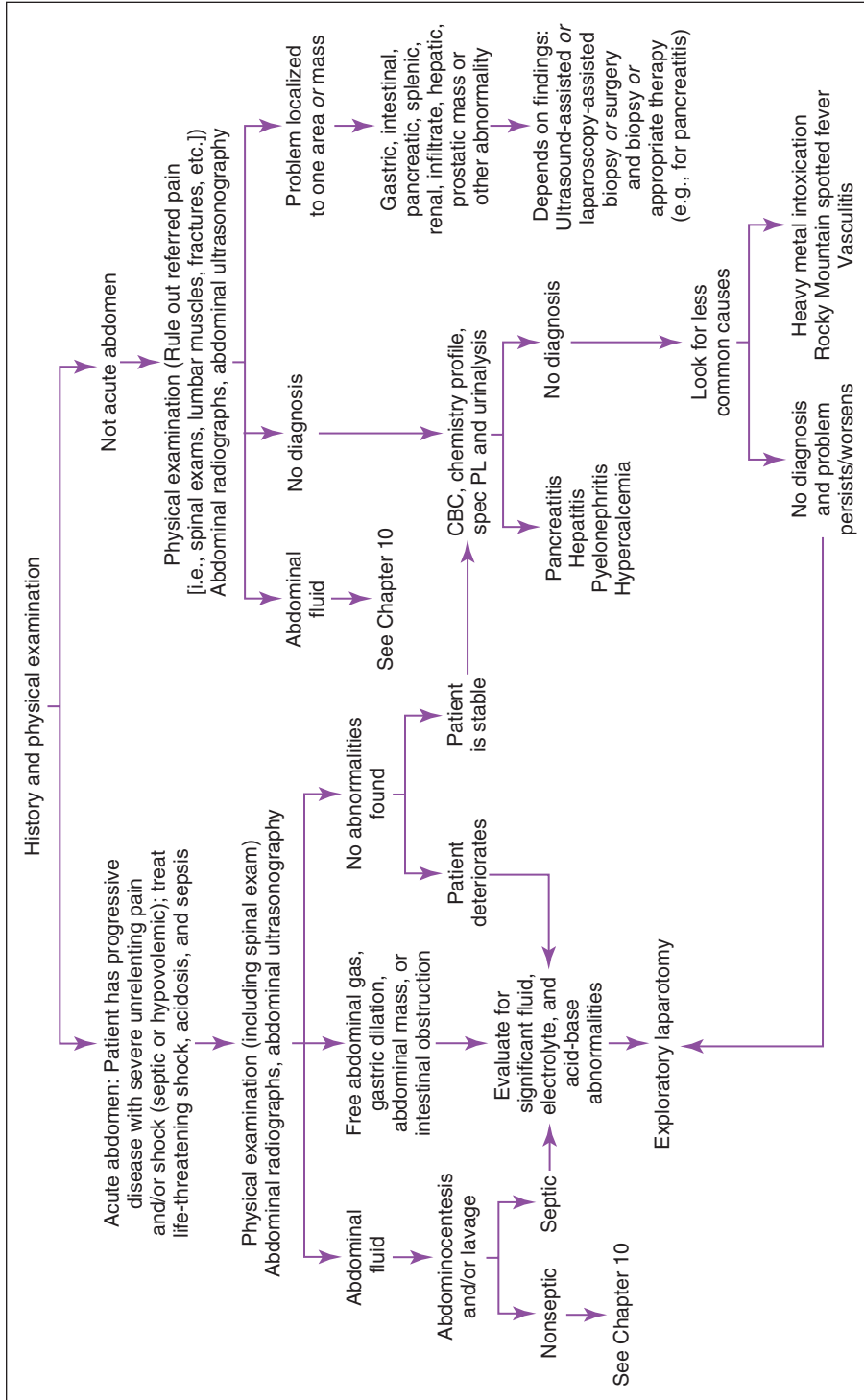


FIGURE 9-14 Diagnostic approach to abdominal pain in the dog and cat. CBC, Complete blood count; spec PL, immunoreactive pancreatic lipase.

hepatic and adrenal gland diseases may require such function tests. It is noteworthy that severe gastric or intestinal disease may cause anorexia or severe weight loss without vomiting or diarrhea, respectively.

Gastroduodenoscopy and ileoscopy plus biopsy are reasonable in patients with severe weight loss of unknown cause. Some cases with gastric neoplasia may present only for anorexia and weight loss. Clinicians without access to endoscopic equipment may consider exploratory laparotomy. If surgery is performed, gastric, duodenal, jejunal, ileal, mesenteric lymph node, and hepatic biopsies are usually appropriate, regardless of a normal gross appearance of the organs. In cats, the pancreas should also be biopsied.

Cancer cachexia can be particularly difficult to diagnose. It is a poorly defined, multifaceted syndrome that may involve loss of taste, malabsorption, increased metabolism with energy wasting, and other mechanisms. Almost any tumor can cause cancer cachexia, and no consistent laboratory findings exist. The causative cancer may be large or small, focal or diffuse; lymphomas and carcinomas are probably the most common causes.

Anorexia of unknown cause is similar to weight loss in being difficult to evaluate if no other identifiable abnormalities are seen. The diagnostic approach is similar to that for chronic weight loss (see Figure 9-13; Box 9-10). Anorexia can be divided into three categories: (1) inability to eat (due to oral, pharyngeal, or esophageal disease), (2) primary anorexia (rare) associated with a primary CNS disorder, and (3) secondary anorexia (the most

common), which is caused by systemic or metabolic disease.

If necessary, one may elect a therapeutic trial to treat for a suspected problem in a patient in whom a diagnosis cannot be made. It is vital that one design such therapy so that it is safe and extremely likely to succeed if the presumptive disease is present. Then, if the trial fails, one may rule out that disease and go on to treat for something else. To do this, the clinician must be sure that the dose and duration of the treatment are sufficient.

ABDOMINAL PAIN

History, physical examination, radiographs, and

ultrasonography are the initial tools in diagnosing the cause of abdominal pain (Figure 9-14). Extra-abdominal diseases (e.g., spinal problems) and patients predisposed to nonsurgical diseases (e.g., pancreatitis) must be identified early.

In patients with severe, progressive, acute abdomen (severe unrelenting pain or shock or stupor in a deteriorating patient), surgery is often indicated as soon as fluid, electrolyte, and acid-base status are acceptable for anesthesia. Imaging is critically important, but extensive laboratory testing is unlikely to identify the more common causes of acute abdomen (e.g., intestinal obstruction, gastric dilation and volvulus, peritonitis, organ ischemia, tumor, sepsis, or bleeding) and usually only delays surgical resolution of disease. Abdominal exploration offers a good chance for definitive diagnosis plus resolution of the disease process.

NOTE: These maladies do not always present as surgical emergencies.

If a patient is not in severe pain and the disease is not progressing rapidly, one must differentiate between problems that ultimately necessitate surgery and those that usually do not (e.g., pancreatitis, hepatitis, cholecystitis, upper urinary tract infection, prostatitis, pansteatitis, heavy metal intoxication, Rocky Mountain spotted fever [RMSF]). Abdominal ultrasonography is useful to examine the liver, spleen, pancreas, kidneys, and prostate, as well as to detect peritoneal fluid. If abdominal fluid is present, abdominocentesis or abdominal lavage with cytologic analysis is indicated. If these procedures are not revealing and the problem continues, exploratory surgery may be necessary. Contrast radiographs are rarely useful because thorough abdominal exploration should diagnose almost anything they reveal; finding an abnormality on contrast radiographs is typically just an indication for surgery. In rare situations the exhibited abdominal pain may be referred from other causes such as pulmonary disease or disk disease.

BOX 9-10. CATEGORIES OF DISEASES THAT CAUSE ANOREXIA

Psychologic (especially cats)

Inability to Smell Food (Anosmia)

Dysphagia

Systemic Inflammation

Etiologic agent (e.g., viral, bacterial, fungal, rickettsial, parasite)

Immune-mediated disease

Neoplasia

Necrosis

Iatrogenic (drugs)

Alimentary or Abdominal Disease Causing Nausea or Pain

Gastritis/enteritis

Neoplasia

Toxins

Exogenous (various)

Endogenous (e.g., primarily due to organ failure)

Endocrine Disease

Hypoadrenocorticism

Hyperthyroidism

Central Nervous System (CNS) Disease

Primary

Secondary

Suggested Readings

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10

Fluid Accumulation Disorders

Sharon A. Center

DIAGNOSTIC APPROACH

Effusions are commonly encountered in veterinary practice and often assist in determining a definitive diagnosis. The term effusion describes inappropriate accumulation of fluid within a body “potential” or “third” space outside of vascular or lymphatic conduits and visceral structures. These are often disclosed on physical examination; for example, overhydrated skin turgor and thickened lip folds, conjunctivae, tarsal webs, or scrotum may indicate localized edema or whole-body edema (i.e., anasarca); joint cavity distention indicates joint effusion often associated with arthritis; dyspnea and/or tachypnea, muted lung sounds, or cough may indicate pleural effusion; irregular femoral pulses, pulses alternans, and muted cardiac sounds concurrent with a jugular pulse may indicate pericardial effusion; and slippery visceral surfaces or a ballotable fluid wave may indicate abdominal effusion. Unfortunately, some effusions evade detection until imaging studies (radiography, ultrasonography) disclose their presence. Furthermore, centripetal adiposity can be confused with abdominal effusion but is easily differentiated on ultrasound examination. Collection, physiocytologic characterization, and chemical evaluation of effusions are crucial for accurate categorization. Classification schemes incorporating these features direct differential diagnoses considering the associated disease pathomechanisms.

FLUID COLLECTION TECHNIQUES

Collection of Fluid • The site of fluid collection is prepared as for aseptic surgery. A 1-inch, 23- to 20-gauge needle, over-the-needle Teflon catheter, or butterfly needle–catheter is recommended. Fluid analysis usually requires a minimum of 3 to 5 ml of fluid. If ultrasound guidance is used, it is important to prevent sample contamination with ultrasound gel that induces artifacts (i.e., blue-appearing smudges on Diff-Quik or Wright-Giemsa-stained preparations). Aspiration methods should relieve negative pressure during needle withdrawal from the site

of sample collection to avoid collection of contaminating and irrelevant tissues (cells).

Abdominocentesis • The abdomen is palpated immediately before abdominocentesis to avoid lacerating visceral structures, and any available images are consulted. The urinary bladder should be emptied before the procedure, particularly when 14-gauge catheters are used. In patients with tense abdominal distention, the abdomen is punctured laterally to avoid gravitational ventral midline seroma formation and adhesions associated with ovariectomy. If septic peritonitis is suspected but unproven with routine abdominocentesis, a four-quadrant tap can be performed. Ultrasonographic guidance is helpful for sampling loculated fluid or fluid collecting in the abdominal gutters. Usually a 22- to 20-gauge, 1-inch needle or Teflon catheter is attached to extension tubing. Mobility afforded by the extension tubing helps avoid visceral laceration should the patient move during the centesis procedure. Alternatively, a butterfly catheter set is used. If an abdominal effusion is difficult to sample, a 14-gauge Teflon catheter is used to puncture the abdomen, and a closed-ended polypropylene Tomcat catheter is inserted through its lumen. The Teflon catheter provides a “sterile stent” through which the Tomcat catheter may be manipulated as the patient’s position is altered. Local anesthesia (lidocaine block) may be needed in some patients to enable nonpainful abdominocentesis.

Abdominal Lavage • Sterile warmed physiologic saline (20 ml/kg) is administered intraperitoneally over 5 to 10 minutes through extension tubing and a Teflon catheter. The abdomen is massaged or the animal moved about for several minutes to mix infused fluid with that trapped within omental recesses and abdominal gutters. Lavage fluid is subsequently aspirated and analyzed. Dogs normally have less than 500 white blood cells (WBCs)/ μ l. Mild leukocytosis occurs after recent abdominal trauma or surgery. Diagnostic guidelines for interpretation of abdominal lavage fluid are listed in [Box 10-1](#). Iatrogenic injury and bacterial contamination are possible

BOX 10-1. SUGGESTED GUIDELINES FOR INTERPRETATION OF ABDOMINAL LAVAGE EFFUSION*

TURBIDITY	
Clear:	No disease or abdominal injury
Bloody:	Iatrogenic or hemorrhage
	Chronic effusion: serosanguineous
Blood darkens on repeat centesis:	Active hemorrhage: acquire packed cell volume (PCV) for relative change
Turbid:	Cannot clearly read newsprint through fluid: cytology indicated
PCV:	
<5%	Mild hemorrhage
>10%	Significant hemorrhage
WBC count:	
<500/ μ l	Normal dogs
>1000/ μ l	Mild to moderate inflammation
>2000/ μ l	Probable peritonitis: cytology indicated
PANCREATIC ENZYMES (lipase or amylase):	If >sera: pancreatic inflammation, injury, necrosis
TOTAL BILIRUBIN:	If >sera: bile spillage or enteric rupture
CREATININE:	If >sera: urinary tract rupture, urine spillage
VEGETABLE FIBERS:	Enteric rupture or sampled enteric lumen
MIXED BACTERIAL FLORA:	Enteric rupture, ruptured abscess, or sampled enteric lumen

*After instillation of 20 ml/kg and mixing throughout peritoneal cavity.
WBC, white blood cell.

consequences of the lavage procedure, and dilutional influences on collected fluid can create diagnostic confusion. The convenient availability of ultrasound in clinical practice has reduced use of this technique by guiding targeted fluid centesis.

Thoracocentesis • Thoracocentesis is usually performed in the seventh or eighth intercostal space at the level of the costochondral junction; however, relevant imaging studies may better guide sample collection. The needle penetrates the middle of the intercostal space, avoiding the caudal rib margin where nerves and vessels are located. Harvesting fluid is optimal with the animal standing or in sternal recumbency. A 1-inch, 18- or 20-gauge butterfly catheter connected to a three-way stopcock and a 20- to 35-ml syringe are recommended. During initial needle placement, negative pressure is maintained

on the syringe so that advancement of the needle immediately discloses effusion, thus avoiding inadvertent pulmonary puncture or laceration. Repeated centesis should be performed only after a local anesthetic block is applied to the puncture site.

Pericardiocentesis • Before pericardiocentesis, samples of blood are used to determine baseline packed cell volume (PCV), total solids (TS) concentration, platelet count, and activated coagulation time (ACT). This procedure is best performed with the patient in sternal recumbency and with local analgesia block. The site of thoracic penetration is surgically prepared and blocked with local anesthetic, and a small incision is made in the skin to facilitate movement of the catheter through the dermis. The right side is preferred to avoid large coronary arteries on the left side. However, echocardiography should assist in determining the optimal site for pericardiocentesis. Without access to echocardiography, the site selected corresponds to the palpable cardiac beat or just caudal to or below the elbow at the level of the costochondral junction (fifth to sixth intercostal space). The catheter is usually passed through the fifth or sixth right intercostal space (i.e., the cardiac notch between lung lobes) after local anesthetic is injected to the level of the pleura. A 12- to 16-gauge, 4- to 6-inch over-the-needle Teflon catheter is used with two or three extra holes aseptically snipped in the lateral aspect of the catheter approximately 1.5 to 3.0 cm from the tip. Extension tubing and a three-way stopcock are necessary in medium- and large-sized dogs. An electrocardiogram (ECG) is simultaneously recorded while the catheter is advanced; touching the myocardium elicits premature ventricular beats. Ultrasound guidance is routinely used for this procedure. Pleural effusion is usually present and first encountered, typically a modified transudate, amber to slightly red in color. Entrance into the pericardium may require an acute thrust associated with a "pop," after which the catheter is slipped over the needle into the pericardial sac, and the needle removed and discarded. Collection of a hemorrhagic effusion is typical and necessitates immediate differentiation of centesis fluid from peripheral blood using comparisons between each fluid in PCV, platelet count, TS concentration, and supernatant color, and an ACT on the fluid to confirm its inability to clot. These assessments avoid inadvertent removal of large volumes of intracardiac blood. Pericardial hemangiosarcomas may initiate a hemorrhagic effusion upon pericardiocentesis that may be difficult to differentiate from iatrogenic cardiac puncture. Ultrasonographic imaging may assist in resolving this conundrum. Complications associated with pericardiocentesis include ventricular premature contractions, laceration of the coronary artery, and sudden death. After removal of pericardial fluid, the patient should be monitored for arrhythmias and acute recurrence of hemorrhagic tamponade (abrupt onset of tachycardia, poor pulse quality, pulsus paradoxus, tachypnea).

Collection of Edema Fluid • A 22- to 25-gauge needle is gently introduced into the affected tissue. Clear watery fluid often drains spontaneously by gravity but can be assisted by gentle massage or aspiration. If lymphatic

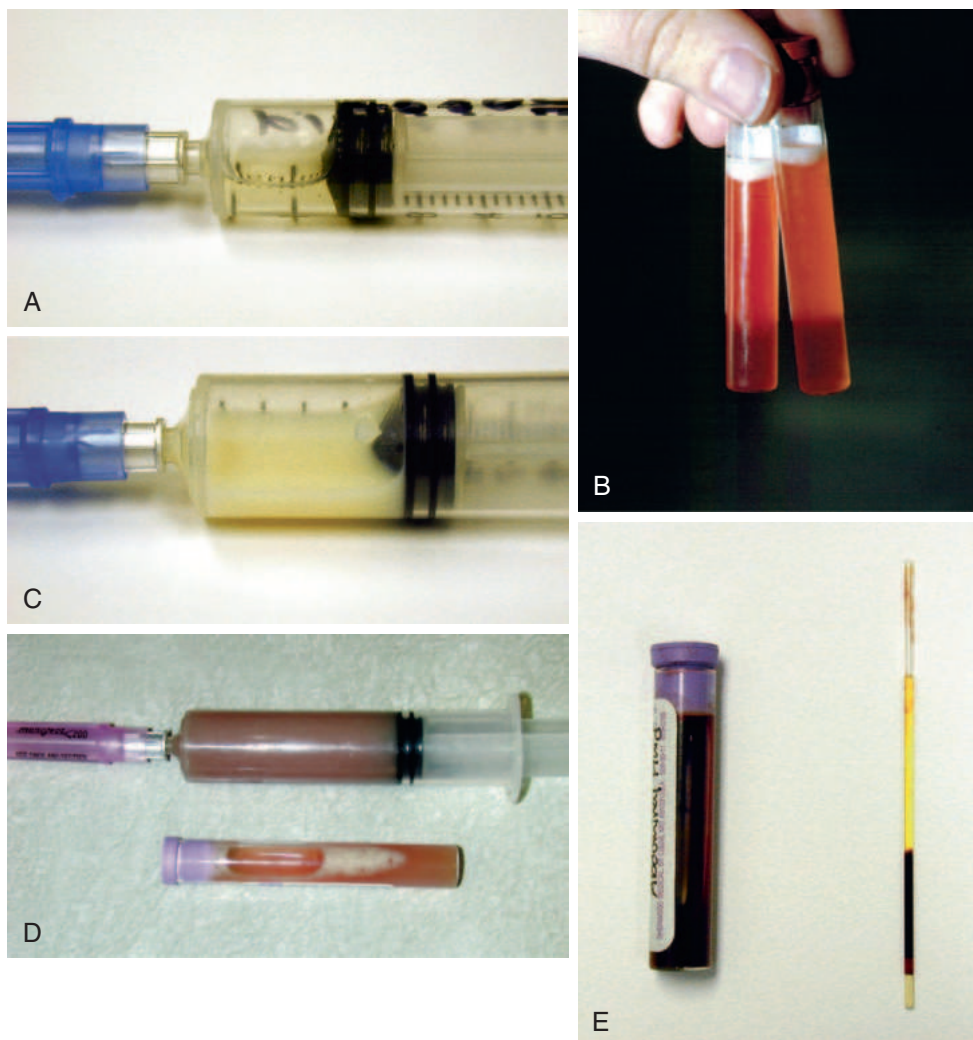


FIGURE 10-1 Examples of effusions with different physical characteristics. **A**, A pure low-protein transudate. It is colorless, clear, and transparent. **B**, A modified transudate that is red tinged and cloudy due to red blood cells (RBCs). It is nearly opaque. **C**, Chylous effusion that is white and opaque. **D**, Septic exudate that is cream colored and opaque. **E**, Hemoabdomen fluid that is dark red and opaque. Note that the supernatant in the microhematocrit tube is xanthochromic (yellow) due to RBC breakdown.

coding is notable, direct puncture for lymph collection is possible.

CHARACTERIZATION OF FLUID

Refer to [Table 10-1](#).

Fluid Analysis • Collected fluids should be analyzed immediately to permit characterization and to direct further diagnostics (e.g., bacterial culture). Three- to 5-ml aliquots of fluid should be stored in an ethylenediamine-tetraacetic acid (EDTA; purple top) tube and a sterile clot tube for cytologic and physicochemical assessments, respectively. A separate sample for culture is stored in a sterile clot tube, a Culturette containing transport medium, or broth culture medium. If only a few drops

of fluid are collected, cytology has first priority. Cultures can be taken from the needle hub with a microtip culturette, or the needle and syringe can be washed with culture broth. PCV, total protein, and appearance of microcentrifuged supernatant of bloody effusions should be compared with peripheral blood. Physicochemical and cytologic assessment of effusions usually permits classification into one of several categories (see [Table 10-1](#)). The scheme presented divides effusions into transudates and exudates and then further subdivides each major category.

Physical Assessment of an Effusion • Color and turbidity of the fluid should be recorded ([Figure 10-1](#)). Turbid fluids contain cells or lipids. Chylous effusions are usually white, pink, or opalescent with a turbid supernatant. A red-tinged or maroon fluid reflects red blood cells

TABLE 10-1. CHARACTERISTICS OF SELECTED TYPES OF EFFUSIONS

	Transudates			Exudates			
	PURE TRANSUDATE	MODIFIED TRANSUDATE	HEMORRHAGIC EFFUSION	NONSEPTIC EXUDATE	SEPTIC EXUDATE	BILIOUS EFFUSION	CHYLOUS EFFUSION
Color	Clear	Serous	Bloody	Serosanguineous	Purulent, creamy	Brown/green	Milky/white/pink
Turbidity	Watery	Serosanguineous			Serosanguineous	Dark yellow/green	Opalescent
Total solids (g/dl)	Clear	Clear to cloudy	Opaque	Cloudy	Cloudy/flocculent	Opaque	Opaque
Specific gravity	<2.5	2.5–5.0	>3.0	>3.0	>3.0	>3.0	>2.5
Nucleated cells/μl	<1.017	1.017–1.025	>1.025	>1.025	>1.025	>1.025	>1.018
Differential	<1000	500–10,000	>1000	>5000	>5000	>5000	Variable
	Mononuclear cells (mesothelial cells, lymphocytes, macrophages)	Mesothelial cells (macrophages, neutrophils, RBCs (few) Lymphocytes	Similar to blood Neutrophils (variable, nondegenerate) Lymphocytes (few) Macrophages (erythrophagocytosis)	Neutrophils (nondegenerate) Macrophages (phagocytized debris) Mesothelial cells (increased in chronic) ± Neoplastic cells	Neutrophils (degenerate, phagocytized bacteria) Mesothelial cells (variable) RBCs (variable)	Neutrophils (predominate in acute) Macrophages (phagocytized and free bilirubin crystals: brown-granular material) Lymphocytes (few)	Lymphocytes (predominate early) Neutrophils (increase in chronic) Mesothelial cells (variable)
Bacteria	No	No	No	No	May see bacteria	±	Rare
Lipid	No	No	No	No	No	No	High triglycerides (fluid > sera) Cholesterol (fluid < sera) Stain positive with Sudan III or oil red O

RBCs, Red blood cells.

(RBCs) or free hemoglobin. Blood-tinged fluids must be centrifuged to determine their PCV relative to systemic PCV and to permit supernatant evaluation. RBCs often accumulate in effusions secondary to inflammation or vascular congestion, where they cause a PCV less than or equal to 8%. If the PCV more closely resembles systemic blood and the supernatant is clear, acute hemorrhage or iatrogenic sample contamination is likely. Fluid PCV may be artifactually lowered by hemolysis caused by very high or low fluid tonicity, by freezing and thawing during sample storage, or consequent to high lipid concentrations or trauma (i.e., forced sample injection into a Vacutainer). Hemolysis should be suspected if a supernatant is maroon colored. Erythrophagocytosis (i.e., RBCs engulfed by macrophages) and macrophages containing hemosiderin (i.e., siderocytes) reflect blood contamination of at least 24 hours (Figure 10-2). However,

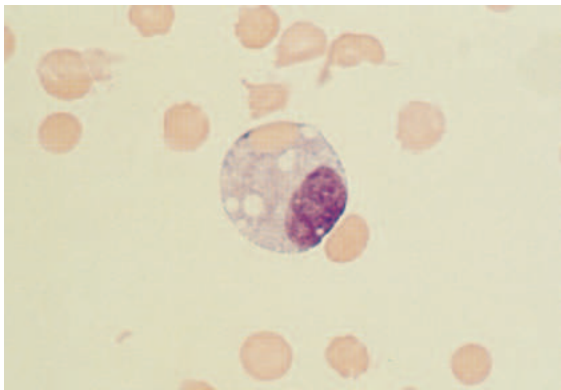


FIGURE 10-2 Erythrophagocytosis, such as in this macrophage, or hemosiderin in phagocytes indicates the hemorrhage in a fluid was, at least in part, from a preexisting disease (i.e., pathologic hemorrhage) rather than an artifact of collecting the sample.

erythrophagocytosis can also develop in fluids during storage (longer than a few hours). Chronically blood-contaminated samples lack platelets but have a xanthochromic (yellow-tinged) supernatant after centrifugation. Xanthochromia reflects presence of hemo-pigments (bilirubin pigments); jaundiced animals are expected to have yellow effusions. Bile peritonitis is usually associated with a brown-green or dark yellow-green effusion with large-volume bile spillage, containing both free and engulfed bilirubin crystals (Figure 10-3). Loculated bile peritonitis in the anterior abdomen (fluid entrapped in the omentum) may be associated with typical effusion near the site of bile leakage and a modified transudate or exudative effusion within the remainder of the abdomen. Septic effusions may emit a foul smell caused by anaerobic bacterial infection from bowel rupture.

Cell Counts and Cytology • Total and differential nucleated cell counts are performed using anticoagulated, noncentrifuged fluid. Total cell counts may be completed using a hemocytometer and manual count or a flow cytometry hematology analyzer. Very-small-volume samples may yield falsely low cell counts as a result of anticoagulant dilution. Poor sample mixing, sample contamination, prolonged storage, and medical therapy may each influence cell counts. Differential counts are best performed from concentrated cellular components. This can be simply done by sample centrifugation, smear preparation, and Diff-Quik staining. Smears of unconcentrated fluid allow estimation of cell numbers when cell counts exceed 1000/ μ l. At least six slides of collected fluid should immediately be made, rapidly air-dried to preserve cell morphology, and stained using a modified Wright stain such as Diff-Quik. If bacteria are visible, Gram stain is applied. If the fluid appears relatively acellular, a portion should be centrifuged and smears made of the sediment as soon as possible. Cytospin centrifugation provides the best cellular morphology. The clinician

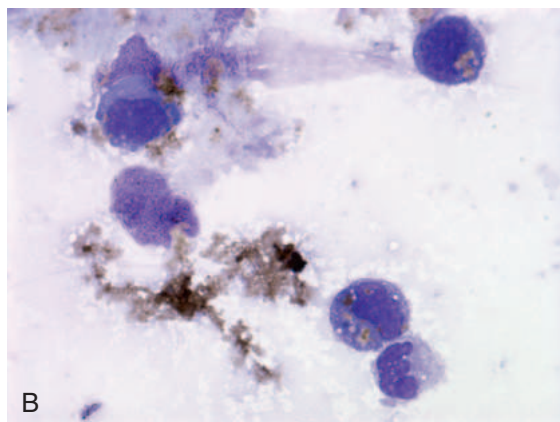


FIGURE 10-3 **A**, Bilious effusion that is golden brown and semi-transparent. **B**, Cytology of bilious effusion showing inflammatory cells that have engulfed bile (brown material). (Courtesy of Dr. Mark Johnson.)

should remain alert for microorganism-contaminated stains, post-sampling degeneration of neutrophils associated with prolonged storage in EDTA or saline, or cell changes induced by exposure to urine or bile. Estimation of total nucleated cell count in a noncentrifuged sample can be done using the following formula and the stipulation that the objective used visualizes 1 to 10 nucleated cells per field of view: (average number of nucleated cells per microscopic field of view [count 10 areas]) $\times$ (objective power)^{2,1} For example, using a 40 $\times$ objective, if 8 cells are counted per field of view: 8 cells $\times$ 40² = 12,800 cells/ml. The total nucleated cell count in fluid from body cavities of healthy dogs and cats is less than 3000 cells/ml (most are <1000 cells/ml).

Determining Fluid Protein Concentration • Protein concentration should be determined on supernatant of centrifuged fluid. Protein concentration can be estimated using a handheld refractometer or biochemically. While a refractometer may underestimate protein in fluid when protein is less than 2.0 g/dl, one study developed conversion values for estimating fluid protein concentration as low as 1.0 g/dl using a handheld refractometer (Table 10-2).²⁵ In normal animals, protein content of body cavity fluid is less than 2.5 g/dl.

DISTINGUISHING DIFFERENT TYPES OF EFFUSIONS

Refer to Table 10-1.

Transudates

These effusions have a low protein concentration and cell count, and are typically clear and colorless (Figures 10-4 and 10-5). Transudates reflect altered fluid dynamics associated with reduced interstitial fluid resorption (into capillaries), increased venous hydrostatic pressure with concurrent hypoalbuminemia, or severe hypoalbuminemia alone.

Pure Transudates

These are poorly cellular (i.e., <1000 cells/ μ l), have TS concentrations less than 2.5 g/dl, and have a specific gravity (SG) less than 1.017. Classic examples include

abdominal effusions associated with hypoalbuminemia plus portal hypertension resulting from hepatic insufficiency, and with severe hypoalbuminemia associated with sodium and water retention in protein-losing nephropathy (PLN) and protein-losing enteropathy (PLE), as well as with iatrogenic fluid overload or uroperitoneum from a ruptured urinary bladder or ureter.

Modified Transudates

These are associated with a higher TS concentration than pure transudates (generally 2.5 g/dl), a SG greater than 1.017, and moderate cellularity. Mesothelial cells are usually plentiful, and modified transudates reflect transudative vascular leakage from normal or noninflamed vasculature (increased capillary hydrostatic pressure or lymphatic obstruction). Modified transudates may be associated with neoplasia and many other disorders leading to transudative effusions, as well as uroperitoneum from a ruptured urinary bladder or ureter.

Hemorrhagic Effusions

These appear bloody, have a measurable hematocrit representing 10% to 25% of the systemic blood PCV, and have a TS concentration greater than 3.0 g/dl (Figure 10-6). If chronic, the supernatant evidences hemolysis or xanthochromia, and cytologic inspection reveals erythrophagocytosis, siderocytes, hematin (a yellow refractile crystalline or amorphous pigment, free of iron, formed from hematin), and lack of platelets. These effusions do not clot. Platelets appear only when bleeding has occurred 1 hour or less before sampling. Peracute or iatrogenic hemorrhage has no or only minor erythrophagocytosis, an absence of siderocytes, a clear supernatant, and platelets, and it may clot. If an acute hemorrhagic effusion is allowed to sit before slide preparation, erythrophagocytosis may occur *in vitro*, confusing the diagnosis.

Exudates

Exudates are characterized by high TS concentration (i.e., >3.0 g/dl), high SG (i.e., >1.025), and increased cellularity dominated by neutrophils and macrophages (i.e., >5000 cells/ μ l). These effusions are characterized as either *septic* or *nonseptic* and may be associated with

TABLE 10-2. CONVERSION FOR ESTIMATING FLUID TOTAL PROTEIN USING A HANDHELD REFRACTOMETER ACCORDING TO GEORGE AND O'NEILL (2001)

REFRACTIVE INDEX	SPECIFIC GRAVITY	BODY FLUID PROTEIN (g/dl)	REFRACTIVE INDEX	SPECIFIC GRAVITY	BODY FLUID PROTEIN (g/dl)
<1.3376	<1.013	<1.0	1.3389	1.017	1.8
1.3376	1.013	1.0	1.3391	1.017	1.9
1.3378	1.014	1.1	1.3393	1.018	2.0
1.3380	1.014	1.3	1.3395	1.018	2.1
1.3382	1.015	1.4	1.3397	1.019	2.2
1.3384	1.015	1.5	1.3399	1.019	2.3
1.3385	1.016	1.6	1.3401	1.020	2.4
1.3387	1.016	1.7	1.3402	1.020	2.5

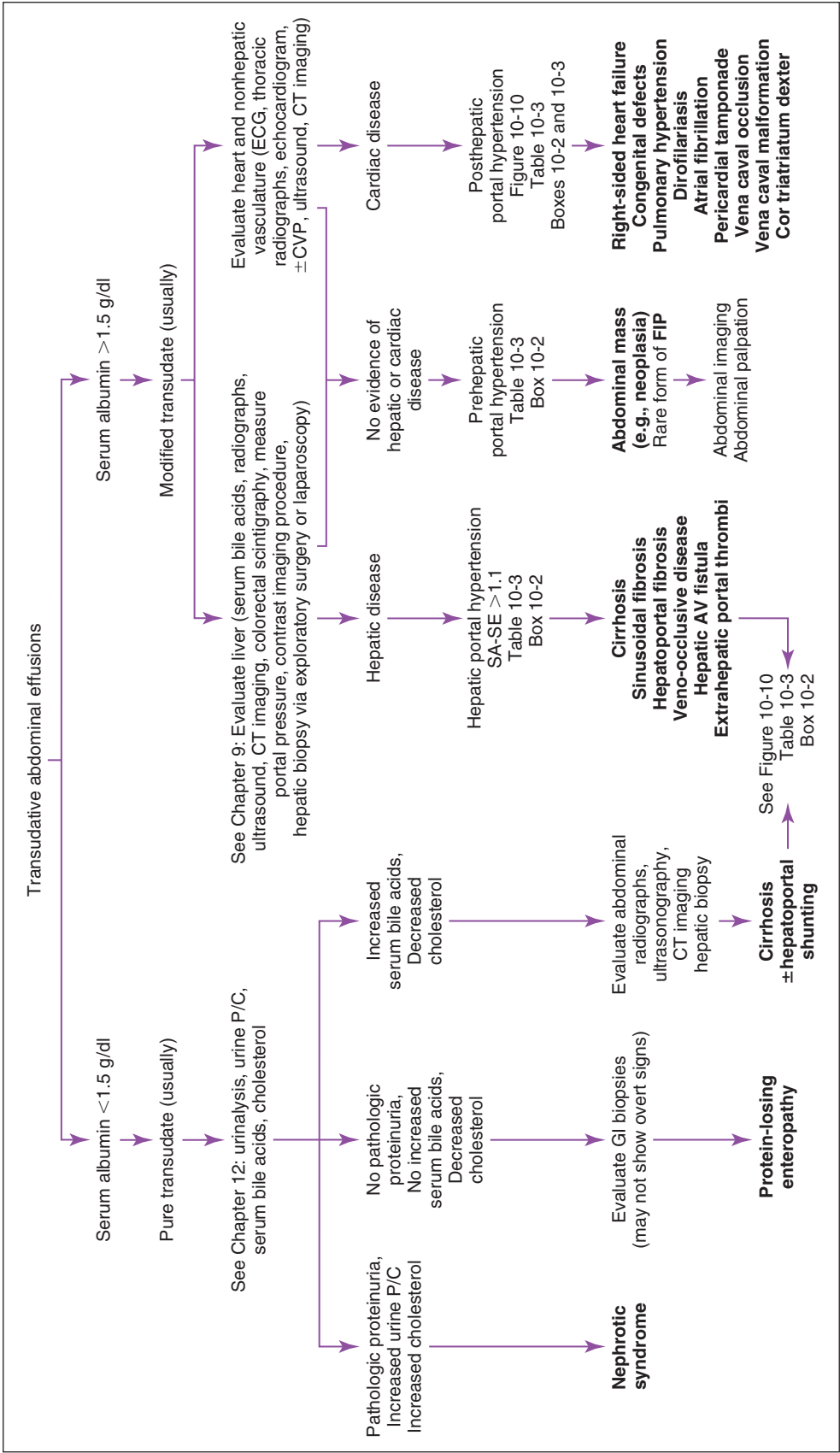


FIGURE 10-5 Diagnostic considerations in animals with abdominal transudates. AV, Arteriovenous; CT, computed tomography; CVP, central venous pressure; EA, effusion albumin; ECG, electrocardiogram; FIP, feline infectious peritonitis; GI, gastrointestinal; P/C, protein:creatinine ratio; SA, serum albumin.

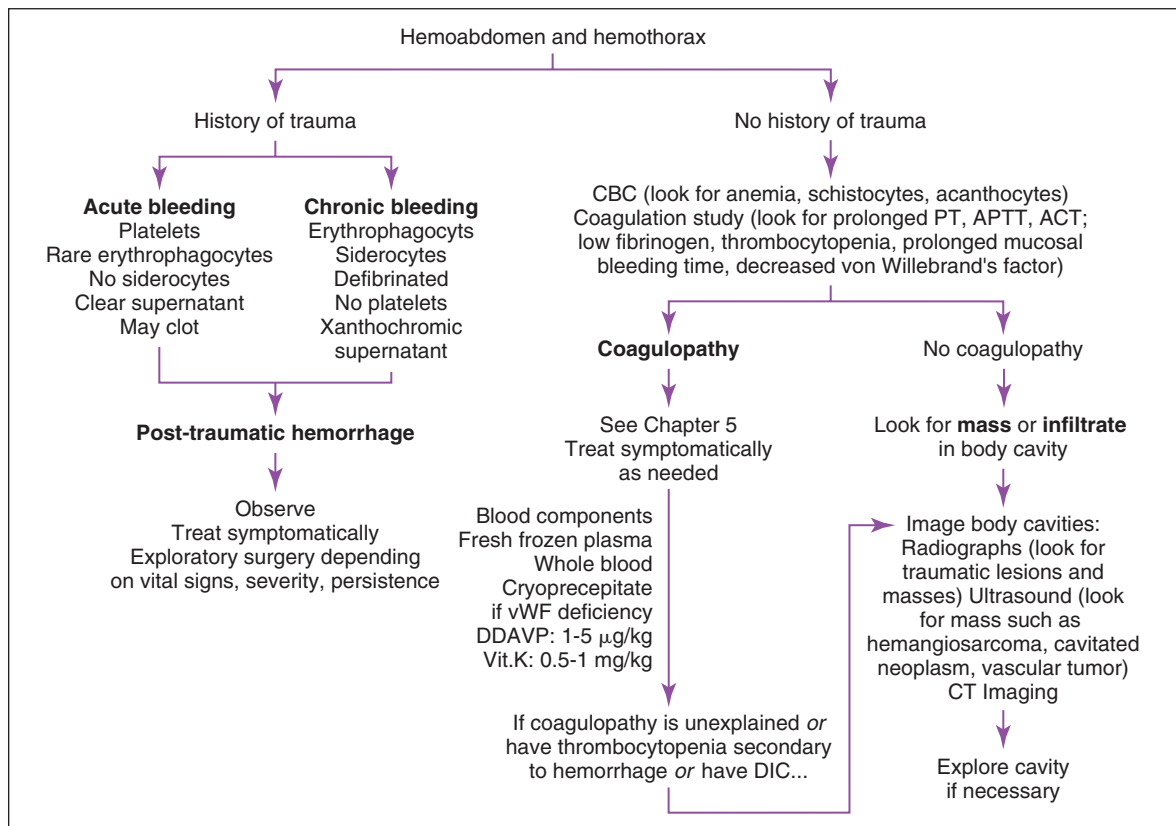


FIGURE 10-6 Diagnostic considerations in animals with hemoabdomen or hemothorax. ACT, Activated clotting time; APTT, activated partial thromboplastin time; CBC, complete blood count; CT, computed tomography; DDAVP, deamino D-arginine vasopressin; DIC, disseminated intravascular coagulation; PT, prothrombin time; Vit. K, vitamin K; vWF, von Willebrand factor.

inflammatory, necrotizing, infectious, or malignant disorders. Exudative effusions should be cultured aerobically and anaerobically for bacteria $\pm$ fungi. Immediate cytologic inspection of effusions is important to recognize septic exudates, which prioritizes bacterial or fungal culture submission. Evaluation of C-reactive protein (CRP) was recently shown to assist in discriminating exudative from transudative effusions; a cutoff value of 4 $\mu\text{g/ml}$ had a sensitivity of 100% and a specificity of 94%.⁴¹

Bilious Effusions

Bilious effusions contain intracellular and extracellular bilirubin crystals (yellow, golden, or brownish debris that may appear refractile or crystalline; see Table 10-1). Large numbers of neutrophils are typical, and these may be highly segmented. Reactive mesothelial cells are common. If septic, bacteria may be visible within phagocytic cells or free in the effusion fluid. Comparing bilirubin concentration in the effusion to that in peripheral blood discloses a 5- to 10-fold higher concentration in the effusion. All effusions in jaundiced animals are yellow colored owing to the solubility and dispersal of bilirubin pigments.

Chylous Effusions

Chylous effusions usually have a TS concentration greater than 2.5 g/dl; a SG greater than 1.018; a predominant population of mononuclear cells (lymphocytes) or high numbers of neutrophils, or both; and a high triglyceride concentration relative to peripheral blood (see Figure 10-4 and Table 10-1). The fluid:serum triglyceride ratio is greater than 2 or 3:1 and commonly exceeds 10:1. Effusion cholesterol concentration is less than in peripheral blood. When centrifuged, chylous effusions have lactescent or opalescent supernatants with a buoyant triglyceride-rich chylomicron layer accumulating at the fluid surface when the sample is refrigerated. A qualitative test for high triglyceride content involves incubation of the suspect effusion pretreated with 1 to 2 drops of 1 N sodium hydroxide and an equal volume of ether. Ether-soluble triglycerides rise to the top of the tube and are discriminated as a white band. Alternatively, a wet mount of fluid may be stained with oil red O or Sudan black and subsequently evaluated for fat droplets (i.e., chylomicrons) (Figure 10-7). Chylous effusions reflect disruption of thoracic duct or smaller lymphatics, obstruction from neoplastic infiltrates within lymphatics or draining lymph nodes (e.g., lymphoma, thymoma), mediastinal

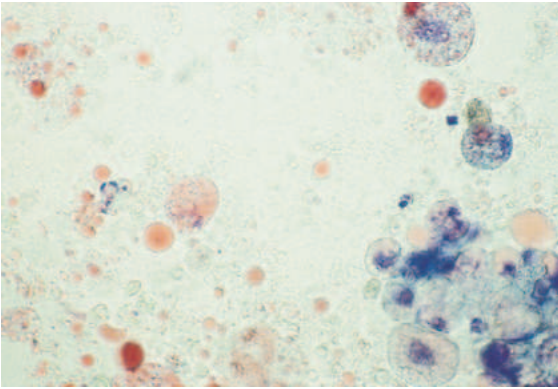


FIGURE 10-7 A simple, cheap test for chylomicrons in thoracic fluid is to stain the smear with fat stain (e.g., Sudan stain or oil red O) to demonstrate neutral fat droplets in the phagocytes and free in the fluid, plus a drop of new methylene blue to stain the nuclei.

inflammation or neoplasia, lymphatic occlusion by diaphragmatic or peritoneopericardial hernia, lung lobe torsion, congenital malformations (e.g., lymphangiectasia), cardiac disorders (including heartworm disease), or idiopathic disease (likely congenital malformations). Diagnosis in patients lacking a history of trauma may require a lymphangiogram (using radiography or computed tomography [CT]) and eventual surgical exploration of the appropriate body cavity after priming the lymphatics with a small high-fat meal (cream ingestion) that elucidates the location of lymphatics.

Pseudochylous Effusions

Pseudochylous effusions as described historically in veterinary literature are either extremely rare or nonexistent. Such effusions grossly resemble chylous effusions but have high cholesterol and low triglyceride concentrations relative to peripheral blood. Effusions associated with dense populations of neoplastic cells may appear “chylous” on gross inspection but fail to demonstrate other diagnostic features of chyle.

Malignant Effusions

Malignant effusions are usually characterized as modified transudates or exudates, are often blood tinged and xanthochromic, and are definitively diagnosed by finding neoplastic cells. However, it is important to recognize that effusions secondary to tumors may or may not contain malignant cells. *Caution:* Reactive mesothelial cells may be misinterpreted as malignant (e.g., binucleated cells, signet ring-shaped cells similar to carcinoma cells, high nuclear:cytoplasmic ratio, large and variably sized nucleus and nucleoli) (Figure 10-8). Immunocytochemical staining assists in differentiation of cell origin in cases in which cytologic characterization of a malignant cell population remains uncertain. Differentiating mesothelioma cells from reactive mesothelial cells is problematic, requiring tissue biopsy for definitive diagnosis. Hemangiosarcomas commonly cause malignant

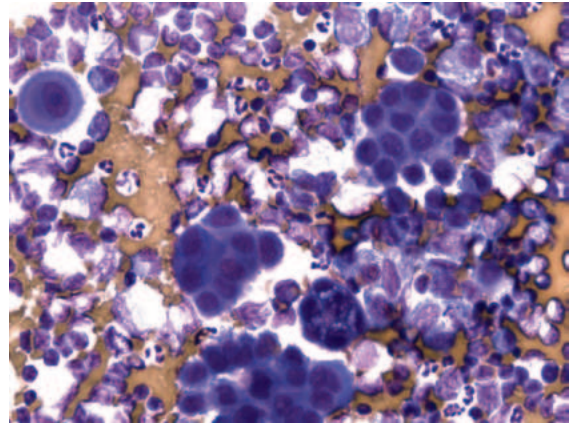


FIGURE 10-8 Cytology of abdominal effusion showing reactive mesothelial cells. These cells have peripheral cytoplasmic blebs resembling a “brush border” and very prominent nucleoli and are found in rafts, closely mimicking carcinoma cells.

effusion in dogs with associated effusions usually containing large numbers of foamy macrophages, reactive and quiescent mesothelial cells, erythrophagocytes, xanthochromic supernatant, and an absence of platelets unless associated with active or iatrogenic hemorrhage. Being vascular tumors, hemangiosarcomas are usually accompanied by circulating acanthocytes and schistocytes (see Chapters 2 and 3). Hemangiosarcomas often produce hemorrhagic effusions lacking cytologic evidence of neoplasia, as these cells do not exfoliate easily. When observed, hemangiosarcoma cells are large spindle-shaped to polyhedral cells, with a round to oval nucleus having one or more prominent nucleoli, a dark-blue cytoplasm (modified Wright-Giemsa staining), and many small, discrete nonstaining vacuoles (Figure 10-9). Carcinomatosis (i.e., miliary tumors implanted on peritoneal or pleural surfaces) frequently cause body cavity effusion. Radiography discloses ill-defined serosal margins with fluid confirmed by ultrasonography that

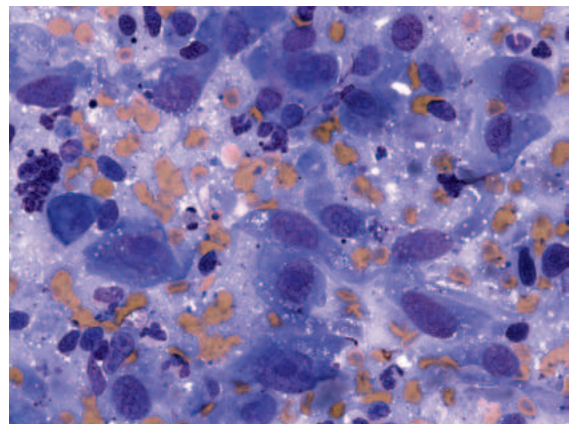


FIGURE 10-9 Cytology of a hemangiosarcoma. Many of the cells are spindloid and contain numerous small punctate cytoplasmic vacuoles. (Courtesy of Dr. Mark Johnson.)

can assist in fluid sample collection. These effusions have a high SG and a large amount of protein and may be hemorrhagic. Cytology may disclose neoplastic cells, but in some cases reactive mesothelial cells and carcinoma cells may be indistinguishable, requiring immunohistochemical differentiation.

Eosinophilic Effusions

Eosinophilic effusions contain greater than 10% eosinophils, with approximately 50% classified as modified transudates and the remainder as nonseptic exudates. Eosinophilic effusions cannot be predicted from circulating eosinophil counts. Canine eosinophilic pleural effusions may be associated with heartworm disease, disseminated eosinophilic granulomatosis, systemic mastocytosis, interstitial pneumonia, lymphoma, hemangiosarcoma, and carcinoma. In cats, eosinophilic effusions may be associated with lymphosarcoma, systemic mastocytosis, and hypereosinophilic syndrome. Pulmonary infiltrates are commonly detected by radiography. In some animals with pulmonary disease, pneumothorax precedes development of the eosinophilic pleural effusion. Animals with multifocal eosinophilic hepatic granulomas may develop a transudative or eosinophilic abdominal effusion.

SPECIFIC BODY CAVITY EFFUSIONS: DIAGNOSTIC CONSIDERATIONS

Abdominal Effusions

Diagnostic considerations in animals with suspected abdominal effusion encompass a wide spectrum of disorders (see Figure 10-4). Pure transudates are typically associated with severe hypoalbuminemia (see Chapter 12; see also Figure 10-5) and are usually caused by PLN, PLE, hepatic failure, protein loss from exudative cutaneous lesions, repeated body cavity lavage, or repeated large-volume or therapeutic abdominocentesis of a large-volume effusion. Anorexia and emaciation alone do not produce hypoalbuminemia severe enough to elicit edema or effusion. Important rule outs include causes of pathologic proteinuria (see Chapter 7) and hepatic insufficiency (see the discussion of bile acids in Chapter 9). Because PLE may occur without signs of enteric disease, enteric biopsy may be needed for diagnosis. Adjunctive scrutiny of the serum cholesterol concentration (see Chapter 8) is helpful in differentiating the cause of a pure transudate associated with hypoalbuminemia: PLE and hepatic failure usually cause hypocholesterolemia, whereas PLN usually causes hypercholesterolemia. The contribution of portal hypertension as an important pathomechanism of abdominal effusion can be discerned by calculating the serum albumin–effusion albumin (SA-EA) gradient, defined by the serum albumin concentration minus the ascitic fluid albumin concentration. Finding an SA-EA value greater than 1.1 indicates that portal hypertension has played an etiopathologic role in that patient's effusion. The gradient correlates directly with only a single physiologic variable (portal pressure) as compared to the ascites fluid total protein

concentration which is influenced by serum protein concentration as well as portal pressure. This ratio is not affected by diuresis, paracentesis, or type of hepatic disease causing portal hypertension, and has a higher utility than traditional effusion classification schemes for differential diagnosis of the cause of effusion in human patients.⁴² The utility of the SA-EA gradient has also been examined in dogs.³⁸ Noteworthy is that detection of portal hypertension with the SA-EA gradient is not synonymous with a diagnosis of hepatic failure, as there are numerous disorders (Figure 10-10; see also Figure 10-4) that can lead to prehepatic, hepatic (presinusoidal, sinusoidal, post-sinusoidal), or post-hepatic portal hypertension. Considerable overlap of SA-EA gradients has been shown in humans and animals for different disorders. In the absence of liver-related causes, malignancy is the second most important cause of an increased SA-EA gradient and portal hypertension. Differential diagnosis of causes of abdominal effusion is best done considering collective physical findings and diagnostic features (Table 10-3).

Abdominal modified transudates are often associated with increased venous (capillary) hydrostatic pressure (see Figure 10-5) and an SA-EA greater than 1.1. Concurrent hepatomegaly suggests impaired blood flow at the level of the hepatic venules, vena cava cranial to the diaphragm, pericardium, right atrium, or pulmonary arterial bed (Box 10-2; see Table 10-3, and Figure 10-10). The clinician should look for jugular pulse, pulsus paradoxus, hepatojugular reflex, poor femoral pulse quality, muffled cardiac sounds, exercise intolerance, and physiologically inappropriate tachycardia, which might indicate pericardial tamponade or pericardial restriction. The hepatojugular reflex is elicited by applying gentle abdominal compression to the liver or cranial abdomen for 10 to 15 seconds (increases venous return to the heart) and observing jugular vein distention or pulsation (indicating reduced right heart function or filling). Hepatomegaly caused by venous congestion may be difficult to palpate because of abdominal distention due to ascites or secondary to patient conformation (deep-chested dog).

Thoracic radiographs evaluate shape and size of cardiac and pericardial silhouettes; the tortuosity and filling of the pulmonary arterial bed; and the shape, distention, and position of the vena cava, an important capacitance vessel. If cardiomegaly is present, echocardiographic evaluations differentiate cardiac from pericardial disease. A vascular interrogation using abdominal ultrasonography (color flow Doppler) may reveal distended hepatic veins and an exaggerated flow pattern because of cardiac outflow obstruction, abdominal masses (i.e., neoplasia, granuloma), or obstructed portal flow (e.g., thrombi causing a luminal filling defect). Central venous pressure (CVP) values greater than 8 cm H₂O are suggestive, and values greater than or equal to 14 cm H₂O are diagnostic of right-sided cardiac dysfunction, filling, or impaired flow of blood into the lungs (e.g., pulmonary hypertension, thromboembolism). CVP may be normal in dogs with cor triatriatum dexter (abnormal congenital occlusive webbing within the right atrium) which causes abdominal effusion secondary to passive congestion. Assessment of CVP is not commonly done because it is subject to many mechanical variables that invalidate its

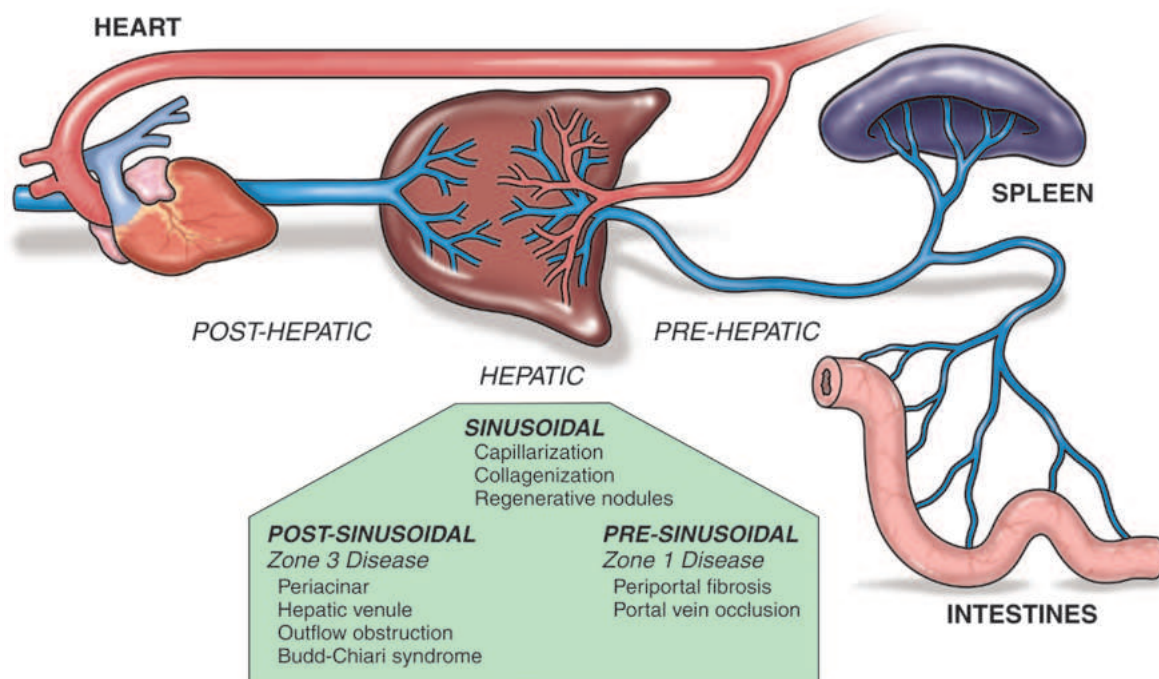


FIGURE 10-10 Diagram of blood flow through the liver identifying the sites of pre-hepatic, post-hepatic, and hepatic causes of ascites, as well as pre-sinusoidal, sinusoidal, and post-sinusoidal causes.

interpretation (e.g., catheter end position variability; kinking or folding of the catheter and catheter occlusion) and because advanced imaging modalities allow better assessment of potential disease mechanisms. Furthermore, it may lead to iatrogenic hemorrhage in patients with coagulopathies (e.g., hepatic insufficiency, rodenticide toxicity, vasculitis, thrombocytopenia).

Modified transudates associated with chronic hepatic disease can develop before severe hypoalbuminemia concordant with onset of presinusoidal, sinusoidal, or post-sinusoidal portal hypertension (see [Box 10-2](#)). Pooling of albumin in the abdominal effusion is one factor contributing to onset of systemic hypoalbuminemia in these patients. Abdominal ultrasonography usually discloses one or more of the following features: microhepatia, irregular lobe margins, altered parenchymal echogenicity, distended extrahepatic portal vasculature and/or retrograde portal blood flow, splenic congestion, or tortuous portosystemic shunts (caudal to the kidneys, adjacent to the splenic vasculature) (see [Table 10-3](#)). Modified transudates may also reflect fibrosis in the porta hepatis, neoplasia occluding (strangulating) portal vasculature, or portal venous thromboembolism (detected by color flow Doppler examination). An SA-EA gradient greater than 1.1 develops in all of these disorders (see [Figure 10-5](#)).

Splenic infarction, thromboembolism, or torsion may produce an abdominal effusion characterized as a modified transudate or exudate. Part or all of the spleen may appear large on ultrasonography, and color flow Doppler interrogation may disclose impaired perfusion (e.g., vascular thrombi, impaired venous or arterial flow). Splenic and other abdominal masses (i.e., neoplasia,

granulomas) may also produce modified transudates; these are usually detected by ultrasonography.

Effusions can be suspected on the basis of radiographic images demonstrating lack of distinct visceral margins. However, ultrasonography is more accurate for fluid detection. Radiographic images made after abdominal fluid evacuation (drainage) allow appraisal of hepatic size, detection of a mass effect, or altered visceral positions. Visceral margins will remain ill defined because of retention of small fluid volumes. After large-volume paracentesis, most effusions reaccumulate within hours to days, so radiographic studies must be done immediately.

Finding an exudative effusion mandates a search for infection, necrosis, or malignancy. Identification of phagocytized organisms (neutrophils or macrophages) is definitive for sepsis but may require careful, tedious inspection of several cytology slides. Finding plant fibers, enteric debris, or a mixed “fecal flora” in an abdominal effusion suggest loss of enteric integrity or gut rupture. Degenerate WBCs ([Figure 10-11](#); see Chapter 16) suggest infection, although some organisms do not alter neutrophil morphology (e.g., *Actinomyces*). Degenerate changes in WBCs also may result from specimen handling (i.e., storage too long before cytology smear preparation). Recent abdominal trauma (e.g., exploratory laparotomy) causes mild, transient, fluid accumulation characterized by a neutrophilia with degenerative changes.

Finding and identifying infecting organisms can be difficult, especially when bacterial numbers are low or bacteria are within granulomas or loculated within abscesses. Certain organisms are notoriously difficult to

TABLE 10-3. CHARACTERISTICS OF DIFFERENT TYPES OF PORTAL HYPERTENSION

DIAGNOSTIC FEATURE	Post-hepatic Portal Hypertension		Hepatic Portal Hypertension		Prehepatic Portal Hypertension
	CARDIAC/ PERICARDIAL (FILLING/PUMP FAILURE)	CVC OCCLUSION	NUMEROUS CAUSES	INTRAHEPATIC AV FISTULA	NUMEROUS CAUSES
Serum Albumin : Effusion Albumin	>1.1	>1.1	>1.1	>1.1	>1.1
CVP	↑	Normal	Normal	Normal	Normal
ECG	Abnormal	Normal	Normal	Normal	Normal
Radiography:					
Cardiac silhouette	↑ /normal	Normal	Normal	Normal	Normal
Caudal vena caval size	↑	↓, normal, mass lesion	Normal	Normal	Normal
Liver Size	↑	↑	Normal, variable, ↓	↑ individual lobe	Normal
Ultrasonography					
Liver pattern	Hypoechoic/ normal	Hypoechoic/ normal	Variable	Anechoic foci	Normal
Size: Doppler confirms flow					
Hepatic vein	Distended	Distended	Normal	Normal	Normal
Portal vein	Prominent	Prominent	Variable	Segmentally larger	Normal
CBC:					
PCV	↑ /normal	↑ /normal	Variable	↓ /normal	Variable
MCV	Normal	Normal	↓	↓	↓ with shunting/ normal
Poikilocytes	Rare	Rare	Common	Common	Variable
Schistocytes, acanthocytes	↑ if vascular lesions	↑ if vascular lesions	Rare	Rare	Rare
Chemistry Profile:					
Albumin	Normal	Normal	↓ /normal	↓ /normal	↓ /normal
Liver enzymes	↑ ALP, ↑ ALT, ↑ AST	↑ ALP, ↑ ALT, ↑ AST	Variable	Variable	Variable
Glucose	Normal	Normal	Normal/ ↓	Normal/ ↓	Normal
Serum Bile Acids:	Normal	Normal	↑ postprandial	↑ postprandial	↑ if shunting
Ascites:	Common	Common	Chronic disease	Common	Variable
Pure transudate	Uncommon	Uncommon	Common	Possible	Possible
Modified transudate	Common	Common	Rare	Possible	Possible

ALP, Alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; AV, arteriovenous; CBC, complete blood count; CVC, caudal vena cava; CVP, central venous pressure; ECG, electrocardiogram; MCV, mean corpuscular volume; PCV, packed cell volume.

find (e.g., *Nocardia* and *Actinomyces*). Therefore all exudative effusions should be cultured aerobically and anaerobically for bacteria (see Chapter 15). Samples should be immediately submitted in sterile clot tubes or transferred to appropriate transport medium. If samples in transport medium cannot be immediately submitted, they should be refrigerated to slow bacterial growth to avoid medium substrate use and microbe death. Finding irrefutable evidence of an infectious organism *may* indicate a need for emergency exploratory surgery. However, animals with suppurative cholangitis or hepatitis may be placed at great risk by surgical exploration that will have no direct survival benefit (aside from tissue biopsy and culture). Animals with obstructive biliary disorders associated with

sepsis should be given intravenous antimicrobials before surgery and intraoperatively; a combination of metronidazole, enrofloxacin, and ticarcillin is recommended. Positive culture results should be reconciled with the antimicrobials in use and the regimen tailored appropriately. Animals with abdominal contamination secondary to surgery or iatrogenic infections (e.g., contamination during paracentesis) should have antibiotic therapy tailored to results of culture and sensitivity; these cases have higher risk for resistant nosocomial pathogens. Infections with fungal agents may also underlie exudative effusions. Fungal cultures should be considered in animals with body cavity effusions characterized as granulomatous and in animals with unexplained effusions within geographic

BOX 10-2. DIFFERENTIAL DIAGNOSTIC CONSIDERATIONS FOR ABDOMINAL EFFUSIONS ASSOCIATED WITH PORTAL HYPERTENSION

POST-SINUSOIDAL/ POST-HEPATIC PORTAL HYPERTENSION Right-Sided Cardiac Disturbance: Cardiomyopathy Tricuspid insufficiency Dirofilariasis Pulmonary thromboembolism Intracardiac neoplasia Atrial, valvular, mural, infiltrative Cor triatriatum dexter Pericardial Disease: Pericardial tamponade: Atrial hemangiosarcoma Coagulopathy Trauma Benign pericardial effusion Infectious Restrictive pericarditis Constrictive pericarditis Obstructed Caudal Vena Cava/ Hepatic Vein: Congenital “kinked” vena cava Post-traumatic vena caval stenosis Vena caval syndrome (dirofilariasis) Vena caval/hepatic vein thrombosis Diaphragmatic hernia: vascular entrapment	HEPATIC/SINUSOIDAL IMPAIRED SINUSOIDAL/PORTAL FLOW PORTAL HYPERTENSION Cirrhosis: Regenerative nodules Collagenization of sinusoids Parenchymal collapse Chronic diffuse hepatitis Chronic cholangiohepatitis Postnecrotic fibrosis Breed-specific hepatopathies Drug-related hepatopathies Biliary Cirrhosis: Severe peribiliary fibrosis Bridging fibrosis—acquired, congenital: Chronic cholangitis/cholangiohepatitis Chronic major bile duct obstruction Malformations: associated with increased extracellular matrix deposition Feline polycystic liver disease = ductal plate malformation Juvenile hepatic fibrosis = ductal plate malformation Miscellaneous Causes: Noncirrhotic portal hypertension Congenital portal atresia Portal/sinusoidal disseminated neoplasia Portal/sinusoidal thromboembolism Diaphragmatic hernia: liver entrapment Hepatic amyloidosis Portal Blood Flow (arterialization of portal vasculature): Hepatic artery/portal vein fistula: Congenital Traumatic Neoplastic Splanchnic Microanastomosis (cirrhosis): intrahepatic sinusoidal shunting	PRESINUSOIDAL/ PREHEPATIC PORTAL HYPERTENSION Prehepatic Portal Vein Occlusion: Portal vein thrombosis Portal vein stenosis: Trauma Congenital Congenital portal atresia Extraluminal portal vein occlusion: Neoplasia Lymph nodes Abscess Granuloma Peritonitis

regions where endemic fungi are recognized (e.g., Southwest United States for *Coccidioides*; regions where blastomycosis or histoplasmosis are common).

Exudates without cytologic evidence of sepsis necessitate review of history for trauma and possible urinary, biliary, or cyst rupture (Table 10-4; see also Figure 10-4). Neutrophilic abdominal effusion can persist for weeks after blunt abdominal trauma or surgery. Radiography of bony structures sometimes reveals evidence of injury. If trauma is considered unlikely, physical assessments should be made looking for other evidence of inflammation. In cats, feline infectious peritonitis (FIP) must be

considered. Although clinical presentations are quite variable, most cats with FIP are chronically ill with systemic signs, hyperglobulinemia (i.e., ≥ 5 g/dl), and a high-protein abdominal effusion (i.e., >3.5 g/dl). Evaluation of the effusion by protein electrophoresis may assist in achieving a diagnosis; one study demonstrated that finding greater than 32% gamma globulins in effusions with a total protein greater than 3.5 g/dl had a 100% positive predictive value for FIP in a suspect population of cats.⁴⁷ Estimation of acute phase proteins also may be helpful. Concentrations of $\alpha 1$ -acid glycoprotein (AGP) greater than 1.5 g/L in plasma, sera, or effusion from cats

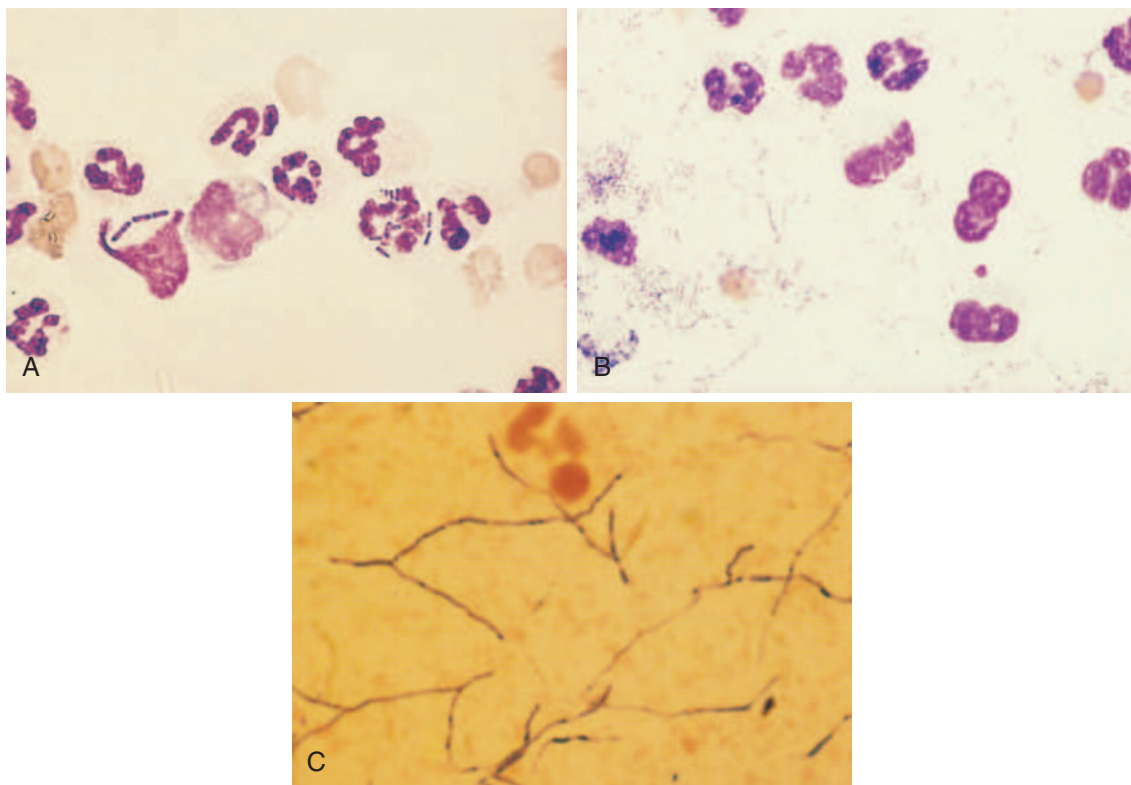


FIGURE 10-11 **A**, Two neutrophils in this canine abdominal fluid contained rod-shaped bacteria. Note that the neutrophils do not look degenerate even though the exudate was septic. One should not consider the lysed neutrophil with bacteria to be degenerate. **B**, The degenerate neutrophils in this thoracic fluid have karyolytic (swollen) nuclei as evidence of degeneration caused by the bacterial sepsis about them. The branching filamentous bacterium was *Actinomyces*. **C**, The gram-positive, branching, beaded organisms were *Actinomyces*. Gram-negative organisms are difficult to find on Gram-stained smears of exudate. Many gram-negative rods are illustrated here, but typically only one bacterium is found per several microscope fields in exudates and, if pale, a rare red bacteria would be easily hidden among red-staining leukocytes or debris.

with spontaneous or “field” FIP were shown in one study to help distinguish FIP-affected cats from cats with similar clinical signs.¹⁵ Unfortunately, not all cats with FIP have raised AGP values. The total nucleated cell count in body cavity effusions from cats with FIP is highly variable, ranging from 1000 to 30,000 cells/ μ L. Cytology reveals nondegenerative neutrophils as the predominant cell type and numerous macrophages and variable numbers of lymphocytes and plasma cells. Inconsistencies in signs and clinical pathologic findings in FIP-affected cats (see Chapter 15) makes conclusive diagnosis impossible in cats with atypical features (e.g., long-term survival, modified transudative effusions) without immunocytochemistry or immunohistochemistry using anti-*Coronavirus* antibody. Serologic polymerase chain reaction (PCR) detection of *Coronavirus* antigen or its antibody does not provide a definitive diagnosis. Other considerations regarding abdominal effusions are summarized in [Figure 10-4](#).

Neoplasia becomes an important differential for modified transudates or exudates after eliminating other causes. Neoplastic cells are sometimes identified cytologically; however, there often are too few or no exfoliated neoplastic cells in small-volume samples. Use of cytopsin

preparations or examination of centrifuged fluid sediment increases the likelihood of finding malignant cells. Sometimes neoplastic cells are only found using sediment derived from large volumes (e.g., >50-ml samples) of effusion. It is important to remember that “reactive” mesothelial cells resemble carcinoma cells and may be erroneously classified without immunocytochemical or immunohistochemical investigations. Ultrasonography can aid in aspiration of mass lesions for cytologic evaluation that may yield a more diagnostic population of cells for definitive identification.

Chylous abdominal effusions usually suggest intestinal lymphangiectasia; lymphoproliferative disease of the gut, mesenteric lymphatics, or lymph nodes; intra-abdominal neoplasia “strangling” the mesenteric root; or idiopathic disease (these may be malformations). Rarely, vitamin E-responsive steatitis or biliary cirrhosis has accompanied feline chyloabdomen (see [Figure 10-4](#)). While small lymphocytes are the initial cell type associated with chylous effusions, neutrophilic inflammation becomes established with chronicity. Repeated large-volume removal of chylous effusions depletes systemic protein concentrations (i.e., chyle contains 1 to 6 g protein/dl), further disrupting Starling’s forces that may

TABLE 10-4. CHARACTERISTICS, CAUSES, AND DIAGNOSIS OF CHEMICAL PERITONITIS

	BILE PERITONITIS	UROABDOMEN	PANCREATITIS	RUPTURED “CYSTS”
Appearance	Golden brown-green, serosanguineous, turbid	Light to dark yellow $\pm$ serosanguineous, clear (some acute), turbid (chronic)	White, yellow, serosanguineous, turbid	Clear to turbid, pale to yellow, colorless
Causes	Blunt abdominal trauma Necrotizing cholecystitis Cholelithiasis	Trauma: avulsed ureter or bladder, ruptured bladder Urolithiasis	Pancreatitis	Perirenal cysts Polycystic renal/hepatic disease Pancreatic cysts Paraprostatic/prostatic cysts
Clinical features	Vague abdominal pain Lethargy Pale or acholic feces Increased hepatic enzymes Jaundice (chronicity) Septic peritonitis Gallbladder: may be difficult to visualize on ultrasonography	Dehydration Azotemia Anuria/oliguria Abdominal distention Hyponatremia Hyperkalemia Hyperphosphatemia Metabolic acidosis	Anorexia Vomiting Abdominal pain Lethargy Fever Jaundice Increased: hepatic enzymes/TLI/PLI cholesterol/ bilirubin Cardiac arrhythmias Pleural effusion Acute renal failure	Vary with underlying tissue involved and severity of lesion
Definitive diagnosis	Free and phagocytized bilirubin crystals Fluid bilirubin > serum bilirubin. May require ultrasound-directed fluid aspiration, CT imaging, or (rarely) hepatobiliary scintigraphy	Intravenous urogram Retrograde ureterocystography, ultrasound or CT imaging Fluid creatinine > serum creatinine	Macrophages contain refractile lipid inclusions Fluid lipase/ amylase/TLI/PLI greater than serum lipase/ amylase/TLI/PLI	Ultrasonography Tissue biopsy Cyst aspiration + fluid analysis/cytology

CT, computed tomography; PLI, pancreatic lipase (species specific); TLI, trypsin-like immunoreactivity.

augment further fluid accumulation. Secondary infections are rare in animals with chylous effusions because chyle imparts a bacteriostatic influence. Animals with chyloabdomen should be evaluated for pleural effusion, lymphadenopathy, and metastatic neoplasia by thoracic radiography. Ultrasonography may reveal mesenteric root masses or mesenteric lymphadenopathy.

Hemoabdomen may be associated with trauma (e.g., ruptured spleen or hepatic parenchyma; avulsed renal pedicle or mesenteric vessels), vascular neoplasia (e.g., hemangiosarcoma, other vascular tumors, or tumors with necrotic centers such as hepatomas and hepatocellular carcinomas), increased hepatic fragility as occurs in feline hepatic amyloidosis, or coagulopathies (see Chapter 5; see also Figure 10-6). Physical examination usually reveals abrasions or pain in traumatized animals, with follow-up radiographs sometimes disclosing broken bones. Inspection for signs of bleeding or coagulopathy should include detection of petechiae (e.g., fundic examination, mucous membranes), rectal and fecal examination looking for melena or hematochezia, palpation for hemarthrosis (i.e., swollen, painful joints), and urinalysis looking for hematuria. Without history or physical findings suggesting trauma, a complete blood count (CBC), including a

platelet count and a coagulation profile, becomes essential. PCV reveals whether the erythron mass is reduced. However, in acute severe hemorrhage, change in PCV is contingent on fluid redistribution and whether a regenerative RBC response (increased reticulocytes, broad RBC distribution width [RDW]) has been realized (3 to 5 days after blood loss). Schistocytes and acanthocytes (see Chapters 2 and 3) suggest microangiopathic damage reflecting vascular neoplasia (e.g., hemangiosarcoma) or disseminated intravascular coagulation (DIC). Scanning a blood smear should detect thrombocytopenia severe enough to cause hemorrhage (i.e., fewer than 3 platelets/400 $\times$ field of view, see Chapter 5). Severe acute hemorrhage not caused by thrombocytopenia initially increases the platelet count. An ACT and buccal mucosal bleeding time (BMBT) (see Chapter 5) detect many hemostatic defects; BMBT is only pursued in animals with an adequate platelet count (>100,000/ μ l). Samples for effusion characterization should be obtained before initiating fluid or blood replacement therapy. Thoracic radiographs may reveal pleural fluid, lymphadenopathy (e.g., sternal lymph node), or frank metastasis. Ultrasonography may discover vascular tumors, usually associated with hepatic or splenic hematomas, and may disclose

the site of active bleeding. Animals with persistent abdominal hemorrhage may require blood component therapy (whole blood, fresh frozen plasma, or cryoglobulin if severe von Willebrand factor [vWF] deficiency is suspected) in addition to empirical vitamin K₁ treatment (0.5 to 1.5 mg/kg subcutaneously [SC] or intramuscularly [IM], 3 doses q8hr) and synthetic vasopressin (1 to 5 µg/kg SC or intravenously [IV] diluted), followed by exploratory laparotomy if hemostatic risks can be attenuated.

Bilious effusions caused by gallbladder or common bile duct rupture may derive from blunt abdominal trauma, necrotizing cholecystitis or choledochitis, cholelithiasis, or gallbladder mucocele (progresses to gallbladder ischemic necrosis). Biliary tree leakage may be immediate or delayed after blunt or surgical injury (see Table 10-4). Affected animals may be asymptomatic or symptomatic. Symptomatic patients demonstrate variable low-grade abdominal pain, lethargy, fever, and jaundice associated with a mild to modest abdominal effusion and may have concurrent bacterial contamination. Patients with aseptic bile peritonitis may be asymptomatic with the exception of jaundice and equivocal cranial abdominal discomfort. Clinical signs may correlate with the extent/severity of bile leakage (e.g., experimentally, the lethal dose of sterile bile injected intraperitoneally into dogs ranged from 20 to 30 ml/kg body weight). Pale or acholic feces indicate deviation of bile from the intestines (extrahepatic bile duct obstruction or severe small bile duct “ductopenia” as develops in cats with immune-mediated cholangiohepatitis). Serum alkaline phosphatase (SAP), gamma-glutamyl transpeptidase (GGT), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) activities and total bilirubin concentrations are invariably increased. Appearance and severity of jaundice depend on underlying cause and severity and chronicity of bile leakage. Bile induces a chemical peritonitis associated with cytokine release and alterations in fluid transport across peritoneal membranes. Cell membranes exposed to high concentrations of bile acids (functionally acting as detergents that disrupt cell membranes), bilirubin, and lysolecithin develop leaky gap junctions permitting translocation of enteric bacteria and endotoxin, leading to endotoxemia and/or septic peritonitis. Focal bile peritonitis may be restricted by omental adhesions to biliary structures. These present as pericholecystic effusions or effusions within the porta hepatis on ultrasonography. Cautious targeted aspiration using a spinal needle and ultrasonographic guidance may successfully collect diagnostic fluid in these cases. A ruptured gallbladder may be indicated by its sudden absence on ultrasound evaluation. In some cases, ultrasound imaging can identify the location of bile leakage (i.e., focal fluid accumulation, hyperechoic foci, adhesions, loss of normal gallbladder wall layering). Focal ileus of small intestine or colon may be identified adjacent to a site of biliary rupture, reflecting chemical peritonitis. Bile peritonitis is cytologically characterized by high numbers of neutrophils and macrophages and the presence of free and phagocytized bile (see Figure 10-3). The fluid specimen is often turbid with a golden-brown or golden-green color. A higher bilirubin concentration relative to peripheral blood is found. Fluid samples for accurate diagnosis of bile peritonitis should be collected from the immediate

area of leakage for best assessment owing to the propensity for omental encapsulation of the most diagnostic fluid (i.e., bile particulates, bacteria).

Uroabdomen occurs when urine leaks and pools within the peritoneal cavity. Affected animals may appear to void normally if the rent reflects a single ruptured ureter or when bladder avulsion surrounded by a fibrous tract provides a voiding conduit. Avulsion of a ureter at the renal pedicle may cause retroperitoneal effusion. Trauma is the major cause of urinary system leakage, but cystocentesis, traumatic diagnostic cystoscopy, or neoplasia may also be causal factors. The degree of azotemia varies depending on severity and chronicity of urinary leakage into the abdomen. If virtually all urine accumulates in the abdomen, rapid-onset azotemia and hyperkalemia are expected. Most patients develop a vaguely painful abdomen, lethargy, fever, and dehydration. Some animals develop pathologic arrhythmias associated with electrolyte aberrations (i.e., severe hyperkalemia and acidosis). Markedly increased blood urea nitrogen (BUN) and creatinine concentrations, hyperphosphatemia, hyponatremia, hyperkalemia, and metabolic acidosis are expected. The effusion is slightly turbid, blood tinged, and yellow. Fluid creatinine concentration is markedly higher relative to peripheral blood, whereas there may be no substantial difference between fluid and serum urea nitrogen concentrations. The small size of the urea molecule allows rapid systemic dispersal in body water, negating its diagnostic utility in uroabdomen. Ultrasonography or an IV urogram (followed by a retrograde urethral cystogram as necessary) usually locates the damaged area. Diagnosis may be more descriptive if contrast studies use CT. Urinary drainage and abdominal lavage rapidly correct electrolyte and acid-base derangements if surgical intervention is delayed.

Pancreatitis sometimes causes diffuse peritonitis and copious effusion. Clinical pathologic changes are discussed in Chapter 9; ultrasonography provides important diagnostic information, such as altered pancreatic echogenicity, marginal irregularity, altered duct morphology (distention), associated distal bile duct obstruction, focal pain on imaging probe pressure application, focal ileus (duodenal corrugation, amotility), and peripancreatic fat hyperechogenicity (saponification). Effusions are grossly turbid and sometimes have a lipid surface interface after refrigeration and centrifugation. Inflammation is characterized by large numbers of neutrophils and macrophages; the latter often contain many small to large, clear or refractile vacuoles (engulfed lipid). Pancreatic enzyme activity in effusion may be markedly higher than in the peripheral blood (i.e., lipase or amylase). This suggests enzyme leakage from pancreatic ducts.

Ruptured “cystic” lesions in the liver, kidneys, pancreas, or prostate occasionally cause transudative abdominal effusions (see Figure 10-4). Fluid within large cysts is sometimes misidentified as free abdominal effusion before leakage. Cystic fluid is evaluated for underlying malignancy or infection but often is characterized as a transudate. Polycystic hepatic or renal disease with well-developed cysts is more common in cats (i.e., Persians, Himalayans) than dogs. Cystadenomatous malformations in cats usually cannot be drained owing to their multicompartmented structure. These may cause

effusions when located adjacent to or within the porta hepatitis due to pressure imposed on vasculature. Perirenal pseudocysts are more common in cats, especially older males. Although rare, pancreatic cysts may be benign, may represent postpancreatitis abscessation or formation of a pseudocyst, or may be the result of malignancy (i.e., pancreatic adenocarcinoma). In intact male dogs, paraprostatic or prostatic cysts may be large and may become infected. If a perirectal cyst is identified, position of the urinary bladder should be confirmed (decompressed by voluntary voiding or catheterization). Thereafter cystic fluid should be aspirated and analyzed for creatinine. Ultrasound-guided cyst aspiration and sampling of suspected abdominal effusion are the least invasive and most cost-effective methods of determining whether resection or drainage of cysts is needed. Examination of cystic fluid relative to any abdominal effusion is important to identify infection or neoplasia or other biochemical components that might incite chemical peritonitis.

Pleural Effusions

The general approach to differential diagnosis of pleural effusion is similar to that described for abdominal effusions (Figure 10-12). However, the pleura is not as readily accessible by exploratory surgery and is less easily visualized by ultrasonography. Animals with pleural effusion often have rapid, shallow breathing with accentuated abdominal effort. Radiographs should include right and left lateral and ventrodorsal views. Radiographically, pleural fluid is characterized as either free (will move upon patient repositioning) or encapsulated (nonmovable with positional change).

Pure transudates are less common in the pleural space than in the abdomen (Figure 10-13). These effusions signal severe hypoalbuminemia and thoracic vascular hypertension. Pure transudates also occur in animals overhydrated with crystalloid fluids; this is most common in overweight animals where calculations are erroneously based on gross body weight. Overhydration causing pleural and pulmonary fluid retention is most symptomatic in patients with incipient cardiac disease (e.g., asymptomatic cardiomyopathy).

Modified transudates are the most common type of pleural effusion (see Figure 10-13). Obstructive effusions can be serous to serosanguineous, have a SG ranging from 1.015 to 1.040, and have a TS concentration greater than or equal to 2.5 g/dl. Cellularity is usually mixed with RBCs, lymphocytes, and fewer neutrophils, eosinophils, macrophages, and mesothelial cells; with chronicity these progressively appear more inflammatory. Physical examination may disclose features suggesting a primary underlying disease (e.g., gallop rhythm, cardiac murmur, loss of normal compression of the anterior chest in cats with an enlarged heart or mediastinal mass). A history of trauma plus a vague “emptiness” of the abdomen and auscultation of borborygmi within the thorax suggest diaphragmatic hernia. Dogs with right-sided cardiac failure usually develop ascites, whereas cats with cardiac failure often develop focal pulmonary infiltrates and/or pleural effusion. Benign or neoplastic pericardial effusion, constrictive or restrictive pericarditis, and right atrial

BOX 10-3. DIFFERENTIAL DIAGNOSIS OF PERICARDIAL EFFUSIONS

Idiopathic Pericarditis (~20% of cases)

Neoplasia (~70%–80% of cases)

Mass Lesion Location and Differential Diagnosis:

Right atrium (most common):

Hemangiosarcoma (~88%; ~25%–30% have splenic neoplasm also)

Neuroendocrine

Thyroid adenocarcinoma

Mesothelioma

Lymphoma (primary & multicentric)

Sarcoma

Heart base (second most common):

Neuroendocrine (~40%)

Thyroid adenocarcinoma

Mesothelioma

Hemangiosarcoma

Pericardial mass

Right ventricular mass

Cranial mediastinal mass

Left atrial mass

Infiltrative Disease

Lymphosarcoma

Pyogranulomatous inflammation:

Coccidioides, *Histoplasma*

Congenital

Peritoneopericardial diaphragmatic hernia

Pericardial cyst

Miscellaneous

Right-sided heart failure

Left atrial rupture: idiopathic (mitral valve insufficiency)

Right atrial rupture: traumatic

Anticoagulant rodenticide toxicosis

Uremic pericarditis

Bacterial or fungal infection

Constrictive pericarditis

hemangiosarcoma cause pleural effusion associated with pericardial tamponade in dogs (Box 10-3). Occasionally, animals with a cranial mediastinal mass and associated pleural effusion demonstrate features of cranial vena cava syndrome (i.e., submandibular edema; jugular engorgement and pulses; injection of the conjunctival blood vessels indicating impaired cranial venous return, lymphatic return, or both). Thymomas, thymic cysts, lymphoma, invasive thyroid adenocarcinoma, and bilateral jugular thrombosis are the most common causes of this syndrome.

Thoracic radiography should be performed before thoracocentesis to minimize the possibility of iatrogenic lung laceration, to identify cardiomegaly (suggesting cardiomyopathy or pericardial effusion), and to detect gas entrapped within visceral structures (diaphragmatic

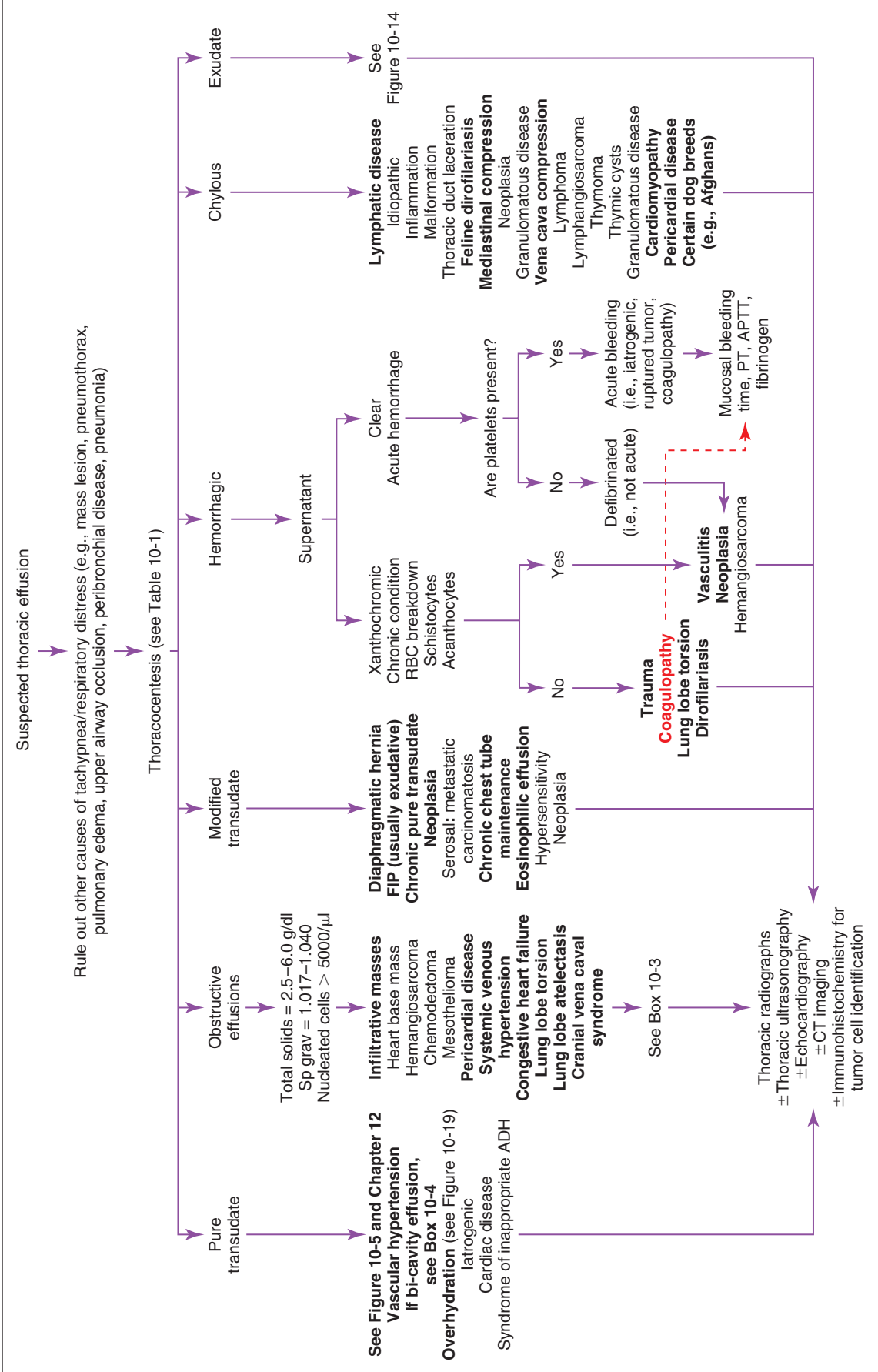


FIGURE 10-12 Diagnostic considerations in animals with suspected thoracic effusion. ADH, Antidiuretic hormone; APTT, activated partial thromboplastin time; FIP, feline infectious peritonitis; PT, prothrombin time; RBC, red blood cell; Sp grav, specific gravity.

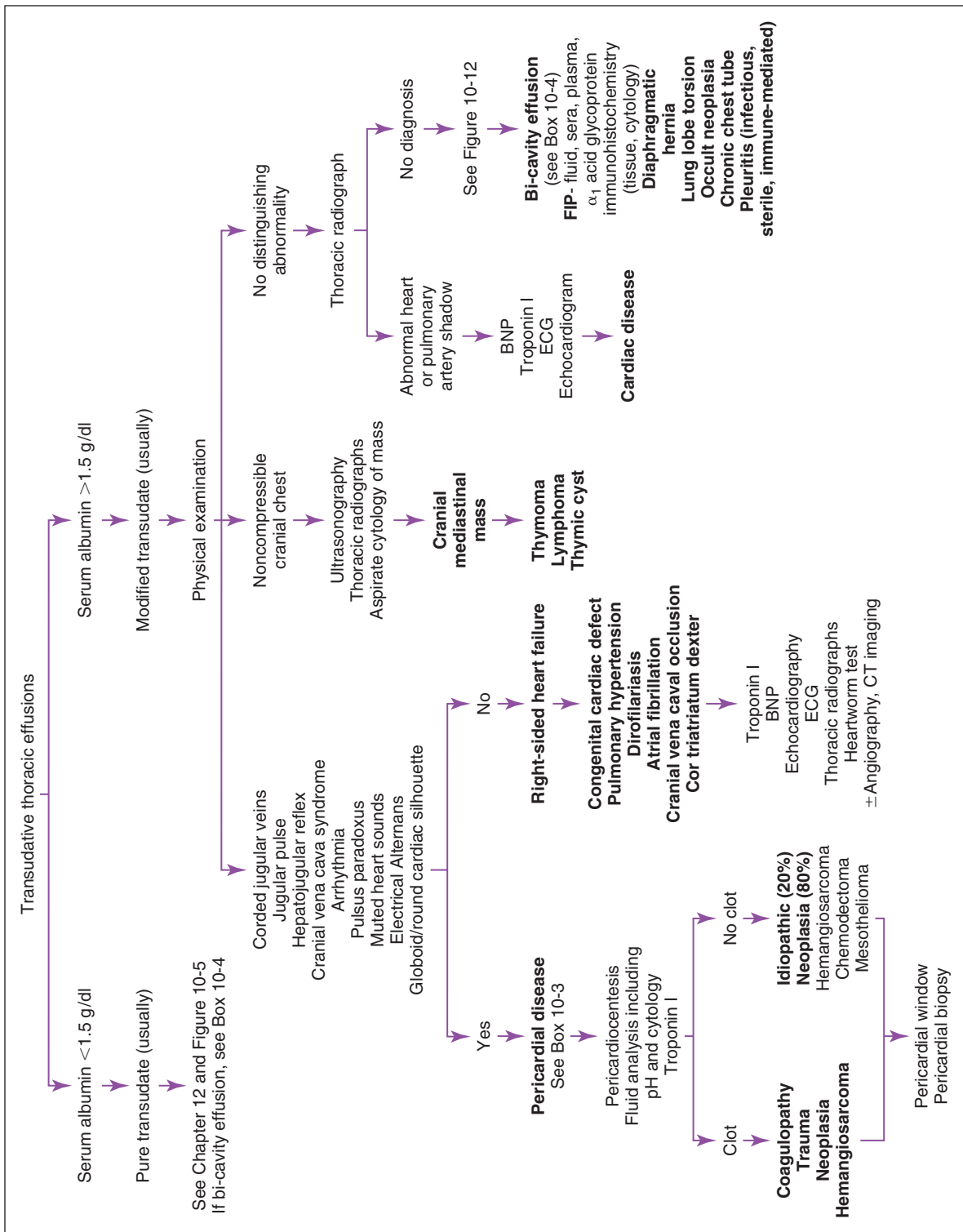


FIGURE 10-13 Diagnostic considerations in animals with pleural transudates. BNP, B-type natriuretic peptide; CT, computed tomography; ECG, electrocardiogram; FIP, feline infectious peritonitis.

hernia) or an abscess. Removing effusion followed by radiography may permit identification of masses and consolidated lung lobes or diaphragmatic incontinuity. Thoracic ultrasonography is enhanced by thoracic effusions, and ultrasound-guided aspiration of masses may provide a definitive diagnosis. Most animals require sedation before needle-targeted thoracic sampling of lesions deeper than the peripheral pleural space.

Thoracic exudates (Figure 10-14) necessitate careful examination for infectious agents. A variety of bacterial organisms may be identified in septic pleural effusions. *Actinomyces* and *Nocardia* are often found in dogs, particularly in geographic locales where foxtail grass awns occur. Multiple bacterial species are commonly found in feline pyothorax. Septic exudates typically are turbid, cream colored, seropurulent or brown tinged and contain degenerate WBCs. Exudates associated with pure *Actinomyces* infection may have minimal or no WBC degeneration; dual infections with *Nocardia* are common, and mixed populations of both organisms may be found in thick or thin red-brown exudates containing degenerate WBCs and "sulfur granules." Inclusion of colored or whitish flecks or "granules" from an exudate on cytologic smears improves identification of *Actinomyces* and *Nocardia* organisms. These are presumptively identified when beaded, branching filaments (see Figure 10-11) are found. Most *Nocardia* spp. stain acid fast with a modified acid-fast stain, whereas *Actinomyces* spp. do not. While both organisms produce exudates containing sulfur granules, only *Actinomyces* produces these within tissues. In animals with pyothorax, thoracic radiography may disclose pulmonary parenchymal involvement. When effusions are managed with chest tube insertion, pleural fluid persists as long as the tube is retained. This necessitates sequential cytologic evaluation of pleural fluid to estimate treatment response and to guide propriety of tube removal.

Nonseptic exudates with a hemorrhagic component are associated with lung lobe torsion (suggested by radiographic evidence of a malpositioned main stem bronchus, persistent air within a twisted bronchus, and usually prolific fluid production). Bronchoscopy may grossly visualize a twisted bronchus. Ultrasonography may confirm lung lobe torsion by interrogation of lung lobe perfusion. Nonseptic exudates also are associated with idiopathic pleuritis, infectious pneumonia (e.g., *Mycoplasma pneumoniae*), and various tumors. FIP can cause a pyogranulomatous pleural effusion that is light yellow and viscous, with a high protein concentration. A background of proteinaceous material (homogeneous pink background on Wright-Giemsa stains) is common on cytologic evaluation.

Thoracic neoplasia often induces pleural effusion associated with exuberant mesothelial cell exfoliation. The most common neoplasm causing pleural effusion in dogs and cats is mediastinal lymphoma. In dogs, lymphocytes are the predominate cell population and these may lack obvious malignant characteristics. Aspiration of mediastinal masses or lymph nodes or other more accessible enlarged nodes may be diagnostic. In cats, exfoliated lymphoblasts are common. It is important to differentiate thymomas from lymphoma, as the former may have a better prognosis. Differentiation of thymoma from lymphoma may require tissue sampling. Malignant or benign

thymic cysts also may cause pleural effusion. Mesotheliomas pose a great diagnostic challenge and commonly require tissue biopsy for definitive diagnosis.

Hemorrhagic pleural effusion (see Figure 10-6) is usually caused by trauma or neoplasia. With trauma, radiographs may reveal rib fractures, pulmonary consolidation, or pneumothorax. Nontraumatic hemorrhagic pleural effusions usually result from bleeding neoplasia; however, coagulopathy also must be considered (e.g., minor trauma can cause substantial bleeding in dogs with severe von Willebrand's disease or vitamin K depletion from warfarin-like rodenticides; see Chapter 5). Other nontraumatic causes of hemothorax include lung lobe torsion, pulmonary abscessation, pulmonary infarction, dirofilariasis, and (rarely in the United States) *Spirocerca lupi*-associated aortic aneurysm. In dogs, hemorrhagic pleural effusion derived from disseminated pulmonary hemangiosarcoma is difficult to diagnose antemortem. Pulmonary aspirates or open-chest biopsy impose high risk for tension pneumothorax and worsening hemorrhage.

Chylous effusions are more common in the thorax than in the abdomen (see Figure 10-12). These may be idiopathic or associated with underlying disease as described previously. Some breeds (e.g., Afghan hounds) may have a congenital propensity for chylous pleural effusions. Thoracic ultrasonography or postdrainage radiography can assist in identifying underlying conditions. Contrast lymphangiography performed by cannulating mesenteric lymphatics or the thoracic duct may elucidate the site of chyle leakage. An alternative strategy is to feed a high-fat (i.e., cream) small meal shortly before surgical exploration to fill lymphatics with grossly identifiable chyle.

Bicavity Effusions

The list of differentials includes several diseases (Box 10-4). Tumors and cardiac disease are particularly common causes.

Pericardial Effusions

The pericardial cavity is a potential space between the parietal and visceral layers of the serous pericardium normally containing from 1 to 15 ml of plasma ultrafiltrate. Normal intrapericardial pressure equates with intrapleural pressure, vacillating by ± 4 mm Hg with ventilation.⁴⁵ Accumulation of fluid within the pericardial sac exceeding its flexible capacitance impairs right atrial/ventricular filling pressures (normally 4 to 8 mm Hg) causing cardiac tamponade. This compromises venous return, ventricular filling, stroke volume, and cardiac output. Initially, a compensatory increase in heart rate and peripheral vascular resistance moderates systemic effects of cardiac tamponade, maintaining normal blood pressure and cardiac output. However, as fluid accumulates and intrapericardial pressure increases, impaired left atrial and ventricular filling lead to left-sided cardiac dysfunction and cardiogenic shock secondary to a marked fall in cardiac output and peripheral blood pressure. The volume of pericardial fluid provoking symptomatic tamponade varies with speed of accumulation, underlying cause, and

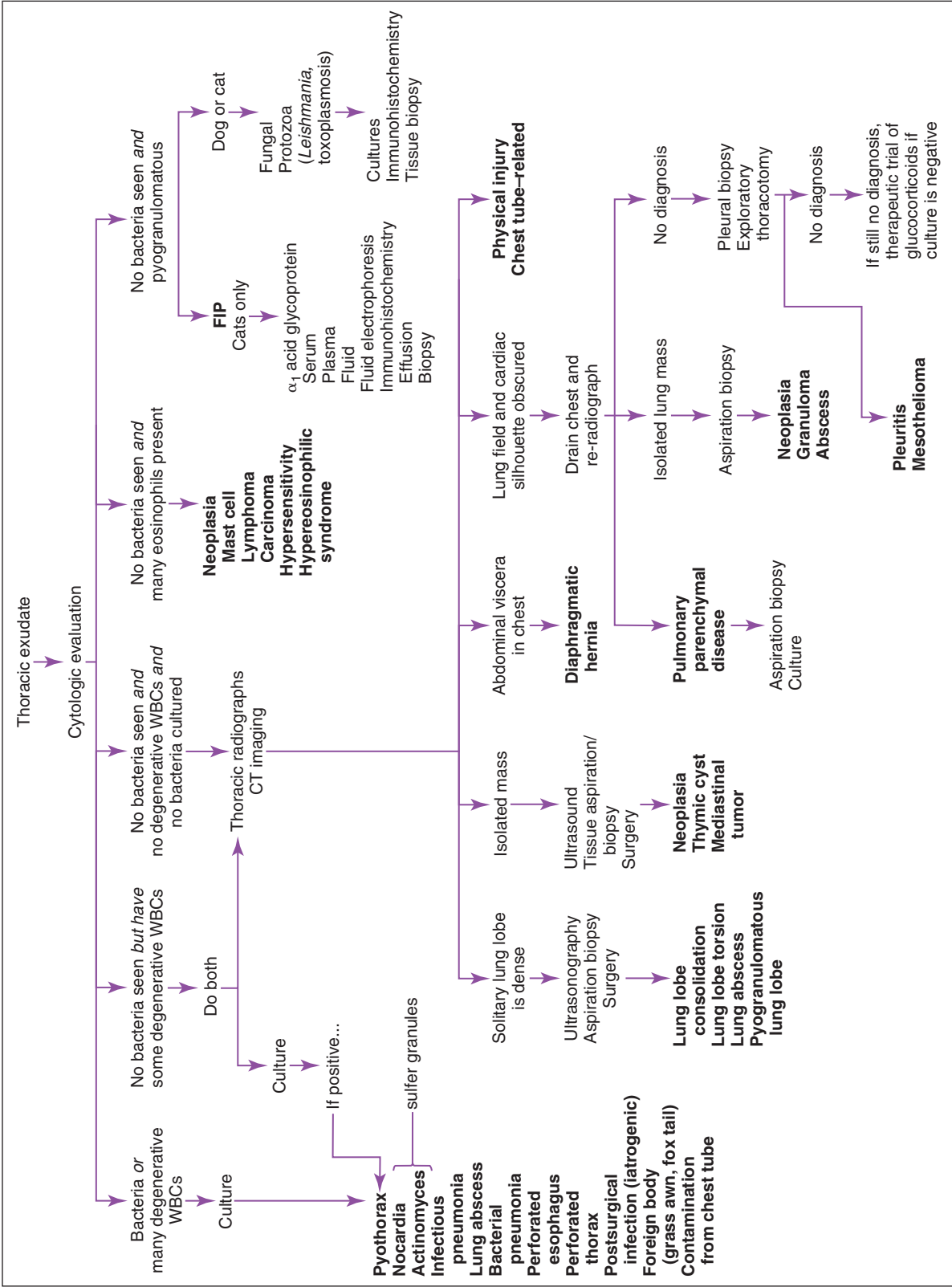


FIGURE 10-14 Diagnostic considerations in animals with thoracic exudates. CT, Computed tomography; FIP, feline infectious peritonitis; WBCs, white blood cells.

BOX 10-4. CONDITIONS ASSOCIATED WITH BICAVITY EFFUSIONS**Cardiovascular Conditions**

Idiopathic hemorrhagic pericardial effusion
 Constrictive pericardial disease
 Biventricular cardiac failure:
 Congestive cardiomyopathy
 Hypertrophic cardiomyopathy
 Idiopathic pulmonary hypertension
 Right ventricular thromboembolism
 Caudal vena cava thromboembolism
 Congenital obstruction: caudal vena cava

Pancreatitis**Bile Peritonitis****End-Stage Hepatic Disease****Feline Infectious Peritonitis****Neoplastic Conditions**

Right atrial fibroma
 Metastatic adenocarcinoma
 Lymphoma
 Hemangiosarcoma
 Mesothelioma
 Cholangiocellular carcinoma
 Chemodectoma
 Prostatic adenocarcinoma
 Diffuse carcinomatosis

total accumulated volume. Rapid accumulation of volumes as small as 25 to 100 ml can abruptly cause tamponade, whereas slower accumulations can generate volumes as large as 2 L in large-breed dogs (which are most commonly affected) before onset of clinical signs. Dogs with chronic pericardial effusion eventually demonstrate clinical signs consistent with right-sided heart failure: lethargy, exercise intolerance, tachypnea, weight loss, and abdominal distention. Signs are progressive in development concordant with fluid accumulation that exceeds compliance of the pericardial sac. Dogs with acute symptomatic pericardial effusion present with acute collapse, syncope, or weakness precipitated by physical exertion and require emergency pericardiocentesis. The clinician should look for jugular pulse, pulsus paradoxus, hepatojugular reflex, poor femoral pulse quality, muffled cardiac sounds, exercise intolerance, and physiologically inappropriate tachycardia consistent with pericardial tamponade or restrictive pericardial disease. Hepatojugular reflex is elicited by applying gentle abdominal compression to liver or cranial abdomen for 10 to 15 seconds (increases venous return to the heart) and observing jugular vein distention or pulsation (indicating reduced right heart function or filling). Hepatomegaly caused by venous congestion may be difficult to palpate because of abdominal distention due to ascites formation or secondary to patient conformation (deep-chested dog). The unique finding of pulsus paradoxus is

best detected with the patient laterally recumbent. This represents an exaggerated change in arterial pressure during respiration: fall in pressure during inspiration and stronger pulse during expiration that coordinate with exaggerated right atrial and ventricular filling during inspiration and reduced stroke volume from reduced left ventricular volume.

Pericardial effusion is usually associated with pericardial irritation and inflammation, neoplasia causing hemorrhage, or central venous congestion. Animals with congestive cardiomyopathy may have small to moderate amounts of pericardial fluid that can lead to diagnostic confusion in delineating the cause of clinical signs. Pericardial effusion is associated with a severe globoid or round enlargement of the cardiac silhouette with the size increasing with the volume of accumulated effusion (large size with chronicity, perhaps near-normal shape and size with rapid onset). The caudal vena cava, an important capacitance vessel, is usually large on the lateral thoracic radiograph reflecting hepatic congestion and hepatomegaly due to passive congestion. Pleural effusion and evidence of metastatic disease may be apparent. Edges of the cardiac silhouette may appear unusually "sharp" due to decreased motion artifact associated with the diminished cardiac contraction.⁴⁶ Abdominal radiographs may disclose hepatomegaly or reduced abdominal detail due to ascites derived from severe passive congestion (right-sided congestive heart failure) caused by pericardial tamponade. Sinus tachycardia is a common electrophysiologic finding along with low-voltage QRS complexes (50% of dogs with pericardial effusion). Electrical alternans (cyclic change in R-wave amplitude) reflects motion of the heart suspended in the pericardial sac. Finding a normal ECG does not dismiss pericardial effusion from considered differential diagnoses. The gold standard for diagnosing pericardial effusion is echocardiography; effusion in the pericardial sac is obvious and can be identified by entry-level ultrasound operators. Fluid volumes as small as 10 to 15 ml can be detected using ultrasonography. Diastolic collapse of the right atrium or ventricle is a classic feature.⁴⁶ Mass lesions may be identified but often require an operator with specialized training. A cavitated mass associated with the right atrium is highly consistent with hemangiosarcoma, the most common neoplasm in large-breed dogs associated with pericardial tamponade linked with a hemorrhagic/xanthochromic effusion. Small hemangiosarcomas located beyond the echocardiographic "window" may remain undetected.

Pericardial effusions characterized as pure transudates may reflect severe hypoalbuminemia. Modified transudates are found with idiopathic pericarditis, right-sided heart failure, pericardial cysts (congenital malformations), uremic pericarditis, and syndromes associated with vascular leakage. Exudates are found with FIP and bacterial or fungal pericarditis. Septic pericarditis is rare but has been reported secondary to migrating grass awns. Apparent chylopericardium also is rare but may develop secondary to mediastinal venous hypertension derived from cardiomyopathy. Trauma, neoplasia, coagulopathy, or rare spontaneous left atrial rupture can cause hemopericardium. Pericardial tamponade is most common in dogs and is most often associated with a

hemorrhagic effusion. However, it also may be associated with a modified transudate or exudate. A benign pericardial effusion is detected in approximately 50% of dogs. In the remainder, the most common diagnosis is neoplasia with right atrial hemangiosarcoma most common (approximately 60% to 75% of neoplastic causes) followed by chemodectoma (approximately 10% of neoplastic causes), mesothelioma (approximately 5% of neoplastic causes), and rarely metastatic adenocarcinoma. Idiopathic pericarditis is associated with extensive fibrosis and a mixed inflammatory response having greatest intensity at the cardiac surface. Perivascular lymphoplasmacytic aggregates are found at the pleural surface and within fibrosed pericardium.¹³ It is notable that some cases of idiopathic pericarditis resolve after a single pericardiocentesis.

Cytologic differentiation of benign from malignant pericardial effusion remains problematic. Irrespective of the definitive diagnosis, hemorrhagic effusions are most common. One study of 50 dogs with pericardial effusion confirmed that cytologic characterizations could not reliably distinguish between neoplasia and other underlying causes.⁴⁸ Use of fluid pH to differentiate benign from neoplastic pericardial effusion in dogs remains controversial. Determining pH requires availability of an accurate portable pH meter and prompt analysis of sample supernatant as a bedside test. In humans, inflammatory pericardial effusions have a significantly lower mean pH than noninflammatory disorders. In dogs, idiopathic effusion tends to have a lower pH compared to neoplastic effusions, but there is broad overlap that thwarts clinical utility of pH as a diagnostic parameter. Measurement of serum cardiac troponin I (cTnI) may assist in differentiating idiopathic and neoplastic causes of pericardial effusion. Dogs with effusion secondary to neoplasia had a median value of 2.77 (range 0.09 to 47.18) ng/dl, contrasting with dogs with idiopathic pericardial effusion that had a median cTnI value of 0.05 (range 0.03 to 0.09) ng/dl. Notably, there was overlap in values between groups that compromises use of cTnI as a stand-alone diagnostic parameter.

Radiographs, ultrasonography, and CT imaging coupled with thoracoscopy or exploratory surgery are usually needed to differentiate benign from malignant disease. Pericardial biopsy performed during laparoscopic pericardiectomy is the least invasive yet reliable method of tissue retrieval for definitive diagnosis. While this procedure may provoke lethal hemorrhage upon sampling of a highly vascular tumor, it remedies pericardial effusion by creating a pericardial window (fluid drains into the pleural space). Most cats with pericardial disease have cardiac disease (i.e., cardiomyopathy, valve abnormalities), neoplasia (i.e., lymphoma, metastatic carcinomas), chronic renal disease (uremic pericarditis), coagulopathies, or more rarely, bacterial infection or restrictive pericarditis. Intrapericardial cysts are a rare cause of pericardial effusion and tamponade. These are associated with a serosanguineous modified transudate or exudate in dogs; diagnosis is achieved by echocardiography. These lesions reflect entrapped omentum or falciform ligament in the pericardial structure during embryonic development and usually are asymptomatic unless associated with effusion.

Joint Effusions

The joint capsule is constructed of three layers: an outermost fibrous layer providing joint stability and flexibility, a subsynovium that serves as the source of joint fluid, and an inner synovial lining comprising two cell types: type A synoviocytes (macrophage-like cells) and type B synoviocytes (fibroblast-like cells that produce hyaluronic acid). Synoviocytes modify synovial fluid, provide viscous hyaluronic acid for joint lubrication, remove large molecules (i.e., plasma proteins), and participate in maintenance of articular cartilage. Synovial fluid, a plasma ultrafiltrate enriched with viscous hyaluronic acid important for joint mobility, nourishes articular cartilage and functions as a boundary lubricant for periarticular tissues.³² Synovial effusions reflect cartilage injury secondary to degenerative joint disease, trauma, immune-mediated mechanisms, and infection. On injury, chondrocytes and synoviocytes release cytokines that induce vasodilation of subsynovial capillaries leading to enhanced vascular permeability and extravasation of protein and inflammatory cells into the joint space. Inflammation is fostered by accumulated leukocytes and release of degradative enzymes and mediators from multiple cell types. Number and cellular composition of leukocytes infiltrating the synovium and migrating into synovial fluid determine characteristics of a joint effusion and clinical features of associated arthritis. Generally, arthropathies are considered inflammatory or degenerative, exudative or transudative, septic or nonseptic, hemorrhagic or nonhemorrhagic, and erosive or nonerosive, and are cytologically classified similar to other body cavity effusions with additional assessment of joint fluid viscosity.

Joint fluid should be analyzed in patients with swollen, fluctuant, or painful joints not historically associated with degenerative joint disease. Because joint pain may be subtle, joints should be carefully palpated for mobility, swelling, and discomfort. Atraumatic arthrocentesis requires familiarity with the anatomic landmarks of the involved joint. Synovial fluid analysis distinguishes noninflammatory from inflammatory conditions, but interpretation of findings must be integrated with realization that many immune-mediated and systemic inflammatory conditions also involve joints. Thus, joint inflammation does not indicate a primary polyarthropathy. Examination and bacterial culture of joint fluid may be helpful in animals with fever of unknown origin associated with sepsis or immune-mediated disorders. Assessment of joint fluid and interpretation of changes in appearance and cytology follow recommendations made for other body fluids with additional assessment of fluid viscosity (Figure 10-15).

Volume

Very small amounts of fluid are available from most normal joints; therefore collecting relatively large volumes of synovial fluid suggests effusion. Arthrocentesis in healthy animals commonly yields less than 0.1 to 0.25 mL. Joint disease can be accompanied by an increase or decrease in synovial fluid volume. Increased volume of synovial fluid is usually detected during physical examination as joint distention in acute and inflammatory

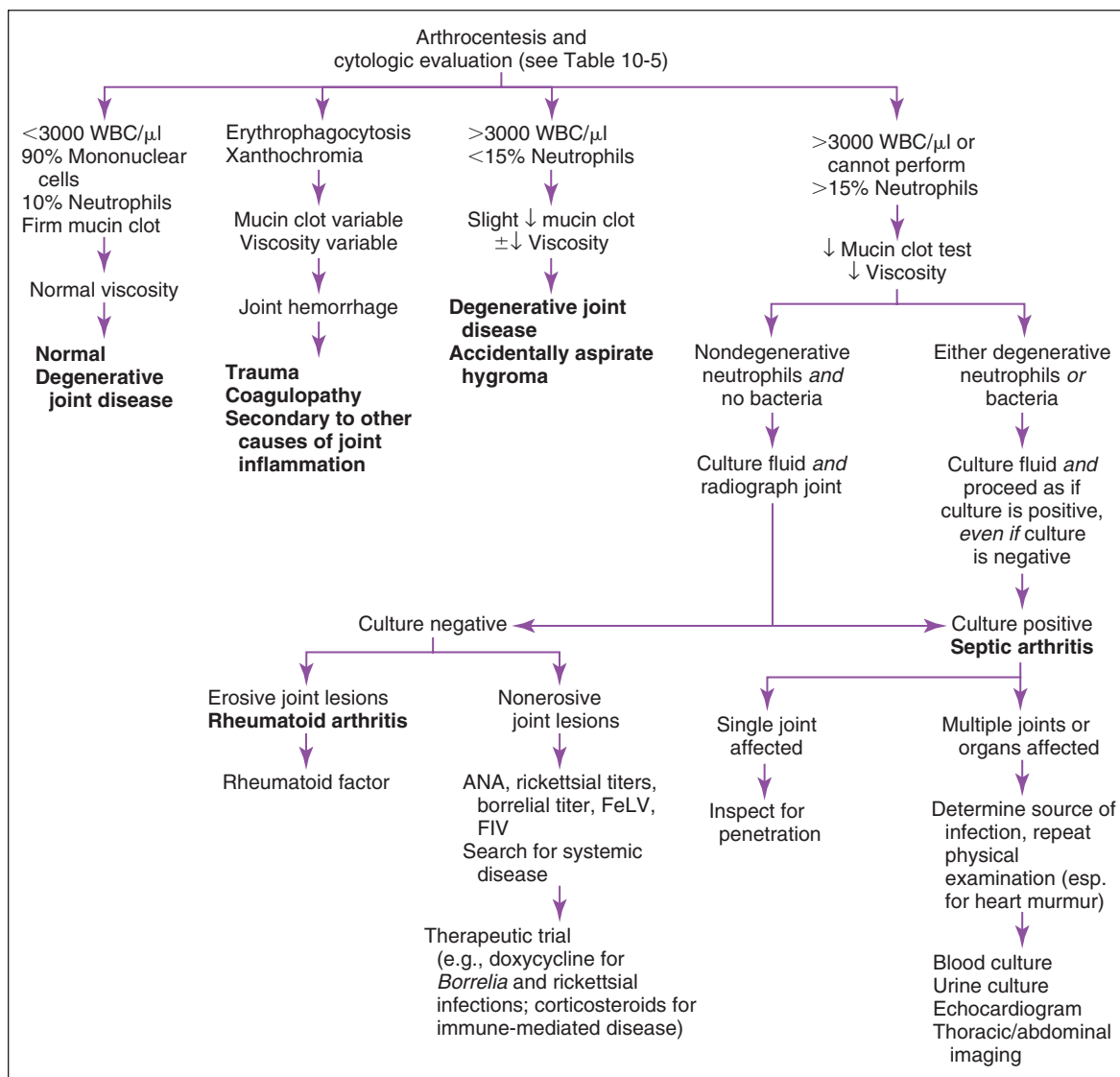


FIGURE 10-15 Diagnostic considerations in animals with joint effusion. ANA, Antinuclear antibodies; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; WBC, white blood cells.

joint conditions, whereas chronic and noninflammatory disorders produce joint enlargement due to soft tissue swelling or thickness despite normal to reduced joint fluid volume. When arthrocentesis is performed to evaluate fever or unexplained lameness in an animal without swollen joints, at least two or three joints should be sampled (carpal and tarsal usually preferred) because of expected low fluid yield.

Gross Appearance

Normal synovial fluid is clear, colorless, viscous, and free of flocculent debris. It does not clot, although it does demonstrate sol-gel reversibility on agitation (thixotropism).^{17,32} Volume is noted subjectively. If greater than 0.25 ml is collected from a single joint, an aliquot should be placed in a pediatric EDTA tube (to avoid clotting) and

in a sterile plain tube (for culture and mucin clot test). Fluid samples for cell counts and cytologic smears can be stored for 24 hours in a refrigerator, but cytologic smears should be promptly made. Homogeneously red or red-tinged fluid implicates joint hemorrhage associated with trauma, inflammation, or bleeding. Fluid containing “streaks” of blood or bleeding only at the end of centesis indicates hemorrhage subsequent to arthrocentesis; platelets should be seen cytologically. Xanthochromia (yellow-orange fluid discoloration) indicates prior hemorrhage and hemoglobin breakdown. White or light yellow fluid or sediment indicates increased nucleated cell counts associated with inflammation, sepsis, or neoplasia (rare). Increased fluid turbidity reflects suspended particulates, including RBCs, WBCs, infectious agents, fibrin, neoplastic cells (rare), and crystals (rare).

Viscosity

Viscosity of synovial fluid should be grossly assessed at the time of collection because this reflects the amount and polymerization of hyaluronic acid and joint lubrication. Decreased viscosity reflects reduced hyaluronic acid production associated with synovial membrane damage, dilution (plasma or fluid), degradation (by WBCs or bacteria), intra-articular injection of drugs, or joint lavage. Crude estimation of viscosity is done by visual observation: a drop of fluid suspended between thumb and opposing finger or suspended from a needle should form a strand at least 1 inch long before breakage. A more qualitative assessment is done using the *mucin clot test* conducted with synovial fluid collected in a plain or heparinized Vacutainer. This test is invalid in EDTA-preserved samples. A mixture of synovial fluid and 2.5% glacial acetic acid (1 part fluid:4 parts acid) is combined in a test tube, mixed well, and the precipitate semiquantitatively graded: good = tight ropy clot with clear solution, fair = soft clot with turbid solution, poor = friable clot with cloudy solution, and very poor = flocculent material in a cloudy solution.⁷ When only a few drops of fluid are collected, hyaluronic acid content (hence viscosity) can also be estimated crudely based on the density of the background staining observed microscopically.³² Normal viscosity is associated with a homogeneous pink, fine to coarsely granular background staining and numerous apparent crescents or folds of this material on cytology smears (Figure 10-16). Fluid viscosity is reduced in most types of joint effusion.

pH

pH of synovial fluid can be determined using narrow-range pH paper immediately after collection (plain tube sample); normal pH ranges from 7.2 to 7.4. Experimental work shows a drop in pH shortly after onset of joint sepsis (to 6.9), but this has not been evaluated for diagnostic accuracy in clinical patients.

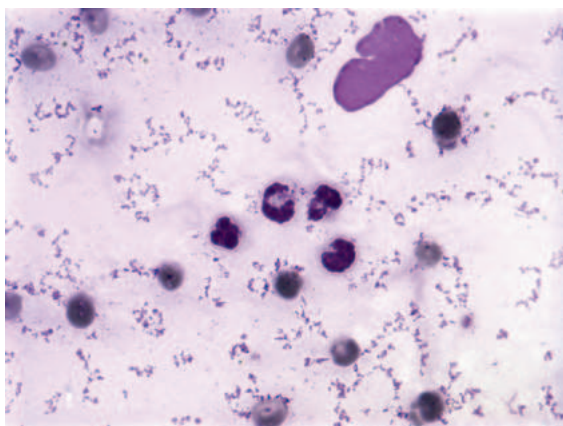


FIGURE 10-16 Cytology of joint fluid from a dog. Note the pink-staining granular background, which represents the mucin in the joint fluid. This is a normal finding. (Courtesy of Dr. Mark Johnson.)

Total Protein

Total protein of synovial fluid is best measured using a quantitative biochemical method; however, refractometry is also used. A normal reference range of 1.5 to 3.0 g/dl is reported.³² An increased total protein concentration reflects inflammation and/or exudation of plasma proteins. However, excessive EDTA relative to joint fluid can falsely increase synovial fluid protein concentrations estimated by refractometry.

Fluid Analysis

Culture, nucleated cell counts, differential cell counts, and estimation of fluid viscosity are recommended (Table 10-5; see also Figure 10-15). Viscosity is reduced in most types of joint effusion. If only small amounts of fluid are obtained (e.g., drops), smears to determine relative cellularity and the predominant cell type have first priority. Determination of the total nucleated cell count/ μ l of fluid is a useful characteristic that helps differentiate inflammatory from noninflammatory arthropathies. Sequential arthrocentesis has been proposed for assessment of response to therapy, although repetitious sampling of a joint can lead to an increase in mononuclear cell count.

Total Nucleated Cell Count

Normal canine stifle joint fluid contains less than 3000 nucleated cells/ μ l, with less than 10% to 12% neutrophils (frequently <5%) in the absence of hemorrhage.³² Mononuclear cells account for the remainder of nucleated cells, comprising 60% to 97% of the total nucleated cell count. RBC quantification provides little useful information. Total nucleated cell counts can be determined by manual methods using a hemocytometer or by electronic cell counters with fluid treated before counting with hyaluronidase to eliminate problems associated with fluid viscosity (pipetting, dilution, acid precipitation of hyaluronic acid) and interactions with normal diluting fluids. One to two drops of a 150-U/ml hyaluronidase suspension added to a small aliquot of synovial fluid diminishes viscosity within a few minutes but increases the rate of cell sedimentation, requiring careful mixing before sample evaluations. Hyaluronidase treatment also invalidates the mucin clot test. Finding greater than 3000 WBCs/ μ l or that greater than 15% of cells are neutrophils indicates inflammation.

Cytologic Features

Cytologic interpretation of joint fluid is hampered by fluid viscosity, which causes cell aggregation and preparation of thick smears in which many nucleated cells appear pyknotic (small, darkly stained, difficult to recognize morphology). As a result, neutrophils may not be distinguishable from lymphocytes. The edge of the smear may be the only location where cell morphology may be distinguishable (area that dried quickly, thin specimen). Because RBCs are rare in normal joint fluid, their presence reflects joint trauma or hemorrhage associated with inflammation, or they are secondary to arthrocentesis. Iatrogenic hemorrhage will display platelets. The chronic presence of RBCs in synovial fluid results in erythrophagocytosis and accumulation of hemoglobin degradation pigments (blue/black granules

TABLE 10-5. ANALYSIS OF JOINT EFFUSIONS

	Noninflammatory Joint Disease			Inflammatory Joint Disease		
	DEGENERATIVE JOINT DISEASE	NEOPLASTIC JOINT INVOLVEMENT	HEMARTHROSIS	INFECTIOUS INFLAMMATION	NONINFECTIOUS INFLAMMATION	NORMAL
Color	Light yellow	Light yellow–blood tinged	Bloody, xanthochromic	Variable: yellow, blood tinged, bloody	Variable: yellow, blood tinged	Straw colored
Turbidity	Clear–slightly turbid	Mild to moderate turbidity	Turbid	Turbid to purulent	Variable: slight to moderate turbidity	Clear
Viscosity	Normal	Normal to reduced	Reduced	Reduced	Reduced	Viscous
Mucin Clot Test	Normal firm	Normal firm	Normal to slightly friable	Friable	Friable	Firm
CYTOLOGY:						
RBCs	Few	Few to many	Many Erythrophagocytosis	Moderate	Few to moderate	Rare
WBCs/μl	<3000	Variable	Variable	40,000–250,000	Many but variable	0–2900
Neutrophils	Few (<20%)	Moderate	Moderate	Many (usually >90%)	Many but variable	0–10%
Degenerative changes	Absent	Absent	Absent	May be present	None to mild	None
Lymphocytes	Few to moderate	Few to moderate	Rare	Few	Few to moderate	Few
Synoviocytes	Common	Few	Rare	Few to moderate	Few to moderate	Few
Macrophages	Few to moderate	Few to moderate	Moderate if chronic	Few to moderate	Few to moderate	Rare
Microorganisms	None	None	None	May be present	None	None
Neoplastic cells	None	Variable	None	None	None	None
Others			Blood contamination deduced by presence of platelets	May not visualize infecting bacteria	May see LE cells, tart cells, ragocytes	Blood contamination noted by blood streaks or blood near end of aspiration; platelets

LE, Lupus erythematosus; RBCs, red blood cells; WBCs, white blood cells.

in macrophages). Neutrophils, lymphocytes, monocytes, and macrophages may be identified in joint fluid from both normal and diseased joints. Normal joint fluid has one to three nucleated cells per high-power field (400×) where each nucleated cell represents approximately 1000 cells/μl; less than 10% of cells are neutrophils and these have a normal cytologic appearance. The remaining cells (90%) consist of small lymphocytes, monocytes, macrophages, and a few synoviocytes. Phagocytosed cytoplasmic debris is common in joint fluid from animals with degenerative joint disease and resolving or chronic inflammation. Infectious agents, including bacteria, fungi, and protozoa, are sometimes found within macrophages in septic arthritis. Infrequent but informative cells in different disorders include multinucleated giant cells, osteoclasts, ragocytes, and lupus erythematosus (LE) cells. Multinucleated giant cells are rarely observed but represent fusion macrophages; these have multiple round to oval nuclei in a gray granular cytoplasm and may demonstrate phagocytosed debris or organisms. Osteoclasts are 5 to 10 times the size of a neutrophil and have an irregular cell margin, abundant fine granular light blue-gray cytoplasm, as well as several round nuclei that contain singular nucleoli; these indicate cartilage damage with exposure of bone.³² Ragocytes are neutrophils containing small, round, purple, variably sized cytoplasmic granules; these are common in joint fluid from animals with inflammatory and immune-mediated arthritis. Cytoplasmic granules are thought to represent phagocytosed droplets of immune complexes.¹² While these may be confused with phagocytosed coccoid bacteria, they are differentiated on the basis of granule size variability. LE cells found in patients with immune-mediated joint disease (e.g., systemic lupus erythematosus [SLE], rheumatoid arthritis) are phagocytes containing engulfed degenerate nucleoprotein with a homogeneous pink appearance that fills the cytoplasmic compartment. Tart cells may be confused with LE cells; these are neutrophils that display phagocytized nuclear material that retains the color and texture of normal chromatin. Tart cells can be seen in joint fluid associated with any inflammatory arthropathy.

Inflammatory Arthropathies

Inflammatory arthropathies share common characteristics of suppurative synovial inflammation (see Figure 10-15). Both infections and immune-mediated disorders generate similar pathologic responses that involve complement activation, vascular fluid leakage, inflammatory mediator release, and increased synovial fluid causing joint capsule distention and pain. Increased total protein, fibrin, and coagulation proteins may cause joint fluid to clot on collection if it is not promptly placed in an EDTA tube. While highest cell counts are encountered with septic effusions, there is broad overlap in neutrophil counts among dogs with septic and immune-mediated arthritis. Neutrophilic inflammation may also be seen in joints affected by degenerative arthritis with as many as 12,000 cells/μl and up to a 56% distribution of neutrophils. Neutrophil morphology may be well preserved in septic and nonseptic inflammatory arthritis, and identification of infectious agents may be difficult. Inflammatory arthritides are associated with turbid synovial fluids (high

cell counts) ranging in color from yellow to orange. Septic joint effusion may appear yellowish-green reflecting large numbers of degenerating neutrophils. Such fluid has reduced viscosity secondary to enzymatic degradation of hyaluronic acid and glycoproteins, a mucin clot test ranging from poor to very poor, and diminished background staining on cytology preparations.

Immune-Mediated Arthropathies

Immune-mediated arthropathies are more common than infectious arthropathies in dogs and cats. Presence or absence of radiographic erosive lesions assists in subclassifying these disorders (see Figure 10-15). Erosive lesions should be followed up with submission of a rheumatoid factor (RF) titer in dogs. Rheumatoid factor is an antibody against the Fc portion of immunoglobulin G (IgG) and requires species-specific antisera. Synovial fluids from animals with immune-mediated arthritis have an increased total nucleated cell count with a neutrophilic or mixed inflammatory cell response. Ragocytes are common, whereas LE cells are rarely observed. Foamy macrophages are common and contain cell debris, RBCs, and disintegrating nuclei. Disintegrating cellular components can also be identified in the background. Finding LE cells and ragocytes supports a diagnosis of SLE-associated immune-mediated arthropathy. Owing to cyclic activity of immune-mediated arthritis, associated joint effusions are widely variable lending confusion to initial diagnosis and during sequential assessments by arthrocentesis. Repeated arthrocentesis for disease monitoring may increase mononuclear cell counts. Similar confusion exists in assessment of canine rheumatoid arthritis, an erosive immune-mediated disorder, because affected animals may present with either a predominantly neutrophilic or mononuclear joint inflammation. Lymphoplasmacytic synovitis, an immune-mediated arthropathy found in some dogs with anterior cruciate rupture and degenerative joint disease, is histologically characterized by synovial hyperplasia and nodular aggregates of lymphocytes. Joint fluid from affected individuals is characterized by a moderately increased cell count (5000 to 20,000/μl) with predominance of neutrophils or small lymphocytes.

Infectious Arthropathies

While most canine polyarthritis is nonspecifically immune mediated, it may reflect response to infection. Sepsis is suggested by degenerative neutrophils and is confirmed by observing or culturing an infectious agent. Neutrophils are not reliably degenerative in septic joint effusion. Perusal of a cytologic preparation should focus initially on clumps of cells and cells on the smear margin. While phagocytosed bacteria confirm septic arthritis, bacteria are only observed in approximately 50% of culture-positive fluid samples. Because joint sepsis and immune-mediated arthritis can produce similar total nucleated cell counts and distribution, bacterial cultures are important for identifying sepsis. If minimal amounts of fluid are aspirated, the syringe and needle can be rinsed with broth-enrichment medium and this rinse material cultured. Synovial fluid preincubated in blood culture medium for 24 hours may be more sensitive for detection of infectious agents (i.e., liquid blood culture medium is

thought to prevent sample coagulation, dilutes bacterial growth inhibitors, inactivates or dilutes antibiotics, and curtails *in vitro* leukocyte phagocytosis of bacteria). If anaerobic bacteria are suspected, the sample must be protected from room air by prompt collection into anaerobic transport medium. If mycoplasma are suspected, specialized culture medium is necessary. In chronic low-grade infections, culturing synovial tissue biopsies may be more productive than joint fluid cultures. In cases in which cultures are negative but infection is still considered likely, blood and urine cultures may yield an infectious agent. Sudden detection of a cardiac murmur may herald the onset of infectious endocarditis and should be explored with echocardiography. Many dogs with septic joints have minimal or no degenerative neutrophils or negative cultures due to concurrent antibiotic therapy. Some infectious agents may not cause degenerative neutrophil changes and are not cytologically detectable (e.g., mycoplasma, rickettsia, L-form bacteria, viruses [calicivirus in cats, postvaccinal arthritides]). A pyogranulomatous response is usually associated with fungal and bacterial L-form infections (L-forms are bacteria lacking cell walls). *Ehrlichia morulae* are only found in very small numbers of neutrophils (~1%) in synovial fluid during the acute stages of infection. Protozoa (e.g., *Leishmania*), fungal hyphae (i.e., *Aspergillus*), or yeast forms (e.g., *Blastomyces*) of fungi causing systemic infections also may be observed in synovial fluid macrophages. Feline polyarthritis (i.e., chronic progressive polyarthritis) is statistically linked with feline syncytium-forming virus (FeSFV) and feline leukemia virus (FeLV) infections. Viral-induced arthritis may present either as a mild or marked mononuclear effusion, as observed in feline calicivirus or as a suppurative response. Omphalophlebitis may be the source of infection in neonatal animals. Serologic testing for borreliosis (i.e., Lyme disease) may help diagnose inflammatory arthritis of unknown cause (see Chapter 15). Response to therapy may be the most compelling basis for diagnosing Lyme disease or rickettsial polyarthropathies. However, administration of doxycycline for these agents also has an anti-inflammatory influence that may modulate joint pain and effusion in the absence of infection.

Exudative Joint Disease

Exudative joint disease without evidence of sepsis is categorized as erosive or nonerosive based on radiographs (see Figure 10-15). Rheumatoid arthritis is classically considered erosive. Nonerosive arthritides are more difficult to definitively diagnose and categorize. SLE and arthritis associated with a variety of underlying diseases are most common. Ragocytes, LE cells, or both indicate immune-mediated disease (Figure 10-17). Submission of sera for antinuclear antibody (ANA) and RF titers (see Chapter 12), blood for an LE cell prep, and synovial biopsies for histologic evaluation may implicate immuno-destructive processes. Chronic, progressive feline polyarthritis usually occurs in males and has two forms. A periosteal-proliferative form is most prevalent, primarily affecting young adult cats causing tenosynovitis followed by non-deforming periarticular periosteal proliferation and subchondral bone erosions. A deforming or rheumatoid-like arthritis form has an insidious onset in older cats where

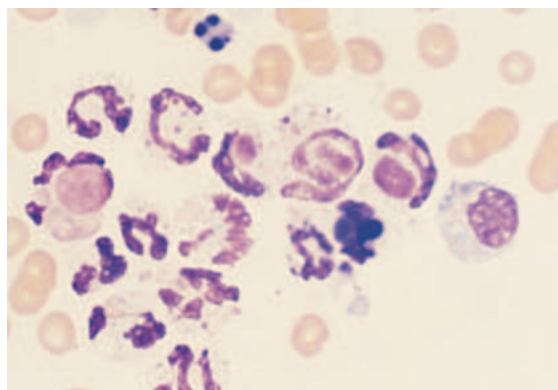


FIGURE 10-17 This canine synovial fluid smear has one large lupus erythematosus (LE) cell at the far left, which is a neutrophil containing a large round violet LE body that is composed of nuclear proteins from a dead lysed cell bound to antinuclear antibodies. Many other neutrophils have multiple, smaller inclusions that are probably antigen-antibody complexes, and these white blood cells are called ragocytes. Both are indicative of immune-mediated joint disease.

it is associated with severe subchondral bone destruction, joint instability, and deformity. Feet, carpi, and tarsi are severely and symmetrically affected.

Noninflammatory/Degenerative Arthropathies

The most common cause of noninflammatory joint disease is degenerative arthritis secondary to trauma or joint instability. Other causes include hemarthrosis, neoplasia, genetic or developmental disorders, dietary or nutritional deficiencies or excesses, and miscellaneous causes (e.g., hypertrophic osteopathy). Noninflammatory arthritides (i.e., degenerative joint disease, traumatic joint injury, hemarthrosis, neoplastic joint conditions) are often characterized by low-grade synovial mononuclear inflammation. Fluid analysis may be normal or show only minor changes: mild vasodilation may slightly increase fluid volume and total protein content, causing mild dilution or no change in hyaluronic acid and fluid viscosity, and a normal or fair mucin clot test. Color of joint fluid in these conditions varies from clear and light yellow to bloody or xanthochromic, reflecting intra-articular hemorrhage. Total nucleated cell counts may be normal to mildly increased but are rarely greater than 5000 cells/ μ l. Cytologic features include a predominance of mononuclear cells and a normal to slightly increased number of neutrophils; total and differential cell counts must be reconciled with blood contamination. Mononuclear cells may appear enlarged and have an abundant foamy vacuolated cytoplasm consistent with phagocytic activity. Traumatic or coagulopathic hemarthrosis often produces erythrophagocytosis and hemosiderin-laden macrophages. Cartilage fragments, chondrocytes, and osteoclasts may indicate severe cartilage damage. Diagnosis of degenerative osteoarthritis is made by reconciling physical findings, radiographic images, and synovial fluid analyses. Bony changes evident radiographically indicate an advanced lesion associated with irreversible cartilage damage.

Edema and Anasarca

Edema is defined as a clinically evident increase in interstitial fluid volume. Anasarca refers to gross, generalized edema. Ascites refers to effusion in the abdominal cavity and hydrothorax to fluid accumulated in the pleural cavity. Depending on cause and etiopathogenesis, edema may be localized or generalized. Forces maintaining homeostatic balance of fluid distribution between the intravascular plasma volume and the interstitial fluid space are referred to as Starling's forces. Finding edema or anasarca signals imbalance of Starling's forces favoring fluid distribution into the interstitial compartment: (1) reduced colloid oncotic pressure (i.e., decreased plasma proteins), (2) altered hydrostatic pressure (i.e., opposing pressures within the interstitium and vasculature) favoring interstitial fluid accumulation, (3) reduced vascular integrity (i.e., leakiness of veins, arteries, capillaries, lymphatics), and/or (4) increased pathophysiologic signals conserving systemic water and sodium (e.g., enhanced angiotensin-converting enzyme activity, aldosterone and antidiuretic hormone [ADH] elaboration, systemic or splanchnic hypotension). To determine the cause of regionalized or local edema, consideration must be given to local anatomy of vasculature and soft tissues and the presence of local inflammatory, structural, or neoplastic conditions. A large number of disease processes can participate in edema formation and development of anasarca (Figures 10-18 and 10-19).

Severe hypoalbuminemia leads to edema when TS concentration is less than 1.0 g/dl, whereas venous congestion, lymphedema, or inflammation generates edema fluid while TS concentration is greater than 2.5 g/dl. In the latter circumstance, total and differential cell counts may distinguish inflammatory (e.g., vasculitis) from non-inflammatory causes.

Regional edema is usually caused by inflammation or vascular or lymphatic obstructions (see Figure 10-18). Lymphadenopathy is an indication for lymph node aspirates for cytology and culture. Congenital or acquired arteriovenous (AV) fistulae are rare but may cause localized edema. These may be detected on the basis of a palpable or auscultable fremitus or bruit, ultrasonography, or CT angiography. Acquired lymphatic insufficiency after trauma, surgery, or regional infections commonly causes regional edema. Lymphangiograms are usually not indicated in acute disorders with a plausible short-term duration. A fine-needle aspiration of involved tissues or regional lymph nodes and aspiration of edema fluid may help distinguish the underlying cause. Lymphatic cording is occasionally palpated in animals with lymphatic obstruction or inflammation; lymph fluid can be easily aspirated from such prominent lymphatics. Congenital malformation or degenerative lymphatic disorders may cause lymphedema in young animals; lymph nodes are sometimes atrophied or absent. Limb edema and tail edema are most commonly recognized. Affected animals develop overtly swollen appendages (single or multiple) but lack significant physical disability.

Generalized edema or anasarca is usually dependent, affecting distal extremities or the brisket (see Figure 10-19). Overhydration, right-sided congestive heart failure, and marked hypoalbuminemia are the most

BOX 10-5. CONDITIONS ASSOCIATED WITH ANASARCA

Iatrogenic Overhydration

Excessive fluid volume calculated or administered
Fluid administration based on whole body weight in morbid obesity leads to overhydration (esp. cats)

Congestive Heart Failure

Failure of sodium restriction
If coupled with crystalloid fluid administration at normal maintenance rate
Increased body sodium and water retention driven by:
Increased ADH elaboration
Increased renin-angiotensin-aldosterone system activity

Acute Renal Failure

Anuria coupled with fluid administration

Hepatic Failure

Hypoalbuminemia
Portal hypertension
Increased body sodium and water retention driven by:
Increased ADH elaboration
Increased renin-angiotensin-aldosterone system activity

Nephrotic Syndrome

Hypoalbuminemia
Increased body sodium and water retention driven by:
Increased ADH elaboration
Increased renin-angiotensin-aldosterone system activity

Vasculitis

Increased vascular permeability: multiple causes
Immune-mediated (e.g., systemic lupus erythematosus)
Infectious diseases (e.g., rickettsial infections)
Hypersensitivity reactions

ADH, Antidiuretic hormone.

common causes (Box 10-5). Iatrogenic overhydration does not typically induce marked generalized edema unless another underlying factor (e.g., hypoalbuminemia, anuria/oliguria) coexists, an inappropriately large volume of fluids has been administered, or a concurrent disease condition or drug therapy has stimulated ADH release. Iatrogenic fluid overload imparts generalized edema associated with an obvious change in skin turgor (e.g., jelly-like consistency); morbidly obese patients have the highest risk when calculation of maintenance fluid requirements is based on gross body weight. Overhydration typically resolves within 48 hours of discontinuing fluid administration, although animals demonstrating cough or tachypnea may benefit from short-term furosemide administration. Animals with a normal serum albumin concentration that are highly sensitive to iatrogenic fluid overload may have incipient cardiac disease (e.g., high-output cardiac failure associated with severe anemia, chronic valvular insufficiency), anuric/oliguric renal failure, or disease processes or drug therapies stimulating excessive ADH release (syndrome of inappropriate antidiuretic hormone secretion [SIADH]).

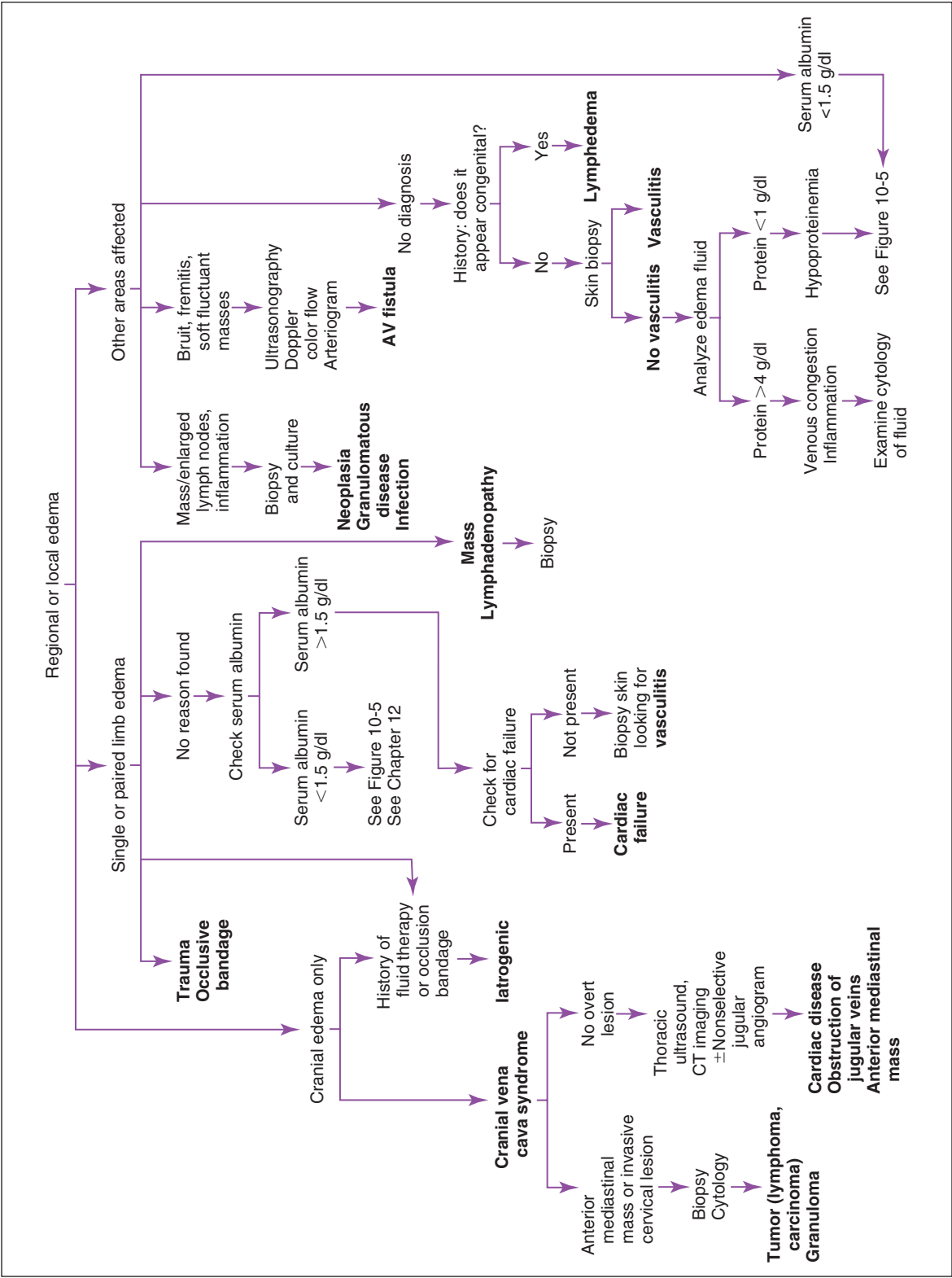


FIGURE 10-18 Diagnostic considerations in animals with regional or localized edema. AV, Arteriovenous; CT, computed tomography.

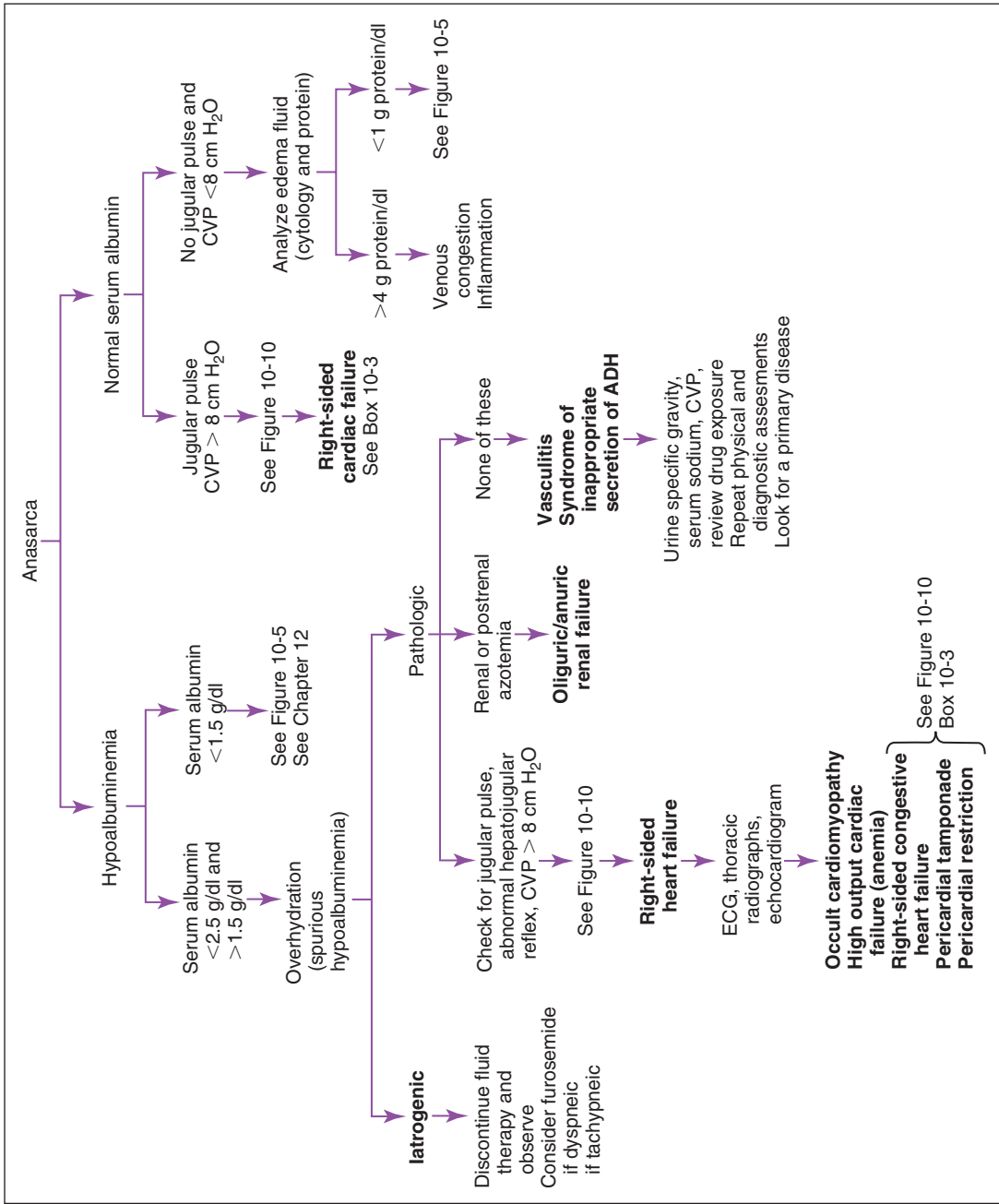


FIGURE 10-19 Diagnostic considerations in animals with anasarca. ADH, Antidiuretic hormone; CVP, central venous pressure; ECG, electrocardiogram.

Serum albumin must be less than or equal to 1.5 g/dl to cause anasarca. This also may generate a pure transudative body cavity effusion. Patients with generalized edema associated with vasculitis usually demonstrate perivascular hemorrhage in some organ system (i.e., petechial hemorrhages, microhematuria, retinal hemorrhages) or microangiopathic effects (i.e., schistocytes, acanthocytes). Total body water retention secondary to SIADH (see Chapter 6) usually coexists with another disorder that overshadows its presence. SIADH is also induced by several drug therapies. With hypoalbuminemia, fluid retention is aggravated by sodium and water retention driven by the renin-angiotensin-aldosterone system, as occurs in severe renal, hepatic, or cardiac disease. Systemic hypertension often reflects abnormal activity of this system. History, physical examination, serum albumin determination, thoracic radiographs, cardiac evaluations (i.e., ECG, echocardiography), systemic blood pressure, CVP determination (not routinely used), urinalysis, abdominal ultrasonography, and CT imaging assist in identifying the cause of anasarca (see Figure 10-19). Finding a jugular pulse or abnormal hepatojugular reflex suggests intrathoracic disease within the cranial mediastinum or involving the heart or pericardium. If an underlying cause is not found, physical examination and laboratory data are reviewed looking for evidence of vasculitis (i.e., petechiation, microvascular lesions on fundic examination, microscopic hematuria, schistocytes on blood smear). Skin biopsies may be performed in areas of bruising or cutaneous lesions to investigate vasculitis; both affected and unaffected tissue and marginal interfaces should be sampled. Collecting edema fluid and analyzing its protein content may help implicate inflammatory causes.

Scrotal Effusions

Scrotal effusion usually develops when abdominal fluid enters the scrotum via the inguinal rings. Severe orchitis or testicular torsion may also be responsible and can be identified by ultrasonography and aspiration cytology. Because scrotal edema may be caused by vasculitis (e.g., Rocky Mountain spotted fever), evaluation for systemic infection is important. If effusion is localized to the scrotum, scrotal ablation and castration may be diagnostic and therapeutic.

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11

Respiratory and Cardiac Disorders

Ned F. Kuehn and Sonya G. Gordon

RESPIRATORY PROBLEMS

Dyspnea

Dyspnea (also called respiratory distress) is an inappropriate degree of breathing effort based on respiratory rate, rhythm, and character. Dyspnea is a common sign associated with a wide variety of respiratory and nonrespiratory disorders. Physical examination is the first step in diagnosis. Patients with obstructive respiratory diseases have a breathing pattern characterized by increased depth, rate, and effort. Dynamic obstruction (e.g., laryngeal paralysis) cranial to the thoracic inlet (i.e., the upper airway) causes increased inspiratory effort. Dynamic obstruction (e.g., collapsing trachea) caudal to the thoracic inlet (i.e., lower airway) causes increased expiratory effort. Fixed obstructions (e.g., tracheal tumor) often are associated with increased inspiratory and expiratory effort. Nasal cavity obstruction only causes an obstructive breathing pattern if the animal does not breathe through the mouth. Auscultation of wheezes suggests obstruction, but wheezes are not always found in obstructed patients. Monophonic wheezes (i.e., single tone) may be noted with an elongated soft palate. Alternatively, wheezes can have multiple tones (i.e., polyphonic wheezes), as heard in asthmatic cats.

Dyspnea due to extra-respiratory causes (e.g., severe anemia, severe metabolic acidosis) can have what appears to be an obstructive breathing pattern with an increased intensity of normal lung sounds (i.e., bronchovesicular sounds) but without wheezes.

Diseases limiting ability of the lungs to expand cause a restrictive breathing pattern characterized by an increased rate but a normal to decreased depth with or without increased inspiratory effort. Auscultation may reveal crackles, particularly inspiratory, or a complete absence of lung sounds with certain restrictive lung diseases (e.g., pneumothorax, hydrothorax). It may be difficult to auscultate pulmonary parenchymal abnormalities, especially in small patients with low tidal volumes. Thoracic radiographs of these patients can reveal substantial pulmonary parenchymal disease despite apparently normal lung sounds. Distinction between obstructive and restrictive breathing patterns is not always clear; the

pattern of breathing exhibited depends on the relative amount of pathologic change in affected tissues. For example, a dog with severe pulmonary edema may have obstructive (airway fluid) and restrictive (interstitial fluid) disease.

Patients with disorders such as flail chest occasionally have paradoxical movements (i.e., a section of the chest wall collapsing during inspiration and expanding during expiration). Pulmonary vascular diseases can be associated with either inspiratory or mixed inspiratory and expiratory dyspnea.

Common causes of dyspnea are listed in [Box 11-1](#).

Useful Tests

After the site of an abnormal breathing pattern has been localized by physical examination, tracheal and thoracic radiographs are typically the most useful next diagnostic step ([Figure 11-1](#)).

NOTE: Although a collapsed trachea can be diagnosed radiographically, it cannot be ruled out by such, even if inspiratory and expiratory films are made. Fluoroscopy or tracheobronchoscopy may be needed to establish the diagnosis.

Transtracheal aspiration (TTA), bronchoalveolar lavage (BAL), or transthoracic fine-needle aspiration (see subsequent sections) may follow if radiography suggests the need for cytologic analysis or culture of the lower airway, especially if coughing is present. Pharyngoscopy, nasopharyngoscopy, and laryngoscopy are useful in patients with upper airway obstruction. Tracheobronchoscopy is useful in tracheal and bronchial disorders, especially for diagnosing obstructive disease such as collapsed trachea, and it is mandatory in most pharyngeal and laryngeal abnormalities (see [Figure 11-1](#)). Pleural effusion is always an indication for fluid analysis (see Chapter 10). If dirofilariasis is suspected, a Knott's test, filter test, or *Dirofilaria immitis* antigen (or antibody in cats) test is indicated (see Chapter 15). Although they rarely provide a diagnosis, fecal flotation and Baermann's fecal analysis are inexpensive and noninvasive and can

BOX 11-1. CAUSES OF DYSPNEA (RESPIRATORY DISTRESS)

RESPIRATORY DISORDERS

Obstructive Disease

Extrathoracic disease

- Nasal (only if patient does not breathe through mouth)
- Stenotic nares
- Neoplasia
- Chronic inflammatory rhinitis
- Fungal granuloma
- Foreign body
- Chondromesenchymal hamartoma (cats)
- Trauma
- Epistaxis (blood clots)

Nasopharynx

- Foreign body
- Neoplasia
- Nasopharyngeal polyps
- Nasopharyngeal stenosis
- Nasopharyngeal turbinates

Pharynx

- Elongated/edematous soft palate
- Pharyngeal edema
- Foreign body
- Neoplasia
- Pharyngeal polyps (cats)

Larynx

- Laryngeal paralysis
- Laryngeal edema
- Laryngeal collapse
- Laryngospasm
- Everted laryngeal sacculles
- Foreign body
- Neoplasia

Trachea

- Cervical tracheal collapse
- Foreign body
- Neoplasia
- Stenosis
- Extraluminal compression
- Traumatic rupture

Intrathoracic disease

Trachea

- Thoracic tracheal collapse
- Foreign body
- Neoplasia
- Stenosis
- Extraluminal compression (tumor or granuloma)
- Parasitic (*Oslerus osleri*)

Principal bronchi

- Bronchial collapse
- Foreign body
- Neoplasia
- Stenosis
- Extraluminal compression (tumor, granuloma, hilar lymphadenopathy, left atrial enlargement)
- Parasitic (*Oslerus osleri*)

Lower airways and pulmonary parenchyma

- Bronchial disease
- Chronic bronchitis (dogs)
- Bronchial asthma (cats)
- Pulmonary edema
- Pneumonia (viral, bacterial, fungal)
- Aspiration pneumonia
- Parasitic pneumonitis
- Hypersensitivity (allergic) lung disease
- Eosinophilic bronchopneumopathy
- Pulmonary hemorrhage
- Neoplasia

Restrictive Disease

Pulmonary disorders

- Pulmonary fibrosis
- Pulmonary edema
- Interstitial pneumonia
- Pulmonary infiltrates with eosinophilia

Pleural space or body wall disorders

- Pneumothorax
- Pleural effusion

Hernia

- Pleuroperitoneal
- Pericardioperitoneal

Cranial displacement of diaphragm

- Abdominal mass or masses
- Abdominal effusion
- Gastric dilation

Trauma

- Rib fracture or fractures
- Flail chest

Neoplasia

- Mediastinum
- Thoracic wall

Obesity

NONRESPIRATORY DISORDERS

Vascular disease

- Dirofilariasis
- Pulmonary embolism

Cardiac disease

Hematologic

- Anemia (severe; not true dyspnea)
- Methemoglobinemia

Metabolic

- Acidosis (not true dyspnea)
- Shock
- Heat stroke

Neurologic and neuromuscular disease (not true dyspnea)

- Central nervous system (CNS)
 - Head trauma
 - CNS inflammatory disease
 - Neoplasia

Neuromuscular diseases involving muscles of respiration

- Myasthenia gravis
- Polymyopathy
- Neuropathy
- Polyradiculoneuropathy

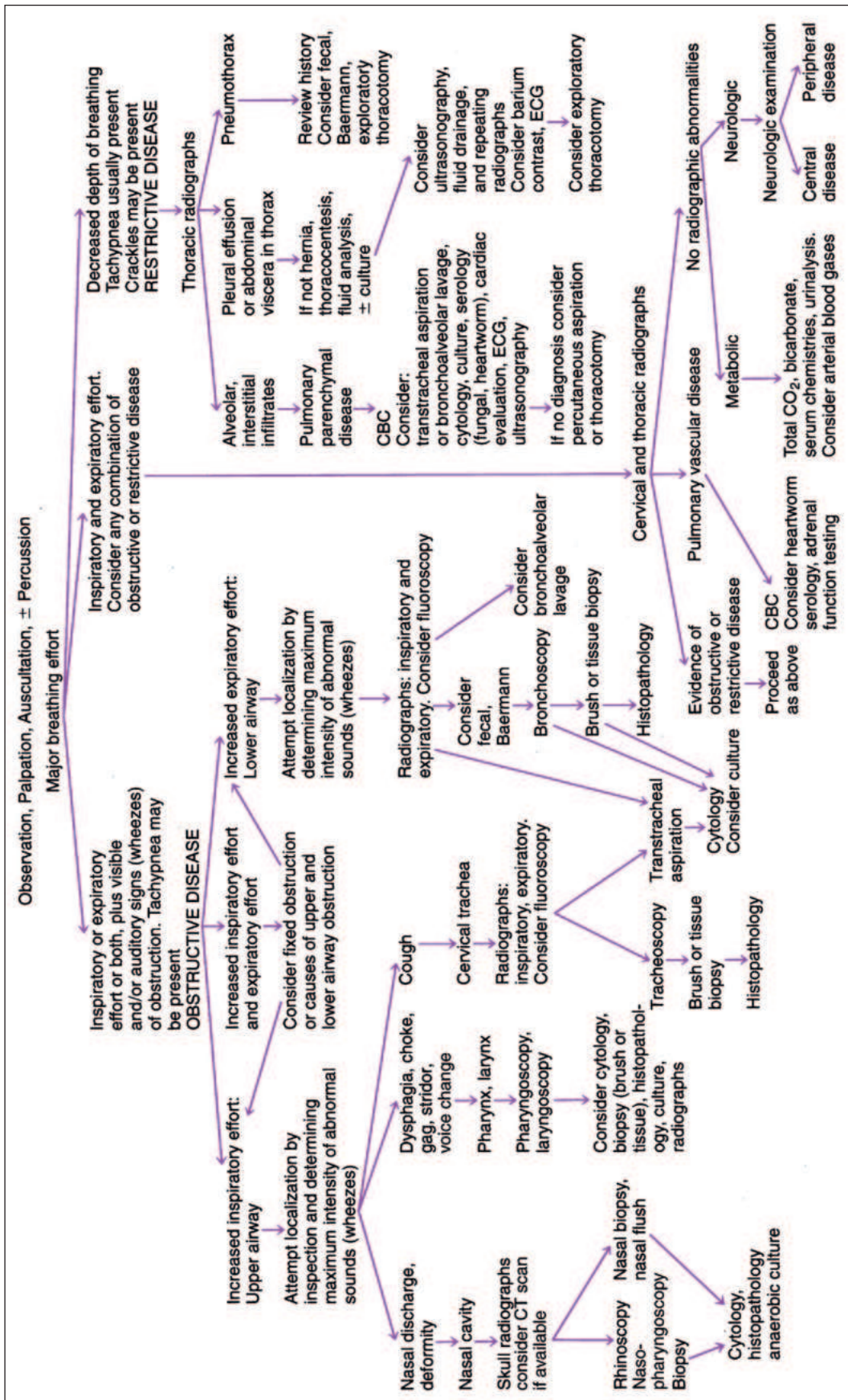


FIGURE 11-1 Approach to respiratory distress and tachypnea in dogs and cats. CBC, Complete blood count; CT, computed tomography; ECG, electrocardiogram.

definitively diagnose respiratory parasites. Serologic testing for systemic mycoses (except histoplasmosis) may be useful in dogs (see Chapter 15). Tests for detecting *Histoplasma* and *Blastomyces* antigens in the urine are now available (<http://www.miravistalabs.com>) and might be better than serology.

A complete blood count (CBC) is useful but rarely diagnostic. Allergic or parasitic disease occasionally causes eosinophilia in a dog, but this is inconsistent in cats. Dyspnea due to severe anemia may also be found. Serum chemistry profile and arterial blood gas analysis are less cost-effective in most of these patients unless the abnormal breathing pattern is nonrespiratory in origin (e.g., severe metabolic acidosis). Percutaneous fine-needle pulmonary aspiration biopsy is occasionally diagnostic in infiltrative disease (e.g., blastomycosis, histoplasmosis, carcinoma, lymphoma), especially if BAL and aspiration of more superficial structures (e.g., lymph nodes) are non-diagnostic. Guiding the needle with radiography, ultrasonography, or fluoroscopy may increase the chance of diagnosis with aspiration of mass lesions. Fine-needle aspiration carries some risk of pneumothorax or hemothorax, however.

Coughing, Including Hemoptysis

Common causes of coughing in dogs and cats are listed in Box 11-2. History is used first to eliminate self-limiting, contagious, infectious diseases. The owner should be

carefully questioned to differentiate cough from gagging, because many owners confuse gagging with coughing. Gagging (not that after a coughing stint) would suggest nasopharyngeal or nonrespiratory disorders (i.e., oropharyngeal, esophageal, or gastrointestinal disease). Differentiation of a productive cough (e.g., bronchopneumonia) from a nonproductive cough (e.g., viral tracheobronchitis) and identification of abnormal breathing patterns (see Box 11-1 and Figure 11-1) are also helpful. Tracheal and thoracic radiographs are essential in patients with chronic cough, hemoptysis, or cardiovascular disease but are less useful in acute infectious tracheobronchial diseases unless secondary pneumonia is suspected. Radiographs can be used to diagnose but not reliably rule out a collapsed trachea. Fluoroscopy may be required to rule in or out tracheal collapse and is often needed for evaluation of extent of tracheal collapse if present. TTA or BAL (especially if combined with bronchoscopy) is often diagnostic of allergic disease, parasitic infections, or bacterial infections and supports a clinical diagnosis of certain chronic bronchial diseases (e.g., chronic bronchitis). Bronchoscopy is often needed to diagnose foreign body or obstructive disease. A CBC is seldom helpful unless marked eosinophilia (e.g., allergic or parasitic disease) is present. Fecal flotation and Baermann's fecal analysis are rarely revealing but are indicated because they are easy and cost-effective. Arterial blood gas analysis is rarely diagnostic or cost-effective in coughing patients without dyspnea.

BOX 11-2. CAUSES OF COUGHING IN DOGS AND CATS

Nasal Cavity/Sinus Disease with Postnasal Drip See Box 11-3

Pharynx/Larynx

Trauma

Foreign body

Infection (bacterial or viral)

Neoplasia

Laryngeal paralysis (congenital or acquired)

Eversion of laryngeal sacculles

Laryngeal collapse

Granulomatous laryngitis

Eosinophilic granuloma

Trachea/Lower Airway

Trauma

Foreign body

Allergy (allergic bronchitis/asthma)

Infection

Viral (see Box 11-3)

Bacterial (*Bordetella bronchiseptica*)

Parasitic (*Filaroides* spp., *Oslerus osleri*, *Capillaria aerophila*)

Anomalies (collapse, hypoplasia, primary ciliary dyskinesia, segmental stenosis, extraluminal compression [left atrium, tumor])

Neoplasia (osteochondral dysplasia [osteochondroma])

Degenerative disease (bronchiectasis)

Pulmonary Parenchymal Disease

Trauma

Allergy (pulmonary infiltrates with eosinophilia)

Infection

Viral (see Box 11-3)

Bacterial

Fungal (*Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Aspergillus* spp.)

Protozoal (*Toxoplasma gondii*, *Pneumocystis carinii*)

Parasitic (*Filaroides hirthi*, *Filaroides milksi*, *Dirofilaria immitis*, *Angiostrongylus vasorum*, *Paragonimus kellicotti*)

Degenerative disease (emphysema)

Neoplasia (primary or metastatic)

Noninfectious granulomatous disorders (eosinophilic pulmonary granulomatosis, pulmonary lymphomatoid granulomatosis)

Cardiovascular Disease

Pulmonary edema

Left atrial enlargement causing bronchial compression

Thromboembolism (dirofilariasis, hyperadrenocorticism, protein-losing nephropathy, neoplasia, cardiac disease)

Mediastinal Disease (causing airway compression)

Lymphosarcoma (especially cats)

Thymoma

BOX 11-3. CAUSES OF NASAL DISCHARGE, SNEEZING, AND EPISTAXIS IN DOGS AND CATS

Structural/Functional Anomalies

Cleft palate
Oronasal fistula
Cricopharyngeal achalasia/asynchrony
Esophageal weakness

Allergic/Immunologic

Allergic rhinitis
Lymphoplasmacytic rhinitis

Bleeding Disorders

Factor deficiency (congenital and acquired)
Thrombocytopenia (infectious and immune mediated)
Vessel wall (trauma and vasculitis)
Foreign bodies/trauma

Infections

Viral: distemper, parainfluenza, adenovirus type 2 (dogs); herpesvirus, calicivirus (cats)
Bacterial: including dental disease, chronic feline rhinosinusitis, *Bordetella bronchiseptica* (dog)
Fungal: *Aspergillus* spp., *Penicillium* spp., *Cryptococcus neoformans*, *Rhinosporidium seeberi*; other opportunistic fungi are rare (e.g., *Trichosporon*)
Rickettsial: *Ehrlichia canis*, Rocky Mountain spotted fever
Parasitic: *Pneumonyssoides caninum*, *Linguatula serrata*, *Capillaria aerophila*, *Syngamus ierei*, *Cuterebra* spp.
Other: *Chlamydia* spp., *Mycoplasma* spp.

Neoplasia/Polyps

Carcinomas, sarcomas, lymphoma, transmissible venereal tumor
Polyp (nasopharyngeal in cats)
Chondromesenchymal hamartoma (cats)

Nasal Discharge, Sneezing, and Epistaxis

Nasal discharge and sneezing may be the result of primary nasal cavity disease or secondary to bronchopulmonary disease (e.g., pneumonia). Epistaxis may be the result of a primary nasal problem (e.g., neoplasia) or a systemic problem (e.g., coagulopathy). Common causes of these problems are listed in Box 11-3.

When epistaxis occurs, evaluation for coagulopathy should be the initial diagnostic step (see Chapter 5). Radiography, but preferably computed tomography (CT) or magnetic resonance imaging (MRI) of the nasal cavity, is usually performed next (Figure 11-2); however, these studies may not be diagnostic in acute disease. If a mass lesion or bone lysis is identified, biopsy via the naris is indicated. Rhinoscopy to look for foreign objects is done following diagnostic imaging studies and can be performed while a patient is anesthetized for imaging. Direct examination may reveal adult *Pneumonyssoides caninum*. Serologic testing for nasal aspergillosis can be falsely

negative and should not be relied on for a diagnosis (see Chapter 15). Direct examination of the nasal cavity (dorsal and ventral meatus) can be performed with rigid or flexible scopes (see under Rhinoscopy). The nasopharynx and posterior portion of the nasal cavity can be visualized with a dental mirror and penlight or more efficiently with a flexible endoscope. Endoscopy of the anterior portion of the nasal cavity in small dogs and cats is limited by the endoscope's diameter. Nasal lavage is rarely diagnostic. Bacterial culture is not routinely recommended; interpretation is difficult because of the large normal bacterial population of the nasal cavity (see Chapter 15). Fungal culture for aspergillosis can have both false-positive and false-negative results (see Chapter 15). Cultures for fungi are generally more reliable if performed on nasal biopsy specimens rather than nasal cavity swabs. Nasal biopsy samples can be obtained with alligator-type clamshell instruments, with uterine or colonic biopsy forceps, or bone curettes (described later). If these procedures are not diagnostic and the condition persists, exploratory rhinotomy should be considered providing nasal CT or MRI has also been performed.

NASAL RADIOGRAPHY

Common Indications • Nasal radiography is indicated for chronic nasal discharge, epistaxis, severe acute undiagnosed sneezing, facial deformity, nasal obstruction, or pawing at the face or nose (see Box 11-3).

Advantages • Nasal radiography is noninvasive. Neoplasia and aspergillosis often cause bone lysis, which is seldom present in other disorders.

Disadvantages • General anesthesia is required, and excessive fluid (e.g., nasal hemorrhage, exudate) may obscure soft tissue abnormalities. In addition, the procedure provides low resolution of fine detail (except occasionally for high-detail dental films in cats and small dogs).

Readers are referred to a radiology text for additional information.²⁰ CT and MRI are preferred imaging modalities and provide superior detail for evaluating the nasal cavity.

COMPUTED TOMOGRAPHY

Common Indications • CT has the same indications as nasal radiography. It is essential for planning radiation therapy for nasal tumors and highly recommended before topical clotrimazole therapy for nasal aspergillosis to ensure that the cribriform plate has not been eroded. MRI can be used instead of CT. It gives more soft tissue detail than CT, but CT is typically sufficient, quicker, and less expensive.

Advantages • CT requires less anesthetic time and provides much more detail than even well-positioned nasal radiographs.^{13,15} CT is excellent at determining extent of disease, finding "caverns" where aspergillosis has caused destruction and loss of turbinate structures, and

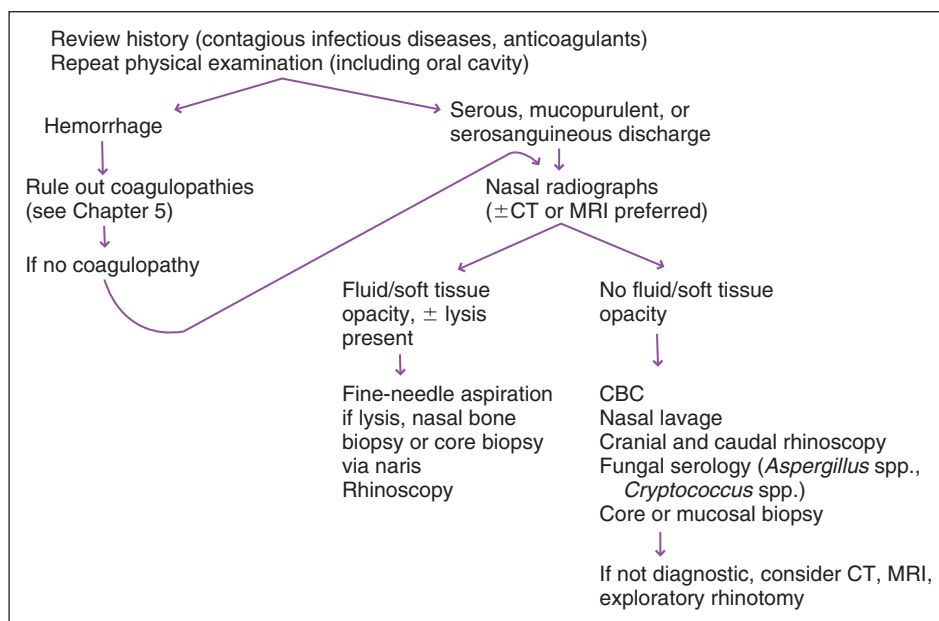


FIGURE 11-2 Approach to nasal discharge and epistaxis in dogs and cats. CBC, Complete blood count; CT, computed tomography; MRI, magnetic resonance imaging.

it is far superior at finding tumors. CT readily detects involvement of the orbital region, frontal sinuses, calvarium, and cribriform plate in both aspergillosis and tumors.

Disadvantages • The need for specialized equipment and the slightly greater cost compared with radiographs are the major drawbacks. Occasionally, contrast media are needed to distinguish between nonenhancing soft tissue density due to nasal discharge and enhancing density due to tumor or inflammation. Readers are referred to a radiology text for additional information.

RHINOSCOPY

Occasional Indications • Rhinoscopy is generally performed for the same reasons as nasal imaging.

Advantages • Rhinoscopy is relatively noninvasive, may be done after nasal imaging during the same anesthetic procedure, and may provide definitive diagnosis.⁶ It is especially useful for diagnosing nasal aspergillosis.

Disadvantages • Anesthesia is required. If an otoscope cone is used, only the rostral nares can be visualized. Even with a fiberscope, arthroscope, or cystoscope, overall visualization is limited, necessitating a careful, methodical examination that does not always allow diagnosis. Copious nasal discharge or hemorrhage will obstruct visualization of nasal structures. In small dogs, rhinoscopy is difficult unless an arthroscope is available. Care must be taken to avoid causing hemorrhage, which can obscure the field of view.

Procedure • Imaging should be performed first. Next, posterior rhinoscopy is performed by placing a flexible scope into the posterior pharynx and retroflexing it to look above the soft palate. If a flexible scope is not available, one may retract the soft palate with an ovarietomy hook and visualize the area with a dental mirror and nasopharyngeal illuminator. Anterior rhinoscopy is then performed, preferably with a rigid arthroscope or cystoscope, or a small-diameter flexible bronchoscope or ureteroscope. If these are not available, an otoscope or nasal speculum will allow some visualization of the rostral nares. Tissue or brush biopsies can be obtained for cytologic analysis, histopathologic examination, or culture.

Analysis and Interpretation • See Nasal Biopsy.

NASAL LAVAGE

Occasional Indications • Nasal lavage can be performed for the same reasons as nasal radiography.

Advantages • It is less invasive and produces fewer complications than core biopsy.

Disadvantage • It is seldom diagnostic.

Procedure • Under general anesthesia with endotracheal intubation, the nasopharynx is packed off with gauze sponges and the nasal cavity is vigorously lavaged with lactated Ringer's solution via a soft rubber tube (Rob-Nel catheter; Sherwood Medical, St. Louis, MO). The fluid is recovered in a dish placed at the nares. A foreign body may occasionally be dislodged and

recovered from the naris or gauze sponges in the nasopharynx.

Analysis • Recovered fluid is centrifuged, and the sediment is stained and examined. A Wright-type (Giemsa) or Gram stain is preferred when looking for organisms (e.g., *Cryptococcus* spp.). If lavaged material appears to be an exudate, it can be cultured for fungi. Bacterial culture is rarely useful. Direct examination of lavage fluid may reveal adult or larval *P. caninum*.

Interpretation • In allergic rhinitis, many eosinophils may be visible (see Nasal Biopsy for additional interpretation of nasal specimens).

NASAL BIOPSY

Common Indications • Indications are the same as for nasal lavage. Nasal biopsy is performed to further characterize the nature of nasal disease present, with or without radiographic evidence of masses or bone lysis. The procedure may also be performed to obtain tissue for culture if indicated.

Advantage • Tissue can be obtained for histopathologic evaluation (and culture if indicated). Diseases other than neoplasia or fungal rhinitis that may be diagnosed by this method are (1) various idiopathic inflammatory diseases,^{8,14} and (2) primary ciliary dyskinesia (i.e., immotile cilia syndrome), although the latter requires electron microscopy to be definitive.

Disadvantages • The procedure often causes bleeding, which may be profuse. Although unlikely, penetration of the cribriform plate is possible if the endoscopist is not careful.

Procedure • If the animal's coagulation status is questionable, a platelet count, mucosal bleeding time, activated clotting time, or prothrombin time (PT) and partial thromboplastin time (PTT) should be performed (see Chapter 5). Nasal biopsy is performed under general anesthesia with endotracheal intubation. The head should be positioned so that the nose points downward. The nasopharynx can be packed off as for nasal lavage. The biopsy instrument can be either small clamshell forceps for smaller patients or uterine or colonic biopsy forceps, or bone curettes, for larger patients. Direct biopsy of lesions may also be accomplished during rhinoscopy using biopsy forceps suitable for the scope. The distance from the naris to the medial canthus of the eye is marked on the biopsy forceps because this approximates the distance to the cribriform plate. The biopsy instrument is advanced with the jaws open slightly in advance of reaching the affected tissue; the jaws are then closed once the area of interest is penetrated, and the biopsy instrument with tissue is withdrawn. *Caution:* The biopsy instrument must not be advanced beyond the level of the medial canthus because of potential cribriform plate perforation.

Specimens are fixed in formalin (or other appropriate fixative if electron microscopy is desired) and

submitted. A portion of the biopsy specimen can be submitted for fungal or bacterial culture if indicated (see Chapter 15). Impression smears can be made for cytologic examination, and the remaining tissue can be submitted for histopathologic evaluation. Bleeding from the naris after biopsy is expected but usually subsides within 30 minutes. Occasionally, bleeding is profuse, prolonged, or both, in which case the affected area can be packed with cotton-tipped applicator sticks dipped in dilute (1:10,000) epinephrine solution while the animal is maintained under anesthesia until bleeding stops. Alternatively, nasal tampons (MeroCel Standard Nasal Dressing with drawstring; Medtronic Xomed, Jacksonville, FL) may be used. In extreme cases, ligation of the ipsilateral internal carotid artery can be life saving.

Interpretation of Impression Cytology

Infection

Because both healthy and diseased nasal cavity contain various bacteria (see Chapter 15), identification of bacteria is rarely helpful. *Aspergillus* spp. and *Penicillium* spp. can occasionally be recovered from nasal cavities of normal animals, as well as from patients with other disorders (e.g., neoplasia); therefore a cytologic diagnosis of aspergillosis or penicilliosis must be confirmed by direct visualization of fungal plaques or occasionally based on characteristic diagnostic imaging changes seen with CT. Finding *Cryptococcus* spp. in a cat with chronic nasal discharge is usually diagnostic (Figure 11-3); however, *Cryptococcus* spp. occasionally are cultured from nasal washings of normal dogs and cats.

Neoplasia

The most common canine nasal tumors are adenocarcinomas and carcinomas (Figure 11-4), although round cell tumors (i.e., transmissible venereal tumor, mast cell tumor, lymphosarcoma) occasionally occur. The most common feline nasal tumors are lymphosarcoma and carcinomas. Other malignant mesenchymal tissue tumors (e.g., fibrosarcoma, osteosarcoma) may occur but are less

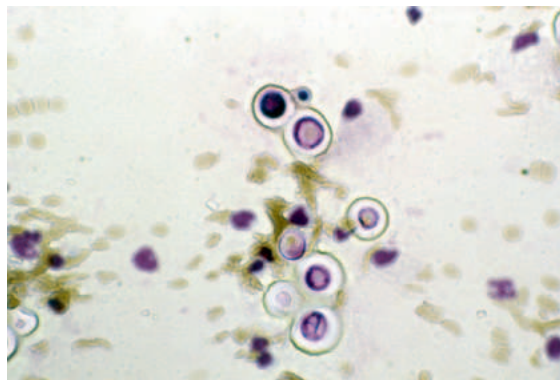


FIGURE 11-3 *Cryptococcus neoformans* from an aspiration from a nasal biopsy specimen.



FIGURE 11-4 Carcinoma cell from a fine-needle aspiration (FNA) smear of a nasal tumor. Note the huge and variably sized nucleoli (black arrows). The cell on the left has a nucleolus larger than a red blood cell (RBC) (red arrow). One cell has two nuclei, another has three nuclei.

exfoliative and more difficult to diagnose cytologically (see the discussion of cytologic evaluation of neoplastic cells in Chapter 16).

Hemorrhage

In nasal hemorrhage, proportions of erythrocytes and leukocytes are approximately equivalent to those in whole blood.

NASAL FINE-NEEDLE ASPIRATION BIOPSY

Occasional Indications • Nasal fine-needle aspiration biopsy is performed if the patient has nasal bone destruction that permits a needle to be introduced into the frontal sinuses or nasal cavity without going through the nares.

Advantages • Anesthesia is not required, and the procedure is minimally invasive.

Disadvantage • Some tumors (e.g., mesenchymal) are poorly exfoliative.

Procedure • The area of bone lysis is identified by palpation or by nasal imaging studies. A 22- or 23-gauge needle is inserted through the lytic area of bone, and aspiration is performed. Specimens are submitted for cytologic examination.

Interpretation • See Interpretation of Impression Cytology under Nasal Biopsy, earlier in chapter.

EXPLORATORY RHINOTOMY

Occasional Indications • Exploratory rhinotomy can be performed on patients with sneezing, nasal discharge, or epistaxis when the cause has not been determined by any of the previously discussed procedures.³ If available; CT should be performed before rhinotomy.

Advantages • It allows excellent visualization, biopsy, and culture of the nasal cavity, as well as identification of foreign bodies.

Disadvantage • It is an invasive, painful procedure.

Procedure • Readers are referred to a surgical text for a procedure description. Impression smears for cytologic examination are made from tissue samples. Tissue is cultured for fungi and fixed in formalin to be submitted for histopathologic evaluation.

SEROLOGY/ANTIGEN DETECTION FOR NASAL FUNGAL DISORDERS

Occasional Indications • Tests for antibodies against select fungi or their antigens is sometimes done for patients with chronic, undiagnosed nasal discharge that may be the result of aspergillosis or cryptococcosis (and rarely other fungi; see Chapter 15).

OTHER TESTS FOR PATIENTS WITH EPISTAXIS

Occasional Indications • Coagulation tests for epistaxis are important in patients with undiagnosed nasal cavity hemorrhage, especially before surgery or aggressive biopsy (see Chapter 5). Chronic unilateral nasal hemorrhage without systemic signs of hemorrhage is usually caused by primary nasal disease; coagulation tests are recommended but not mandated. Acute bilateral hemorrhage necessitates coagulation times and platelet count, however. Mucosal bleeding time is also appropriate. Severe hypertension can also cause epistaxis; therefore repeated measurement of systemic blood pressure is appropriate in these cases.

LARYNGEAL, NASOPHARYNGEAL, AND PHARYNGEAL EXAMINATION

Occasional Indications • Nasopharyngeal, laryngeal, and pharyngeal examination is important for patients with stridor or gagging suggestive of an upper airway obstructive disorder (e.g., nasopharyngeal polyp, laryngeal paralysis, tumor, nasopharyngeal stenosis) or foreign body.^{7,16}

Advantage • It permits definitive diagnosis and treatment if polyps, laryngeal paralysis, pharyngeal or laryngeal tumors, nasopharyngeal stenosis, or foreign bodies are present.

Disadvantage • It requires appropriate anesthesia.

Procedure • Under a light plane of anesthesia (i.e., the patient is spontaneously breathing), movement of the laryngeal cartilages is observed. If the patient is not breathing spontaneously or is taking very shallow breaths, one may administer doxapram in order to stimulate respiration. The corniculate processes of the arytenoid cartilages and vocal folds normally should abduct with inspiration and passively adduct with expiration. With laryngeal paralysis, these structures do not abduct with inspiration but remain in a partially adducted position or collapse (i.e., adduct) on forceful inspiration. The oropharynx is examined by pulling the tongue forward. The nasopharynx is examined as described for posterior rhinoscopy.

TRACHEAL AND THORACIC RADIOGRAPHY/FLUOROSCOPY

Common Indications • Tracheal and thoracic radiography and fluoroscopy are important as early diagnostics for patients with chronic or severe cough or other bronchopulmonary disease.

Advantages • These procedures are noninvasive and often localize problems.

Disadvantage • They are rarely of value in acute inflammatory disorders (e.g., viral tracheobronchitis) or thromboembolism not caused by dirofilariasis.

Procedure and Interpretation • The reader is referred to a radiology text for additional information. Evaluation of both right and left lateral views improves visualization of pulmonary masses.

TRANSTRACHEAL/TRACHEAL ASPIRATION

Common Indications • Indications for tracheal aspiration (TA) and transtracheal aspiration (TTA) are generally the same as for thoracic radiographs.

Advantages • TTA is relatively noninvasive, yet samples the tracheobronchial tree without anesthesia. TA is relatively noninvasive but does require approximately 2 to 3 minutes of anesthesia. Therefore TA is more commonly performed in cats and smaller dogs in which it can be hard to safely penetrate the trachea with a needle.

Disadvantages • Complications are uncommon with TTA and can include subcutaneous (SC) emphysema originating at the site of needle penetration, esophageal perforation, hemorrhage, and lower airway catheter stylet trauma. Samples may not accurately reflect lower airway or lung disease (e.g., bronchiolitis, interstitial pneumonia). TTA is contraindicated in patients with severe respiratory distress because restraint can worsen the distress and cause death. Severe coagulopathy is a relative contraindication. Possible complications with TA include

making respiratory distress worse and anesthesia-associated problems.

Procedure • Dogs often tolerate TTA without sedation, but tranquilizers (e.g., acepromazine) can be used. For cats, sedation (e.g., 1 to 2 mg ketamine/kg intravenously [IV]) is routine; oxygen should be available if respiratory distress occurs. The patient is restrained in sternal recumbency. After clipping and surgical preparation of the skin over the larynx, a bleb of lidocaine is injected over the cricothyroid membrane. A through-the-needle type of catheter (Intracath; Deseret Medical Inc., Sandy, UT) (20 gauge for cats and small dogs, 16 gauge for medium-sized and large dogs) is inserted through the cricothyroid membrane and advanced to approximately the level of the main stem bronchi. Alternatively, a sterile 3.5-French polypropylene urinary catheter can be inserted through a 14-gauge needle in large dogs. In anesthetized animals, the catheter can be inserted through a sterile endotracheal tube (recommended for cats if anesthesia is tolerated).

Depending on the animal's size, 0.5 to 1.0 ml/kg of sterile lactated Ringer's solution (0.9% saline is acceptable but causes more cellular distortion) is injected into the catheter. After the animal coughs, aspiration is performed. Chest coupage after fluid instillation may improve recovery of debris from the airways. Usually only a small amount of injected material is recovered. If a low-pressure (i.e., <5 mm Hg) suction pump is available, yield is higher. Using the suction pump method, material can be aspirated into a suction trap (Dee Lee suction catheter; American Hospital Supply, McGraw Park, IL). Multiple aliquots of lactated Ringer's solution can be injected until a sample is obtained. Aliquots of aspirate may be submitted for cytologic analysis and aerobic, anaerobic, or fungal culture. The use of "roll preparations" of the cellular pellet of spun samples is advised because cellular disruption often results if unspun fluid samples cannot reach the laboratory within 1 hour of collection. To make a roll preparation, the wooden end of a cotton-tipped applicator stick is used to gently roll cells from the pellet onto clean glass slides. The decision to culture is based on cytologic findings (see the following text under Interpretation).

For TA, the patient is anesthetized (e.g., propofol) and intubated. A urinary catheter is passed through the endotracheal tube to the level of the intrathoracic trachea or bifurcation, and a wash is performed as described for TTA.

Interpretation • Occasional ciliated columnar or cuboidal epithelial cells, occasional undifferentiated macrophages with few or no vacuoles, rare neutrophils, and small amounts of mucus are normal. Some normal epithelial cells may appear smudged or lack cilia because of trauma during sample preparation. TTA aspirates from animals with bronchopulmonary disease may be classified as mucopurulent inflammation, nonpurulent inflammation, neoplastic, or hemorrhagic (see Chapter 16).

Mucopurulent Inflammation • This aspirate is a mixture of neutrophils (see the discussion of neutrophilic inflammation in Chapter 16) and abundant mucus, often plus a few macrophages (Figure 11-5). In mucopurulent inflammation, the mucoid material may stain basophilic

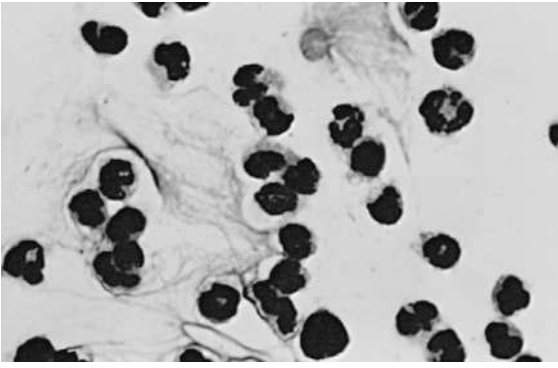


FIGURE 11-5 Mucopurulent inflammation from a transtracheal aspirate of a dog with chronic obstructive pulmonary disease associated with collapsed trachea. Note the large numbers of nondegenerate neutrophils and abundant mucus.

(blue) or become eosinophilic (pink) as the inflammation becomes more severe. A thorough search for bacteria (especially intracellular) should be made if degenerate neutrophils are seen (Figure 11-6). Causes of mucopurulent inflammation include bacterial, fungal, viral, mycoplasmal, and protozoal infection, as well as chronic bronchitis, tumors, foreign bodies, and aspiration. Occasionally, bacteria (e.g., *Bordetella bronchiseptica*) can be found attached to ciliated columnar epithelial cells (Figure 11-7).

A portion of the TTA sample should be placed in a culture transport medium (see Chapter 15) before cytologic evaluation. If mucopurulent inflammation is found, this portion should be submitted for culture (see Chapter 15).

Nonpurulent Inflammation • This aspirate includes a higher percentage of macrophages (see the discussion of granulomatous and pyogranulomatous inflammation in

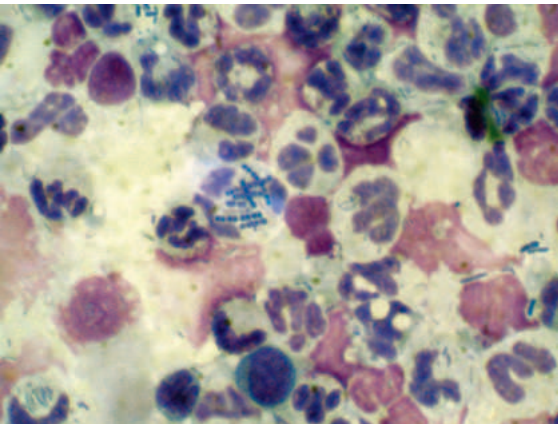


FIGURE 11-6 Septic inflammation in a transtracheal aspiration (TTA) specimen from a dog with bacterial pneumonia. Note the large number of degenerate neutrophils, some containing intracellular bacteria.

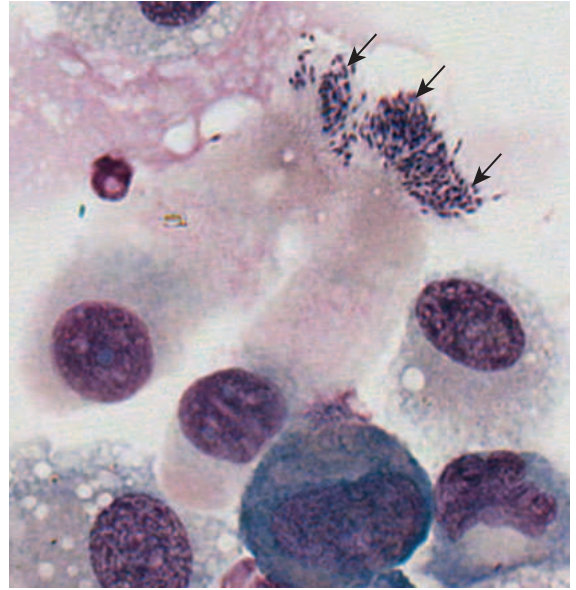


FIGURE 11-7 Ciliated columnar epithelial cell from a tracheal washing. There are numerous rod-shaped bacteria (i.e., *Bordetella bronchiseptica*) attached to the apical end of these cells (arrows).

Chapter 16), eosinophils (see the discussion of eosinophilic inflammation in Chapter 16), or both than is found in mucopurulent exudates. Nonpurulent exudates may contain a predominance of eosinophils or macrophages or a mixture of eosinophils, macrophages, and neutrophils. Eosinophilic inflammation (Figure 11-8) suggests hypersensitivity caused by inhaled allergens, parasites, or eosinophilic pulmonary granulomatosis. Parasitic causes include *D. immitis*, *Capillaria aerophila* (Figure 11-9), *Paragonimus kellicotti* (Figure 11-10), *Aelurostrongylus abstrusus* (Figure 11-11), *Oslerus osleri*, *Filaroides* spp., or migrating parasites such as *Toxocara* spp. or *Ancylostoma* spp. Small numbers of mast cells are common

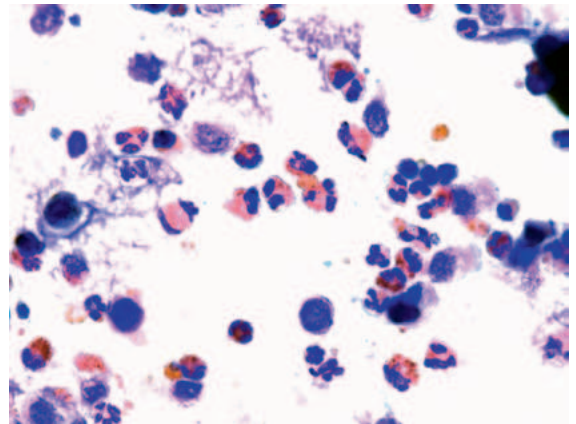


FIGURE 11-8 Eosinophilic inflammation in a transtracheal aspiration (TTA) specimen from a dog.

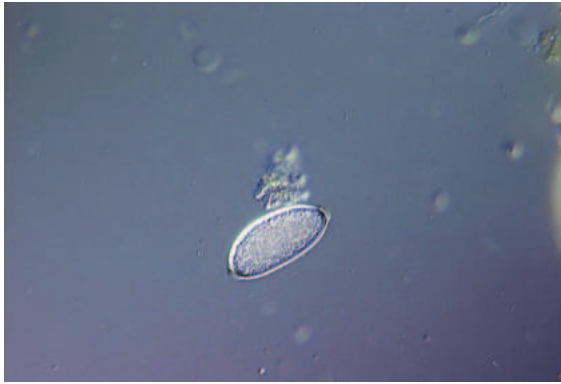


FIGURE 11-9 *Capillaria aerophilia* egg in a fecal specimen from a dog with chronic cough. (Courtesy of Dr. Tom Craig, Texas A&M University.)

in eosinophilic inflammation. A predominance of differentiated macrophages (i.e., larger macrophages with abundant cytoplasm and numerous cytoplasmic vacuoles) suggests subacute to chronic disease, such as granulomatous pneumonia caused by fungi or lipid. Fungal organisms are rarely recovered because they tend to be interstitial instead of in the airways.

Other Cells in Transtracheal Aspirates • Reactive epithelial cells may be found with any inflammatory process, especially in cats. The cytoplasm is more basophilic (blue) than normal epithelial cells, and nuclei have fine chromatin and visible nucleoli. Cells may be single or clustered.

Goblet cells are occasionally visible in inflammatory disease. They contain granules of intracellular mucus and often occur in conjunction with abundant extracellular mucus.

Neoplastic cells are occasionally recovered from animals with primary lung tumors, particularly adenocarcinomas. Primary lung tumors, however, are much less

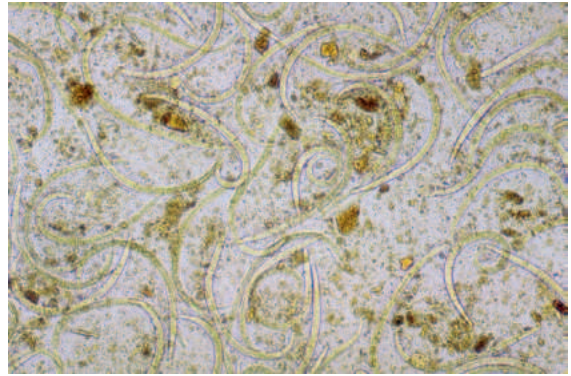


FIGURE 11-11 *Aelurostrongylus abstrusus* larvae from a Baermann's fecal examination. (Courtesy of Dr. Tom Craig, Texas A&M University.)

common than metastatic tumors. Because of their interstitial location, cells from metastatic pulmonary tumors are rare in TTA specimens.

Aspirated material from the oral cavity is suggested by squamous epithelial cells (which may be coated with bacteria) or certain large bacteria such as *Simonsiella* spp. (Figure 11-12).

Lymphocytes may be visible in acute viral tracheobronchitis, and lymphocytes plus plasma cells may be visible in chronic, progressive, septic bronchopneumonitis; sterile bronchopneumonitis; or pulmonary lymphoid granulomatosis.

Ova of *C. aerophilia* (see Figure 11-9) *P. kellicotti* (see Figure 11-10) and *Filaroides hirthi* occasionally are found, as are larvae of *O. osleri*, *Crenosoma vulpis*, *A. abstrusus* (Figure 11-11), *Toxocara canis*, *T. cati*, and *Strongyloides stercoralis*, as well as microfilariae of *D. immitis*.

Anthraxotic pigment appears as dense black granules within macrophages and is an incidental finding in dogs in industrial areas.



FIGURE 11-10 *Paragonimus kellicotti* egg in a fecal specimen from a cat with chronic cough. (Courtesy of Dr. Tom Craig, Texas A&M University.)

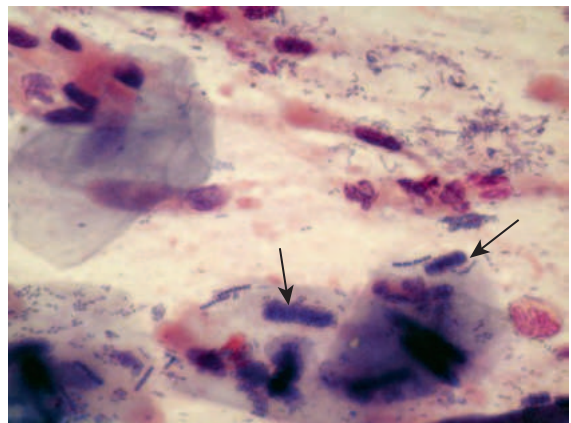


FIGURE 11-12 *Simonsiella* spp. (arrows) and other bacteria in a transtracheal aspiration (TTA) specimen from a dog.

BRONCHOALVEOLAR LAVAGE

Common Indications • Indications for BAL are generally the same as for TTA.

Procedure • BAL is an invasive technique, requires general anesthesia, and causes temporary respiratory compromise. Although not technically difficult, practice is required to acquire confidence and skill in performing BAL. The procedure can be performed through an endotracheal tube; however, endoscopic BAL is recommended because of improved site selectivity for collection of specimens and enhanced retrieval of lavage fluid.

Animals undergoing BAL should receive atropine as a preanesthetic and be anesthetized with ketamine/acepromazine or ketamine/diazepam IV, with isoflurane tank induction (cats), or with other IV short-acting (e.g., propofol) anesthetic agents (dogs). Additional anesthetic can be given IV if needed to maintain anesthesia. BAL may be performed directly through a sterile endotracheal tube (4 mm internal diameter) in cats and very small dogs. The endotracheal tube is placed rostral to the carina, and the cuff is inflated. Oxygen (100% O₂) is administered for 1 to 2 minutes before lavage. After preoxygenation, the patient is placed in lateral recumbency with the most affected side down and a syringe is attached to the endotracheal tube. Three aliquots (5 ml/kg) of warm, sterile lactated Ringer's solution (0.9% saline is acceptable but causes more cellular distortion) are gently infused into the lung and immediately retrieved with gentle suction. Elevating the patient's hindquarters improves retrieval of BAL fluid. Each aliquot is kept in a separate syringe for analysis. After the procedure, O₂ (100%) is administered continuously until the animal is fully awake and breathing is no longer suppressed.

If an endoscope is used, an endotracheal tube is not necessary. Rather, the endoscope can be passed into the trachea while O₂ is delivered through the endoscopic biopsy channel (be careful to avoid barotrauma) or through an adapter adjacent to the endoscope. In larger dogs, the endoscope can be passed through an endotracheal tube and O₂ and inhalant anesthesia administered around the endoscope with a T-adapter. The distal end of the endoscope is "wedged" in a main stem bronchus or airway of interest. Three aliquots (i.e., 2 ml/kg) of warmed, sterile lactated Ringer's solution are flushed through the endoscopic biopsy channel and immediately retrieved as previously described. Pre- and post-BAL oxygenation is performed as previously described.

BAL fluid often appears foamy because of pulmonary surfactant recovered by the BAL procedure. Unspun BAL specimens should be transferred immediately to the laboratory for analysis. If rapid transport of BAL fluid to the laboratory is impossible, cytologic specimens should be prepared immediately by cytocentrifugation or roll preparations (Cytocentrifuge II; Shandon Southern Instruments, Sewickley, PA). Roll preparations are made from the cell pellet resulting from centrifugation. The wooden end of a cotton-tipped applicator stick is used to gently roll cells from the pellet onto clean glass slides. These consistently produce excellent cytologic specimens.

The cellular character of BAL preparations is vastly different from TTA specimens. The predominant cell type

is the alveolar macrophage; however, large numbers of eosinophils (up to 30%) can be present in clinically normal cats. Other cell types and infectious organisms (e.g., yeast, fungal hyphae, bacteria) may be present, depending on the patient's disease. Interpretation is similar to that described for TTA.

Because of inevitable oral contamination of instruments used for BAL procedures, BAL specimens are not useful for accurate bacteriologic culture (unless sterilization of the scope and associated equipment is feasible). Specimens for culture are ideally collected by TTA or with a guarded swab.

TRACHEOBRONCHOSCOPY

Occasional Indications • Tracheobronchoscopy is primarily performed in patients with persistent undiagnosed coughing, hemoptysis, or a suspected obstructive lesion; whenever direct visualization of larger airways is required to look for obstruction or collapse; or to selectively sample an area of the tracheobronchial tree. It is the procedure of choice to diagnose collapsed trachea if radiography or fluoroscopy has not established the diagnosis. Flexible bronchoscopes are preferred.

Advantages • It provides direct visualization of major airways and allows biopsy of specific sites. This is the technique of choice to diagnose *O. osleri* infection. Cytologic specimens obtained by brush biopsy are usually superior to those obtained by TTA. Pulmonary biopsy can be performed via a transbronchial biopsy.

Disadvantages • It requires general anesthesia; pulmonary biopsy has the potential risk of tracheal rupture (especially in cats), pneumomediastinum, pneumothorax, and pulmonary hemorrhage. If oxygen is being insufflated through the biopsy channel, care must be taken to avoid barotrauma.

Procedure • The endoscope is passed either through an endotracheal tube (large dog) or directly into the trachea (small dog or cat). A thorough systematic examination is made of all accessible parts of the tracheobronchial tree.

Interpretation • In collapse of the trachea, main stem bronchus, or bronchi, severity of the lesion is assessed. Other lesions are visualized and may be sampled by either brush or endoscopic forceps. Specimens obtained may be submitted for bacteriologic culture or for cytologic and histopathologic examination.

FECAL EXAMINATION

Occasional Indications • Fecal examination can be helpful for patients with undiagnosed coughing or dyspnea, particularly if unexplained eosinophilia is present or if radiographic signs suggest pulmonary parasitism.

Advantage • It is noninvasive.

Disadvantage • Ova of some pulmonary parasites (e.g., *F. hirshi*) are not reliably recovered.

Procedure • Fecal flotation is performed using zinc sulfate solution or other flotation media (see Chapter 9).

Interpretation • Ova of *C. aerophilia* (see Figure 11-9), *F. hirshi*, *Eucoleus boehmi*, and *P. kellicotti* (see Figure 11-10) may be detected.

BAERMANN'S FUNNEL APPARATUS

Occasional Indications • A Baermann's funnel apparatus can be used for the same reasons as fecal flotation for respiratory parasites.

Advantage • It is noninvasive.

Disadvantages • It is inconvenient and cumbersome; some parasites (e.g., *O. osleri*) shed larvae erratically.

Procedure • Fresh feces are placed on cheesecloth in a strainer and then in the Baermann's apparatus (a funnel with a clamped rubber tube at the stem end). Water is added to the funnel to cover the feces, which are then broken up into small pieces. After a few hours, a small aliquot of water is drained through the rubber tube and examined microscopically for larvae.

Interpretation • Larvae of *Filaroides milksi*, *F. hirshi*, *O. osleri*, *C. vulpis*, *A. abstrusus* (see Figure 11-11) and *S. stercoralis* may be identified, but a negative test result does not eliminate these parasites. The method of choice to diagnose *O. osleri* infection is bronchoscopy.

PULMONARY ASPIRATION BIOPSY

Occasional Indications • Pulmonary aspiration biopsy is primarily used in patients with masses or diffuse infiltrative disease, and to procure material for culture.

Advantage • It samples pulmonary parenchyma without thoracotomy.

Disadvantages • Possible complications include pneumothorax, pulmonary hemorrhage, hemoptysis, and (if the myocardium is inadvertently penetrated) cardiac arrhythmias, any of which may rarely cause death.

Contraindications • It is contraindicated in uncooperative patients and those with thrombocytopenia, bleeding disorders, severe uncontrolled coughing, and pulmonary bullae or cysts.

Procedure • Coagulation status should be screened (see Chapter 5) with platelet count and mucosal bleeding time. For diffuse pulmonary disease, the recommended aspiration site is between the seventh and ninth intercostal spaces, two thirds of the distance from the costochondral junction to the vertebral bodies. A 25-gauge hypodermic or spinal needle (Spinal needle;

Becton-Dickinson, Rutherford, NJ) with stylet removed and a 12-ml syringe are used. After clipping and surgical preparation of the skin, the needle is attached to the syringe and inserted into the pulmonary parenchyma, and aspiration is performed. The procedure should be done quickly, actual aspiration not taking more than a few seconds. Very little material is usually aspirated, and what is present typically remains in the needle hub. The needle should be removed from the syringe, air introduced into the syringe, the needle reattached, and the aspirated material quickly "blown" onto a clean glass slide. Another clean slide is used to prepare a horizontal "pull-apart" specimen for cytologic examination. Pulmonary aspiration should be performed early in the day so that the patient can be monitored for dyspnea caused by pneumothorax, pulmonary hemorrhage, or hemoptysis. Ultrasonography is generally not useful for evaluating the pulmonary parenchyma. If a mass lesion is present, however, ultrasonographic or fluoroscopic guidance may help in needle placement.

Larger lung tissue specimens for histopathologic examination can be obtained with a similar technique using a modified Menghini's aspiration biopsy needle (Modified Menghini Biopsy needle; Becton-Dickinson) or Bard Monopty biopsy needle (C.R. Bard, Inc., Murray Hill, NJ).¹ Complications are similar to those for pulmonary aspiration biopsy.

Interpretation • Fine-needle aspiration biopsy specimens are examined cytologically and classified as inflammatory, neoplastic, or hemorrhagic (see Chapter 16). Larger specimens are submitted for histopathologic examination.

Inflammatory • Neutrophils, monocytes, or eosinophils predominate. Abundant erythrocytes are common. In fungal pneumonia, especially if caused by blastomycosis (Figure 11-13) or histoplasmosis (see Figure 9-3A), free or engulfed yeasts may be visible. The number of eosinophils can be increased in aspirates from animals with pulmonary infiltrates with eosinophilia, as well as other hypersensitivities and parasitism.

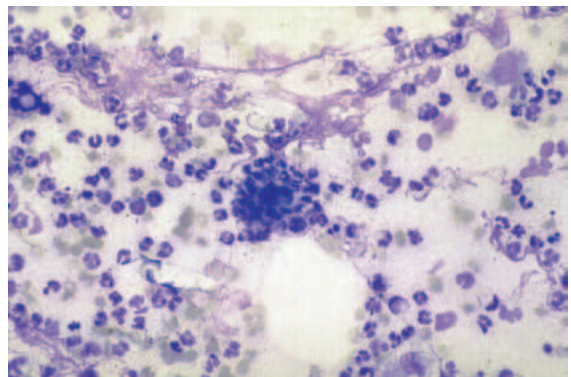


FIGURE 11-13 *Blastomyces dermatitidis* in a fine-needle pulmonary aspiration specimen from a dog with weight loss and respiratory distress.

Neoplastic • Neoplastic cells occasionally are visible. Malignant epithelial cells tend to appear in clusters (e.g., rafts), and inflammatory cells may be present.

Hemorrhagic • Hemorrhage frequently occurs and is usually iatrogenic.

Parasitic • Adult *F. hirshi* organisms are rarely recovered.

SEROLOGY/ANTIGEN TESTS FOR PULMONARY DISEASES

In histoplasmosis, blastomycosis, cryptococcosis, and coccidioidomycosis, finding organisms (yeast forms) is diagnostic. Pulmonary disease caused by these organisms is usually interstitial, and TTA rarely demonstrates the organism. Serology for antibodies or antigen tests (i.e., coccidioidomycosis, blastomycosis, cryptococcosis, histoplasmosis) may establish a tentative diagnosis when TTA, BAL, and pulmonary aspiration biopsy do not (see characteristics of individual tests in Chapter 15). Serologic testing for *Toxoplasma gondii* is rarely indicated in patients with pulmonary disease.

AMINO-TERMINAL PRO-B-TYPE NATRIURETIC PEPTIDE (NT-proBNP)

Indications • Current indications are based on available data, but this is an evolving area and indications are likely to change significantly in the near future. This test should be used in conjunction with other appropriate tests which it does not replace; however, NT-proBNP may improve both accuracy and confidence when used appropriately. Evaluation of NT-proBNP may be useful in screening for cardiac disease in selected asymptomatic populations of cats and dogs, including Dobermans, boxers, cats with murmurs or gallops or arrhythmias, and purebred cats with a high risk of cardiomyopathy. Evaluation may help differentiate if clinical signs suggestive of heart failure (e.g., dyspnea, tachypnea, orthopnea, exercise intolerance, weakness, collapse/syncope) are related to heart disease versus other noncardiac etiologies in both cats and dogs. More recently, data suggest that trends in asymptomatic dogs with chronic valve disease may help identify dogs at increased risk of developing heart failure.

Advantages • NT-proBNP is a simple blood test, and relatively inexpensive.

Analysis • Enzyme-linked immunosorbent assay (ELISA) testing is done on EDTA plasma that is submitted in a protease tube supplied by the laboratory (IDEXX). The blood sample must be spun and plasma put in the protease tube within 1 hour. Once in the protease tube, the sample is stable.

Normal Values • Based on current available data, normal values for cats are typically less than 50 pmol/L and for dogs are less than 800 pmol/L. However, this is an

evolving area of research and current interpretive criteria provided by the diagnostic laboratory (IDEXX) should be used when interpreting test results in individual patients.

Danger Values • Currently values greater than 1800 pmol/L in asymptomatic dogs (270 pmol/L in cats) with known acquired heart disease are considered indicative of risk for developing heart failure. In dogs and cats with clinical signs suggestive of heart failure, levels greater than 1800 pmol/L and 270 pmol/L, respectively, support that current clinical signs are related to heart disease and should be considered an indication for additional cardiac testing (e.g., thoracic radiographs, echocardiogram).

Artifacts • False positives are possible; they are more common if inappropriate populations are tested (e.g., all dogs).

Drugs That May Alter Results • Appropriate heart failure therapy can result in a reduction in NT-proBNP level, but it will not normalize.

Causes of Decreased Serum NT-proBNP • Unknown.

Causes of Increased Serum NT-proBNP • Occult and symptomatic acquired and congenital heart disease, pulmonary hypertension, and severe arrhythmias can cause increased NT-proBNP; false positives can occur.

CARDIAC TROPONIN I (cTnI)

Indications • Cardiac Troponin I cTnI is a marker of significant myocyte injury and has been demonstrated to be elevated in veterinary patients with both primary cardiac disease and systemic diseases known to cause cardiac damage. Thus it may be useful to identify patients at increased risk of significant cardiac damage, and elevations should be cause for concern and additional (more specific) diagnostic testing.

Advantages • It is a simple blood test, relatively inexpensive, and readily available at many laboratories.

Analysis • Typically analysis utilizes an immunoassay and is performed on serum. However, it is best to check with the laboratory to which the sample is being submitted, since there could be some variation in sample submission. In addition, the assay must be validated at each lab and reference ranges established for both cats and dogs.

Normal Values • In general, values above 0.2 ng/ml are associated with reduced survival time in dogs and cats. However, specific reference ranges from the laboratory that runs the assay should be used.

Danger Values • In general, higher values are considered to reflect more severe cardiac damage. Elevations in cTnI are greater with ischemia (approximately 10-fold increase) versus myocarditis (5-fold increase) and may be lower in other acquired cardiac diseases.

Artifacts • An elevated cTnI is suggestive of myocardial damage but does not diagnose a specific cause. Elevated cTnI should be considered an indication for additional cardiac diagnostic tests. A negative test does not rule out all cardiac diseases.

Drugs That May Alter Results • Appropriate cardiac therapy may result in reductions in cTnI.

Causes of Decreased cTnI • None recognized.

Causes of Increased cTnI • Dogs: gastric dilation and volvulus, pyometra, cardiac contusion, babesiosis, acquired and congenital heart disease, arrhythmogenic right ventricular cardiomyopathy in the boxer, infarction, cardiac and noncardiac dyspnea, pericardial effusion, age.

Cats: hyperthyroidism, cardiomyopathy.

ARTERIAL BLOOD GASES

Alveolar ventilation refers to ability of inspired air to enter and leave alveoli. *Ventilatory failure* refers to inadequate airflow into and out of alveoli and results in an inability to maintain carbon dioxide (CO_2) homeostasis in the body. *Respiratory failure* refers to failure of ventilation, perfusion, or diffusion.

Rare Indications • Measurement of arterial blood gases can be done in patients with airway or pulmonary parenchymal disease causing respiratory insufficiency or ventilatory failure of any cause.

Advantages • It quantitates the degree of respiratory impairment, evaluates compensatory changes, and monitors response to treatment.

Disadvantages • It does not allow the clinician to determine focal versus disseminated respiratory disease, to diagnose, or to prognosticate. In addition, it requires arterial puncture and prompt submission of the specimen.

Sample Procurement • See Chapter 6.

Analysis • An arterial blood sample is necessary for evaluation of the respiratory system. Use of venous blood gas analysis for determination of acid-base status is discussed in Chapter 6; venous O_2 tension (PvO_2) is discussed in the next section.

Normal Arterial Values • See Table 6-1.

Danger Values • Arterial partial pressure of oxygen (PaO_2) less than 60 mm Hg; arterial partial pressure of carbon dioxide (PaCO_2) greater than 70 mm Hg.

NOTE: Danger values depend on duration of the problem (chronicity allows compensatory mechanisms, enabling tolerance of greater abnormalities).

Artifacts • Improper sample storage before analysis can decrease the PaO_2 , while failing to remove air bubbles can increase the PaO_2 (see Chapter 6).

Drug Treatment That May Alter Values • Excessive heparin decreases both pH and PaO_2 , whereas citrate, oxalate, or ethylenediaminetetraacetic acid (EDTA) may decrease pH (see Chapter 6). Any drugs altering control of respiratory drive (e.g., anesthetic agents) may alter PaO_2 .

Partial Pressure of Oxygen

Although measurement of PaO_2 helps evaluate the degree of respiratory dysfunction, it is only one factor affecting O_2 delivery to tissues. Other parameters, such as cardiac output, blood pressure, regional blood flow, position of the hemoglobin dissociation curve, and hemoglobin concentration, are also important (Box 11-4). PaO_2 in atmospheric air at sea level may range between 149 and 159 mm Hg (19.7% to 20.8%), depending on humidity. The corresponding PaO_2 in alveolar air (104 mm Hg or 13.6%) is lower because of increased PaCO_2 and H_2O respiratory gases.

Causes of Hypoxemia • Causes include inadequate oxygen in inspired air, alveolar hypoventilation, impaired diffusion, ventilation/perfusion mismatching, and vascular shunting (see Box 11-4). Characterization and evaluation of hypoxemia and accompanying PCO_2 and HCO_3^- changes are discussed later under Diagnostic Evaluation of Blood Gases.

BOX 11-4. CAUSES OF HYPOXEMIA IN DOGS AND CATS

Inadequate Oxygen in Inspired Air

High altitude

Failure of oxygen source during anesthesia

Alveolar Hypoventilation (associated with increased PaCO_2)

Depression/arrest of respiratory center

Failure of muscles of respiration

Third-space disease (pneumothorax, pleural effusion, flail chest, diaphragmatic hernia)

Airway obstruction

Impaired Diffusion

Interstitial pulmonary disease (pneumonia, edema, neoplasia, embolism)

Airway disease

Vascular Shunting

Right-to-left cardiac shunts (tetralogy of Fallot, right-to-left shunting from patent ductus arteriosus or ventricular or atrial septal defect)

Intrapulmonary arteriovenous shunts

TABLE 11-1. ASSESSMENT OF VENTILATION BASED ON BLOOD GAS ANALYSIS

Decreased PaCO_2 (Hyperventilation):			
pH ↓↓↓ HCO_3^- ↓ Partially compensated metabolic acidosis	pH normal HCO_3^- ↓ Compensated metabolic acidosis	pH ↑ HCO_3^- ↓ Chronic hyperventilation (partially compensated respiratory alkalosis)	pH ↑↑ HCO_3^- normal Acute hyperventilation (uncompensated respiratory alkalosis)
Normal PaCO_2 :			
pH ↓↓↓ HCO_3^- ↓ Uncompensated metabolic alkalosis		pH ↑↑↑ HCO_3^- ↑ Uncompensated metabolic acidosis	
Increased PaCO_2 (Hypoventilation):			
pH ↓↓↓ HCO_3^- normal Acute ventilatory failure (uncompensated respiratory acidosis)	pH normal HCO_3^- ↑ Chronic ventilatory failure (compensated respiratory acidosis)	pH ↑ HCO_3^- ↑ Partially compensated metabolic alkalosis	

Explanation of pH (dogs): pH normal: 7.36 to 7.44; pH ↑: 7.45 to 7.50; pH ↑↑: >7.50; pH ↓: 7.30 to 7.35; pH ↓↓: <7.30. No attempt is made to quantify HCO_3^- changes.

Partial Pressure of Carbon Dioxide

PaCO_2 in alveolar air at sea level is 40 mm Hg (5.3%), whereas that of atmospheric air is only 0.3 mm Hg (0.3%). An increase or decrease in PaCO_2 is caused by a decrease or increase in ventilation, respectively (Table 11-1). Because increased PaCO_2 decreases pH, the condition is also known as respiratory acidosis. The reverse occurs with a decrease in PaCO_2 (i.e., respiratory alkalosis).

Causes of Hypercapnia (Hypercarbia) • See Chapter 6.

Causes of Hypocapnia (Hypocarbica) • See Chapter 6.

Compensatory Responses to Alterations in Blood Gases • Decreased PaO_2 is associated with either increased or decreased PaCO_2 . In dogs, predictable changes in HCO_3^- are associated with alterations in PaCO_2 , and similar changes may occur in cats (see Table 6-2).

Diagnostic Evaluation of Blood Gases

First, the clinician should decide if the abnormality is significant. Minor elevations in PaO_2 are not an indication for additional evaluation other than characterization of the underlying disorder. In patients breathing an enriched O_2 mixture, a PaO_2 less than approximately five times the inspired O_2 concentration is an indication for additional diagnostics. If the change is significant, one should first characterize the abnormality as a primary or secondary, compensated or uncompensated disorder and then seek to determine the underlying cause (see Table 11-1). A three-step process is recommended to characterize the

abnormality. A brief overview of the three steps is given next. For additional information, readers are referred to DiBartola.^{5,11}

Step 1: Evaluation of the Ventilatory Status

The clinician should evaluate PaCO_2 . Ventilation is classified as acceptable (i.e., normal PaCO_2), hyperventilation (i.e., decreased PaCO_2), or hypoventilation (i.e., increased PaCO_2). From Table 11-1, the abnormality can be assessed as respiratory or metabolic in origin. For additional information, see Boxes 6-8 and 6-10 and the discussions of respiratory acidosis and respiratory alkalosis in Chapter 6.

Step 2: Assessment of the Hypoxemic State

Decreased PaO_2 confirms arterial hypoxemia and suggests tissue hypoxia (see Box 11-4).

Step 3: Assessment of the Tissue Oxygen State

Normal tissue oxygenation requires perfusion by adequately oxygenated blood. Therefore this step involves assessment of cardiac status, peripheral perfusion, and blood O_2 transport (see later).

PARTIAL PRESSURE OF OXYGEN IN VENOUS BLOOD

Occasional Indications • Measurement of PvO_2 helps evaluate adequacy of O_2 delivery to tissues and monitor cardiac output.

Analysis and Interpretation • If pulmonary edema is present, PaO_2 should be measured. If PaO_2 is greater than 65 mm Hg, partial pressure of oxygen in venous blood (PvO_2) will reflect cardiac output. The sample should be taken from the jugular vein. Occlusion of the vein for longer than 5 to 10 seconds artifactually decreases PvO_2 . Normal PvO_2 is greater than 40 mm Hg. Precautions for obtaining and storing the blood sample before analysis are the same as for arterial blood gases.

If PvO_2 is less than 30 mm Hg, O_2 delivery to the tissues is inadequate. Cardiac output, O_2 saturation, hemoglobin concentration, and peripheral arteriovenous shunts should be considered as causes of low PvO_2 and evaluated as described previously. In patients with cardiac disease, a PvO_2 cannot be correlated to a specific cardiac output, but increases or decreases in PvO_2 reflect improving or worsening cardiac output (respectively) relative to the initial value.

PULSE OXIMETRY

Occasional Indications • Pulse oximetry evaluates pulse rate and hemoglobin saturation in arterial blood (SaO_2). Measurement of SaO_2 is nearly as informative as PaO_2 , because each is a measure of ability of the lungs to deliver oxygen to the blood. Pulse oximetry is valuable as an ongoing monitor to detect hypoxemia, especially during anesthesia.

Procedure • A pulse oximeter electrode is attached to the patient (e.g., tongue, lips, ear). Arterial blood gas measurements can be made to verify accuracy of the pulse oximeter.

Interpretation • Reasonable pulmonary and cardiovascular functions are required to achieve accurate measurements of pulse rate and hemoglobin saturation. Peripheral vasoconstriction may result in a poor reading of pulse rate but has value in identifying hypoxemia. Relationships between PaO_2 and SaO_2 with respect to hypoxemia are as follows: normal, SaO_2 greater than 90 mm Hg and PaO_2 greater than 80 mm Hg; severe hypoxemia, SaO_2 less than 90 mm Hg and PaO_2 less than 60 mm Hg; very severe hypoxemia, SaO_2 less than 75 mm Hg and PaO_2 less than 40 mm Hg. SaO_2 would be expected to be normal with anemia but reduced with methemoglobinemia or severe cardiopulmonary disease. It is critical to interpret the SaO_2 in view of the patient's respiratory status and not blindly believe the numbers; it is easy to have erroneously low SaO_2 because of poor placement of the probe.

THORACOCENTESIS

Occasional Indications • Thoracocentesis is performed on patients with pleural effusions or mass lesions of the pleural cavity or mediastinum.

Advantage • It is relatively noninvasive.

Disadvantages • It has the potential to produce pneumothorax, hemothorax, or cardiac arrhythmias.

Procedure • Skin over the right 5th to 11th intercostal spaces (or elsewhere if effusion is localized) is clipped and prepared surgically. The needle of a butterfly-type catheter is inserted in the seventh to eighth intercostal space at approximately the level of the costochondral junction, and fluid is aspirated into a syringe. A three-way stopcock can be attached to the syringe if the procedure is to be both therapeutic and diagnostic. Fluid analysis is described in Chapter 10. Masses may also be aspirated for cytologic evaluation, ideally with fluoroscopic or ultrasonographic guidance. When fluid has been analyzed (see Chapter 10), it may be useful to aspirate as much fluid as possible and radiograph (or re-radiograph) the thorax to look for structures (e.g., mediastinal masses) not previously evident.

Interpretation • See Chapter 10.

THORACOSCOPY/THORACOTOMY

Rare Indications • Thoracotomy and thoracoscopy can be performed to look for a foreign body (e.g., grass awn) in patients with nocardiosis or actinomyces or to look for infiltrative disease (e.g., mesothelioma) causing chronic or progressive pleural effusion. Thoracotomy is also used for lobectomy or biopsy (lung or masses) in patients with infiltrative disease that cannot be diagnosed with other tests. Thoracoscopy has the advantage of less morbidity and shorter postoperative recovery times compared with thoracotomy; however, thoracoscopy does not allow for as thorough an examination as thoracotomy. In general, imaging (i.e., radiographs or CT) should be done before thoracotomy or thoracoscopy.

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12

Immunologic and Plasma Protein Disorders

Mark C. Johnson

SERUM TOTAL PROTEIN AND ALBUMIN

Commonly Indicated • Indicated in most ill patients, but especially those with known or suspected anemia, edema, ascites, trauma, coagulopathies, diarrhea, weight loss, and hepatic or renal disease.

Advantages • Test is technically easy to perform.

Disadvantages • Changes are often nonspecific, and additional testing is required to establish cause of altered protein concentrations.

Analysis • Total protein can be estimated in fluid, serum, or plasma (ethylenediaminetetraacetic acid [EDTA] or heparinized) by refractometry. Temperature-controlled refractometers calibrated for total protein are preferable to those that read only total solids (see comments under **Artifacts**).⁶ Total protein and albumin can be measured in serum, urine, or fluid by spectrophotometric or dry reagent methods. Serum globulin concentration is calculated by subtracting the serum albumin from the serum total protein.

Normal Values • Listed in [Table 12-1](#).

NOTE: Lower values are normal for perinates and very young animals. Values gradually increase until adulthood; higher values in [Table 12-1](#) are average normal values for adults. Both albumin and globulin tend to decline with advancing old age.

Danger Values • Albumin less than or equal to 1.0 g/dl can be associated with major fluid shifts, but animals with concurrent increased portal hypertension can be at risk for abdominal effusion formation at higher values (i.e., >1.5 g/dl). Patients with severe hypoalbuminemia may also have decreased antithrombin III (AT III) activity (see under [Causes of Hypoalbuminemia](#)), thus also putting them at risk for thromboembolism of pulmonic, mesenteric, or portal vasculature.

Artifacts • Falsely increased values (refractometer) can result from lipemia, severe hyperglycemia, azotemia, and significant hypernatremia and hyperchloremia. Hyperbilirubinemia is historically listed as a cause for falsely increased serum total protein, but this likely occurs only with marked elevations.¹⁵ Likewise, hemolysis interferes with visual interpretation of total protein reading but does not directly interfere with measurement.⁶

NOTE: Certain methodologies that measure human albumin give falsely low values for canine albumin.

Drug Therapy That May Alter Protein Values • Hormonal changes generally have a slight effect on serum proteins, even though physical changes (e.g., body weight, muscle mass) may be marked. Hyperproteinemia may be caused by anabolic steroids, progesterone, insulin, and thyroid hormones in people, but a similar effect is not expected in dogs and cats. Prolonged, high-dose corticosteroid therapy can cause hyperproteinemia and hyperalbuminemia in normal dogs, but values return to normal within weeks after cessation of therapy.¹⁵ Hypoproteinemia may be due to estrogens; hypoalbuminemia may be due to anticonvulsants, acetaminophen, estrogens, and various antineoplastic agents in people. Anticonvulsants and antineoplastic drug administration in dogs and cats are not expected to cause similar changes by themselves without associated underlying causes (e.g., hepatic cirrhosis).

Causes of Alteration in Plasma and Serum Protein • Serum total protein concentration is a direct reflection of cumulative serum albumin and globulin values. Therefore the value only gives an overview of the general state of protein homeostasis.

Causes of Hyperalbuminemia • Only clinically relevant cause is dehydration.

Causes of Hypoalbuminemia • The first consideration is typically to concurrently determine the serum globulin concentration and determine if it is also similarly

TABLE 12-1. NORMAL SERUM TOTAL PROTEIN AND ALBUMIN VALUES (g/dl)

	DOGS	CATS
Plasma total protein	6.0–7.8	6.0–7.5
Serum total protein	5.5–7.5	5.5–7.8
Serum albumin*	2.5–4.0	2.5–4.0

*Serum globulin concentration should mirror serum albumin concentration.

decreased (nonselective), or only albumin is decreased (selective). If both are decreased (i.e., panhypoproteinemia), nonselective causes for hypoproteinemia such as hemorrhage, exudation from severe skin lesions, protein-losing enteropathy (PLE), and hemodilution are usually more likely (Box 12-1). Overt bleeding should be apparent, but gastrointestinal hemorrhage can be difficult to determine if it is relatively mild and chronic. GI hemorrhage can be due to many types of gastrointestinal diseases (see Chapter 9), including PLE, but other nonspecific disorders such as hypoadrenocorticism are also of consideration. Causes of PLE are numerous and are discussed in Chapter 9. Although both serum albumin and globulin are often decreased in PLE, globulin concentrations may be normal to increased in some cases (especially those with other concurrent diseases such as heartworm infection, ehrlichiosis, or chronic skin disorders).

Hemodilution rarely causes hypoalbuminemia but can occur due to intravenous fluid overload or plasma expanders (e.g., hetastarch), diseases causing edema (e.g., congestive heart failure), and rarely from excess antidiuretic hormone (ADH) secretion. Hemodilution usually causes mild decreases (albumin 2.1 to 2.4 g/dl), whereas PLE can cause moderate (1.5 to 2.0 g/dl) to severe (<1.5 g/dl) hypoalbuminemia.

Decreased albumin plus normal to increased globulins can be referred to as selective hypoalbuminemia. The most common and clinically significant causes are decreased albumin production from chronic hepatic insufficiency, increased loss from protein-losing nephropathy (PLN), or sequestration in a body cavity due to major effusion (see Box 12-1). Chronic hepatic insufficiency can produce marked hypoalbuminemia (<1.0 g/dl) if the liver is severely affected. Causes for hepatic insufficiency are numerous and can be congenital (e.g., congenital portosystemic shunt) or acquired (e.g., cirrhosis, neoplasia) (see Chapter 9). Hypoalbuminemia from PLN can be substantial (e.g., <2.0 g/dl, even <1.5 g/dl). If renal protein loss is detected, urinalysis $\pm$ urine protein:creatinine ratio (see Chapter 7) are typically indicated to confirm presence and severity of albuminuria. Causes for PLN are discussed in Chapter 7.

NOTE: PLN of very severe magnitude can sometimes cause concomitant hypoglobulinemia.

BOX 12-1. CAUSES OF HYPOALBUMINEMIA IN DOGS AND CATS

Decreased Production

Chronic hepatic insufficiency^{*,1}

Inadequate protein intake^{†,‡}

Maldigestion[†]

Malabsorption[†]

Hyperglobulinemia

Sequestration

Body cavity effusion¹

Vasculopathy

Increased Loss

Protein-losing nephropathy (PLN) because of glomerular disease^{*,1}

Gastrointestinal: protein-losing enteropathy (PLE)^{*,2}

Exudates from cutaneous lesions²

Hemorrhage²

Hemodilution²

*Most common and important causes of serum albumin ≤ 2.0 g/dl. Other causes rarely, if ever, cause serum albumin ≤ 2.0 g/dl.

[†]Doubtful importance as a sole cause of serum albumin ≤ 2.0 g/dl. Probably more important as a contributing factor when there is another problem that results in hypoalbuminemia.

[‡]Can be important in very young animals or animals fed diets that are extremely restricted in protein for prolonged periods.

¹Important selective causes.

²Important nonselective causes.

Sequestration of albumin may occur in pleural or peritoneal cavities or subcutaneous (SC) tissues. Patients with effusion caused by hypoalbuminemia may further lower their serum albumin concentration via sequestration. Alternatively, sequestration can be secondary to increased hydrostatic pressure (e.g., portal hypertension, right-sided cardiac failure). Immune-mediated or infectious vasculopathies (e.g., endotoxemia and bacteremia, ehrlichiosis, Rocky Mountain spotted fever [RMSF]) also allow albumin loss from the vascular compartment. Hypoalbuminemia as the result of sequestration or vasculopathy is usually mild.

Severe malnutrition, malabsorption, or maldigestion leading to poor protein intake can be associated with hypoalbuminemia, but this generally causes very mild hypoalbuminemia. Likewise, hyperglobulinemia from inflammation can cause mild hypoalbuminemia due to down-regulation of albumin production to offset the increased globulin levels or because albumin is a negative acute phase protein that decreases during inflammation. Significant hypoalbuminemia (i.e., albumin < 2.1 g/dl) should not be attributed solely to decreased nutrition or down-regulation of albumin production until hepatic insufficiency and protein-losing disorders have been eliminated by definitive tests (not just history and physical examination), because starvation and down-regulation rarely cause serum albumin concentrations less than 2.1 g/dl, except perhaps in very young animals.

Basic diagnostic approach to hypoalbuminemic patients is outlined in Figure 12-1. Clinical pathology testing should include a complete blood count (CBC), clinical chemistry, and urinalysis on animals with

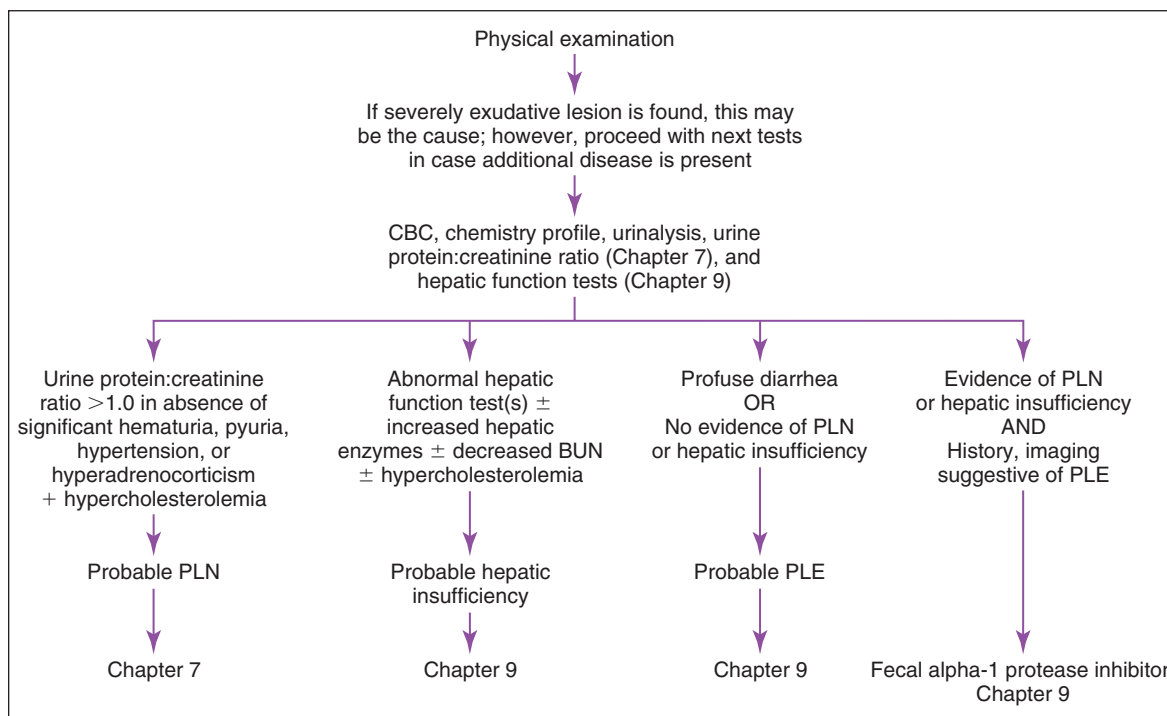


FIGURE 12-1 Diagnostic evaluation of hypoalbuminemia in dogs and cats when the serum albumin is less than or equal to 2.0 g/dl. BUN, Blood urea nitrogen; CBC, complete blood count; PLE, protein-losing enteropathy; PLN, protein-losing nephropathy.

physical exam findings suggestive of hypoalbuminemia. Serum bile acids and blood ammonia are indicated if hepatic insufficiency is suspected (see Chapter 9). If proteinuria is found without pyuria or hematuria and hypoalbuminemia is present, a urine protein:creatinine ratio (see Chapter 7) should be performed. Pyuria and hematuria can cause proteinuria, making it impossible to determine if there is glomerular loss; therefore follow-up urinalysis following resolution of pyuria or hematuria is indicated. An attempt should be made to categorize the degree of hypoalbuminemia (2.1 to 2.4 g/dl, mild; 1.5 to 2.0 g/dl, moderate; <1.5 g/dl, marked) in order to establish initial differential diagnoses. However, definitive exclusion of potential causes by this categorization should not occur until additional testing has been performed, because mild hypoalbuminemia can be observed in some cases of PLE and PLN and early hepatic insufficiency.

Initial approach in hypoalbuminemic patients begins with clinical evaluation. Severe cutaneous exudative lesions may be diagnosed by physical examination, but the possibility of renal, hepatic, and alimentary disease should still be investigated. Subcutaneous edema and body cavity effusions associated with hypoalbuminemia are usually transudates. Hypoalbuminemia associated with PLN or PLE, chronic hepatic insufficiency, and immune-mediated or infectious vasculitis may cause body cavity effusion, primarily transudates. However, one should always evaluate fluid accumulations to be sure that they are in fact transudates as opposed to unexpected modified transudates or exudates (which would strongly

suggest that more than hypoalbuminemia is causing the effusion).

Next, recognizing certain patterns on diagnostic samples can be suggestive of different disease processes. Hypercholesterolemia plus hypoalbuminemia suggests PLN. Significant proteinuria without pyuria and hematuria indicates a diagnostic workup for PLN (see Chapter 7). Hypocholesterolemia plus hypoalbuminemia is suggestive of hepatic insufficiency or PLE. Hypoalbuminemia associated with hepatomegaly; microhepatia; neurologic signs; icterus; decreased blood urea nitrogen (BUN) with or without increased alanine aminotransferase (ALT), serum alkaline phosphatase (SAP), or both; or abnormal hepatic function test results (e.g., serum bile acids) indicates a diagnostic workup for hepatic insufficiency (see Chapter 9).

NOTE: ALT and SAP may be normal in many patients with severe hepatic disease, and decreased mean corpuscular volume (MCV) (see Chapter 2) is sometimes present in dogs with congenital portosystemic shunts.

A congenital portosystemic shunt is more likely in young animals; however, congenital shunts can be diagnosed in animals more than 10 years old. Acquired hepatic disease is more common in adults and requires hepatic biopsy for diagnosis; however, some dogs less than 1 year old have severe, acquired hepatic disease with acquired

shunting. Hypoalbuminemia with normal hepatic function tests and absence of proteinuria or cutaneous lesions allows one to diagnose PLE by exclusion (see Chapter 9), even if feces are normal. If the patient has renal or hepatic disease and PLE is still a concern, then measurement of fecal α_1 -protease inhibitor concentrations (Chapter 9) may allow diagnosis of PLE by inclusion. Intestinal biopsy may then provide a definitive diagnosis of which intestinal disease is causing PLE. Endoscopic biopsies are safer than surgical biopsy, but it is critical that excellent-quality tissue samples be obtained; many endoscopically obtained samples are poor quality and nondiagnostic. If exploratory laparotomy is performed, hepatic biopsy should generally be performed along with intestinal biopsies. It is important to obtain biopsy specimens at several sites along the small intestine, even when no apparent gross lesions are found.

Causes of Altered Globulins • Changes in globulin levels are most often attributed to alterations in immunoglobulin values. Nonselective causes for hypoglobulinemia are similar to nonselective causes for hypoalbuminemia (see previous discussion of [Causes of Hypoalbuminemia](#)). True selective hypoglobulinemia (i.e., normal or increased albumin) occasionally occurs in dogs and cats from congenital or acquired immunodeficiencies. However, neonatal immunodeficiency patients are likely to succumb to this disorder early in life, and definitive diagnosis is often not established. Acquired immunodeficiencies are often secondary to chemotherapy or radiation therapy or directly from neoplastic transformation of lymphocytes (i.e., lymphoproliferative disorder) where antibody production is impaired or deficient. Hyperglobulinemia can be either nonselective (i.e., albumin elevated concurrently) from dehydration or selective due to three main processes, (1) acute phase protein increase (usually only induces mild elevation in globulins), (2) increased immunoglobulins from generalized antigenic stimulation with chronic inflammation, or (3) paraproteinemia (abnormal immunoglobulin production in blood) from a lymphoproliferative disorder (see following discussions).

ACUTE PHASE PROTEINS

Occasionally Indicated • Acute phase protein analysis is performed in certain clinical situations where more specific information regarding inflammation or coagulation is needed. Collectively, acute phase proteins are part of the α and β globulins measured in protein electrophoresis (see [Protein Electrophoresis](#) later) and, in conjunction with gamma globulins, compose the globulin fraction of the total protein analysis. Acute phase proteins typically include fibrinogen, haptoglobin, C-reactive protein (see Chapter 9), complement (C3a), serum amyloid A, α_1 -acid glycoprotein, α_1 -antiprotease, transferrin, α_2 -macroglobulin, and ceruloplasmin.

Advantages • Some acute phase proteins (e.g., fibrinogen) can be measured rapidly and provide specific information regarding coagulation and inflammatory status of the animal.

Disadvantages • Additional testing is required for these proteins, and some require specialized testing procedures. Results of testing may not provide any more useful information as to the health status of the animal compared to total protein, albumin, and globulins.

Analysis • Most acute phase protein assays can be performed with serum samples with the exception of fibrinogen, which requires plasma. Methodologies of the various assays include heat precipitation (fibrinogen), immunoassays, enzyme-linked immunosorbent assay (ELISA), and spectrophotometry.

Normal Values • Normal reference intervals for fibrinogen in dogs and cats are generally accepted as approximately 100 to 300 mg/dl but are slightly variable depending on the methodology used to measure fibrinogen. Normal values on other acute phase proteins are variable depending on published data, so it is important to use reference intervals established at each laboratory performing the analysis.³

Danger Values • Alterations in values reflect only a change in health status (i.e., values increase with inflammation and dehydration or decrease with consumption or loss of protein); therefore there are no danger values.

Artifacts • Hemolysis and lipemia can interfere with certain testing methodologies. Heat precipitation methods for fibrinogen are not as accurate as instrument-based measurements. Dehydration will cause relatively increased values of some acute phase proteins such as fibrinogen. Laboratory or sampling error can cause spurious results.

Causes of Increased Acute Phase Proteins • Some acute phase proteins increase with associated inflammation and are appropriately termed positive acute phase proteins. Hyperfibrinogenemia is one of the best indicators of acute inflammation in large animal species, but has traditionally not been utilized in small animal medicine. This concept may change because of more accurate methods for fibrinogen analysis being employed by reference laboratories. Regardless, elevated levels of fibrinogen are frequently seen in infectious disease (e.g., bacterial, viral, protozoal, fungal), trauma, neoplasia, and necrosis. C-reactive protein is increased in pregnant dogs, glucocorticoid therapy increases haptoglobin in dogs, and phenobarbital treatment in dogs can cause elevated α_1 -acid glycoprotein.^{8–10,15}

NOTE: Care must be utilized in excluding inflammation in animals with normal or low values of acute phase proteins because these proteins can decrease with hepatic insufficiency (similar to albumin), may be lost in generalized protein-losing disorders (PLE), or may be consumed in cases of coagulopathies.

Causes of Decreased Acute Phase Proteins • Acute phase proteins can decrease in patients as well and are often referred to as negative acute phase proteins when the decrease is associated with inflammation. Albumin (see previous discussion on [Causes of](#)

Hypoalbuminemia) and transferrin are considered the classic negative acute phase proteins. However, other mechanisms such as lack of production or consumption can be associated with decreases in acute phase proteins that are not a result of the protein representing a negative acute phase protein. Hepatic insufficiency, if severe enough, can lead to decreased production levels of most acute phase proteins, similar to albumin. Fibrinogen, while most frequently considered a positive acute phase protein related to inflammation (see previous discussion on Causes of Increased Acute Phase Proteins), is equally clinically relevant in patients with decreased values. Primary consideration with hypofibrinogenemia is consumptive coagulopathy, such as disseminated intravascular coagulation (DIC). While not present in every case (in some instances fibrinogen is normal or increased), hypofibrinogenemia in conjunction with significant thrombocytopenia, decreased AT III, schistocytes, high D-dimer, and prolongation of activated partial thromboplastin time (aPTT) or prothrombin time (PT) is highly suggestive of DIC (see Chapter 5). Rare reports of inherited or congenital hypofibrinogenemia in dogs are documented.¹⁵

PROTEIN ELECTROPHORESIS

Occasionally Indicated • Protein electrophoresis is performed when hyperglobulinemia is not caused by dehydration or known antigenic stimulation but is high enough to make monoclonal gammopathy from a lymphoproliferative disorder a reasonable possibility. Electrophoresis is also rarely performed on patients with suspected humoral immunodeficiencies.

Advantages • Protein electrophoresis is a useful screening test to differentiate monoclonal gammopathies from other causes of hyperglobulinemia.

Disadvantages • A specific diagnosis is seldom obtained from electrophoresis.

Although a specific diagnosis is seldom obtained, electrophoretic patterns can be valuable when interpreted with clinical signs and other laboratory data. Two general types of electrophoresis are utilized, protein electrophoresis and immunoelectrophoresis. Protein electrophoresis is quantitative, can be performed on blood and urine, and is usually the first step in determining if a monoclonal gammopathy is present. Immunoelectrophoresis is qualitative, identifying specific classes of immunoglobulins present in a monoclonal gammopathy. Immunoelectrophoresis is the method of choice to detect urinary and serum Bence Jones protein, a monoclonal protein equivalent to immunoglobulin light chains that occasionally occurs in multiple myeloma and macroglobulinemia. Protein electrophoresis performed on a concentrated urine sample occasionally detects an isolated monoclonal peak in urine (e.g., Bence Jones protein). Finding a urine electrophoresis pattern mimicking that of serum indicates a glomerular lesion substantial enough to allow leakage of serum proteins including the serum monoclonal heavy chain peak; therefore, it is not evidence of Bence Jones light chains. Canine Bence Jones proteinuria is only rarely

detected by heat precipitation. Positive results for Bence Jones proteins by an acid precipitation screening test should be confirmed by concentrated urine electrophoresis or immunoelectrophoresis because of the possibility of false-positive results.

Analysis • Serum or urine may be analyzed, and it may be refrigerated or frozen.

ELECTROPHORESIS

Analysis • The cellulose acetate technique is the method of choice. Interpretation of electrophoretograms is based on densitometric measurements of intensity of staining of protein bands on cellulose acetate strips. Serum separates into four fractions: (1) albumin, (2) alpha (α) globulins, (3) beta (β) globulins, and (4) gamma (γ) globulins (Table 12-2). Canine and feline α- and β- globulins are usually divided into two subfractions each: α₁, α₂; β₁, β₂. Gamma globulins are usually listed as having one fraction composed primarily of immunoglobulin G (IgG), although older references do list subfractions γ₁ and γ₂. *Important:* Immunoglobulin M (IgM) and immunoglobulin A (IgA) antibodies will often appear in the β₂ region or “bridge” the β₂-to-γ region on electrophoresis. Normal-appearing electrophoretograms from dogs and cats are presented in Figures 12-2 and 12-3.

Artifacts • Albumin concentration is usually underestimated by electrophoresis compared with a chemical

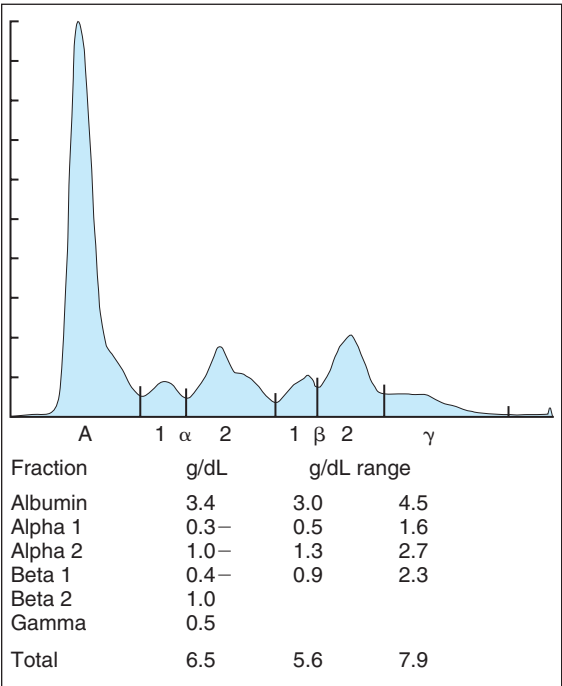


FIGURE 12-2 Electrophoretogram of normal canine sera. A, Albumin; 1 α 2, alpha₁ and alpha₂ globulins; 1 β 2, beta₁ and beta₂ globulins; γ, gamma globulins. (Courtesy of Susan Fielder, TVMDL.)

TABLE 12-2. NORMAL VALUES (MEAN $\pm$ 1 SD) FOR SERUM PROTEIN ELECTROPHORESIS IN DOGS AND CATS

DOGS	Breitschwerdt et al. (1987)		Kaneko (1980)*	
	MEAN	LIMITS	MEAN	LIMITS
Total protein (g/dl)	6.84 $\pm$ 0.66	(6.0–7.6)	6.10 $\pm$ 0.52	(5.4–7.1)
Albumin [†]	3.20 $\pm$ 0.34	(2.72–3.67)	2.91 $\pm$ 0.11	(2.6–3.3)
α_1 -globulin	0.33 $\pm$ 0.11	(0.25–0.60)	0.30 $\pm$ 0.03	(0.2–0.5)
α_2 -globulin	1.13 $\pm$ 0.25	(0.72–1.40)	0.62 $\pm$ 0.21	(0.3–1.1)
β_1 -globulin	0.74 $\pm$ 0.10	(0.63–0.89)	0.82 $\pm$ 0.23	(0.7–1.3)
β_2 -globulin	0.79 $\pm$ 0.14	(0.59–0.96)	0.89 $\pm$ 0.33	(0.6–1.4)
γ -globulin	0.64 $\pm$ 0.15	(0.49–0.83)		
γ_1 -globulin			0.80 $\pm$ 0.25	(0.5–1.3)
γ_2 -globulin			0.70 $\pm$ 0.14	(0.4–0.9)
A:G ratio	0.89 $\pm$ 0.10	(0.79–1.02)	0.83 $\pm$ 0.16	(0.59–1.11)

CATS	Turnwald and Barta (1989)		Kaneko (1980)*	
	MEAN	LIMITS	MEAN	LIMITS
Total protein (g/dl)	7.66 $\pm$ 0.10	(7.3–7.8)	6.60 $\pm$ 0.70	(5.4–7.8)
Albumin [†]	3.41 $\pm$ 0.18	(2.82–4.18)	2.70 $\pm$ 0.17	(2.1–3.9)
α_1 -globulin	0.47 $\pm$ 0.03	(0.30–0.64)	0.70 $\pm$ 0.02	(0.2–1.1)
α_2 -globulin	0.55 $\pm$ 0.04	(0.41–0.68)	0.70 $\pm$ 0.02	(0.4–0.9)
β_1 -globulin	0.91 $\pm$ 0.06	(0.77–1.25)	0.70 $\pm$ 0.03	(0.3–0.9)
β_2 -globulin	0.40 $\pm$ 0.02	(0.35–0.47)	0.70 $\pm$ 0.02	(0.6–1.0)
γ -globulin	1.92 $\pm$ 0.12	(1.39–2.22)		
γ_1 -globulin			1.60 $\pm$ 0.77	(0.30–2.50)
γ_2 -globulin			1.70 $\pm$ 0.36	(1.40–1.90)
A:G ratio	0.80 $\pm$ 0.11	(0.63–1.15)	0.71 $\pm$ 0.20	(0.45–1.19)

*Numbers do not add up to the total protein values and A:G ratios as given in the table.

[†]Concentration of albumin is usually underestimated by electrophoresis compared with a chemical determination. Therefore, the A:G ratio is usually higher by chemical determination than by electrophoretic determination.

Data from Breitschwerdt D, et al: Monoclonal gammopathy associated with naturally occurring canine ehrlichiosis, *J Vet Int Med*, 1:2, 1987; Kaneko JJ: Serum proteins and the dysproteinemias. In Kaneko JJ, editor: *Clinical biochemistry of domestic animals*, ed 3, San Diego, 1980, Academic Press; Turnwald GH, Barta O: Immunologic and plasma protein disorders. In Willard MD, Tvedten H, Turnwald GH: *Small animal clinical diagnosis by laboratory methods*, Philadelphia, 1989, WB Saunders.

determination. Therefore albumin:globulin ratio (A:G) is usually higher by chemical determinations than by electrophoretic determination.

IMMUNOELECTROPHORESIS

Analysis • After electrophoresis in agar gel, polyclonal antiserum to specific proteins (including immunoglobulins) is added to a trough parallel with the separated serum proteins. Reagents are allowed to diffuse. To obtain quantitation of individual immunoglobulins, radial immunodiffusion (RID), electroimmunodiffusion (i.e., rocket electrophoresis), or laser nephelometry is performed; these procedures can also be used to quantitate immunoglobulin subclasses.

Normal Values • Values vary among laboratories and the different techniques for quantitating individual immunoglobulins (see Table 12-2). Immunoglobulins migrate in the β_2 and γ regions of electrophoresis. Average concentrations of immunoglobulin classes are listed in Table 12-3.

NOTE: Normal values for puppies differ substantially from those for adults. Age-matched controls are recommended when submitting samples for immunoglobulin quantitation from young dogs because of great variation in adult values among different antibody classes. It is likely that breed-specific variations also occur.

Artifacts • Electrophoretic bands with high-intensity staining (e.g., albumin) are underestimated, and bands of low-intensity staining are overestimated. Immunoglobulins migrate in β_2 - and γ -globulin regions; therefore, immunoglobulin concentrations determined by RID are usually higher than the γ -globulin fraction determined by electrophoresis. This discrepancy can increase when immunoglobulin hyperproduction occurs, such as in some myelomas, canine ehrlichiosis, feline infectious peritonitis (FIP), and other chronic infections, especially if IgM or IgA is the primary antibody.

Causes of Altered Electrophoretic Patterns • Diagnostic evaluation of patients with abnormal electrophoretograms is discussed next under Causes of Hyperglobulinemia.

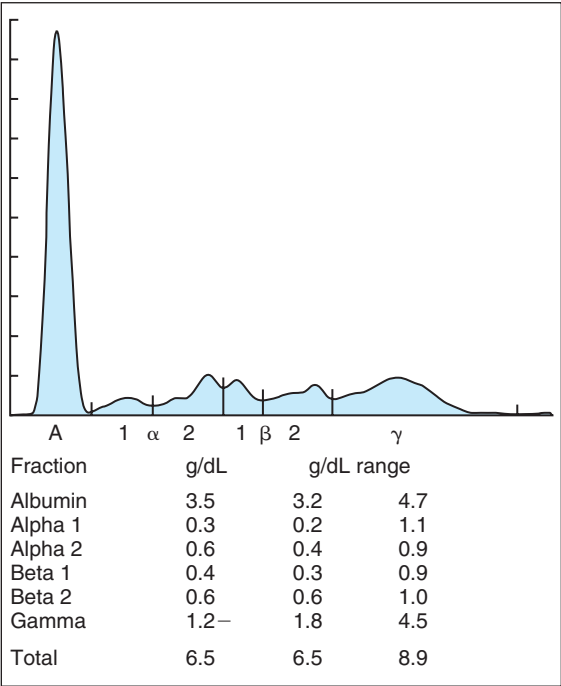


FIGURE 12-3 Electrophoretogram of normal feline sera. A, Albumin; 1 α 2, alpha₁ and alpha₂ globulins; 1 β 2, beta₁ and beta₂ globulins; γ, gamma globulins. (Courtesy of Susan Fielder, TVMDL.)

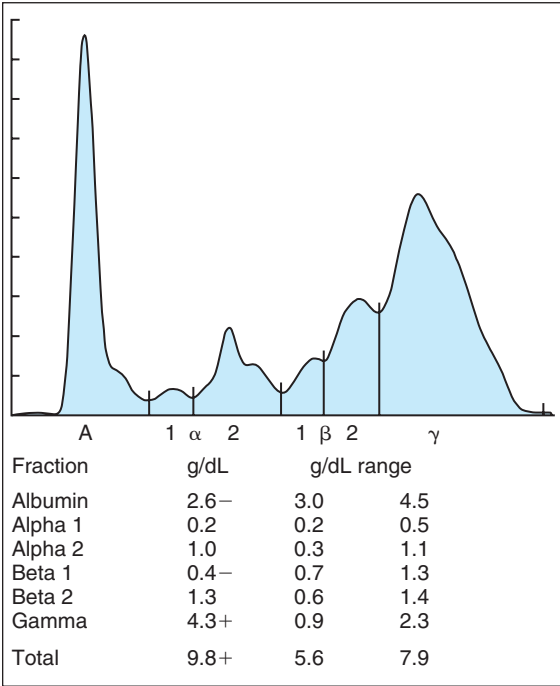


FIGURE 12-4 Electrophoretogram of a dog with suspected systemic fungal disease showing a polyclonal gammopathy. A, Albumin; 1 α 2, alpha₁ and alpha₂ globulins; 1 β 2, beta₁ and beta₂ globulins; γ, gamma globulins. (Courtesy of Susan Fielder, TVMDL.)

Causes of Hyperglobulinemia • Polyclonal hyperglobulinemias (i.e., polyclonal gammopathies) have a broad-based peak encompassing β and γ regions (sometimes α) and suggest persistent antigenic stimulation and inflammation secondary to infectious diseases (chronic bacterial, viral, fungal, protozoal, rickettsial, or parasitic disorders), neoplasia, or immune-mediated disease (Figure 12-4 and Box 12-2). The most common causes in dogs are cutaneous parasitism, pyoderma, dirofilariasis, and ehrlichiosis, depending on geographic location (Figure 12-5; see also Box 12-2). In cats, the most common cause of severe polyclonal gammopathy is FIP (Figure

12-6). Increases in feline globulins are commonly in the γ region but can bridge in the β region as well.

Monoclonal hyperglobulinemias or monoclonal gammopathies generally have a tall and narrow-based electrophoretic peak (i.e., “spike”) in the β or γ region, normally no wider than the albumin peak. Rare cases of monoclonal gammopathies with biclonal peaks occur.⁷ Monoclonal immunoglobulin elevations are also called paraproteins or M proteins and are usually due to lymphocyte and plasma cell neoplasia (e.g., multiple myeloma, macroglobulinemia, lymphosarcoma; see Box 12-2). Monoclonal or oligoclonal spikes are occasionally

TABLE 12-3. SERUM IMMUNOGLOBULIN CONCENTRATIONS IN DOGS AND CATS

	Mean Concentration (mg/dl)				
	PUPPY (2 weeks)	PUPPY (2 months)	ADULT MONGREL DOG	ADULT PUREBREED DOG	ADULT CAT
IgA	Undetected	30	79	83	ND
IgG	56	143	1445	925	2400
IgM	73	118	45	156	ND

ND, Not done.
Data from Reynolds HY, Johnson JS: Quantitation of canine immunoglobulins. *J Immunol* 105:698, 1970; Heddle RJ, Rowley D: Dog immunoglobulins: immunochemical characterization of dog serum, saliva, colostrum, milk and small bowel fluid. *Immunology* 29:185, 1975; Schultz RD, Adams LS: Immunologic methods for the detection of humoral and cellular immunity. *Vet Clin North Am* 8:721, 1978; Reimann KA, et al: Immunologic profiles of cats with persistent naturally acquired feline leukemia virus infection. *Am J Vet Res* 47:1935, 1986.

BOX 12-2. CAUSES OF HYPERGLOBULINEMIA IN DOGS AND CATS**POLYCLONAL****Infections****Bacterial*†**

- Brucellosis
- Pyoderma
- Bacterial endocarditis

Viral

- Feline infectious peritonitis (FIP)[‡]
- Feline immunodeficiency virus (FIV)
- Feline leukemia virus (FeLV)

Fungal*†

- Systemic fungal infections (e.g., blastomycosis, histoplasmosis, coccidioidomycosis)

Rickettsial†,‡

- Ehrlichiosis

Parasitic

- Dirofilariasis*†
- Demodicosis
- Scabies

Immune-Mediated Disease**Infections (immune complex)**

- Dirofilariasis*†
- Feline cholangitis/cholangiohepatitis
- Pyometra

Systemic lupus erythematosus (SLE), including glomerulonephritis, immune-mediated hemolytic anemia (IMHA) and thrombocytopenia (IMT), and polyarthritis*

IMHA, IMT (not because of SLE)*

Pemphigus complex, bullous pemphigoid*

Rheumatoid arthritis*

Neoplasia†,‡**MONOCLONAL****Infection**

Ehrlichiosis†,‡

Leishmaniasis†,‡

FIP (rare)

Idiopathic†,‡

Benign monoclonal gammopathy

Neoplasia†,‡

Multiple myeloma[‡]

Macroglobulinemia

Lymphosarcoma

Extramedullary plasmacytoma (rare)

Miscellaneous

Cutaneous amyloidosis

Plasmacytic gastroenterocolitis*

*Mild (4 to 5 g/dl).

†Moderate (5 to 6 g/dl).

‡Severe (>6 g/dl).

NOTE: Effect of age should be considered when assessing globulin values (see [Causes of Hypoglobulinemia](#) in text).

caused by infectious (e.g., ehrlichiosis, leishmaniasis) (Figure 12-7) or idiopathic disorders, but they are likely to be of polyclonal origin with similar but closely related immunoglobulin types (i.e., IgG₁ and IgG₂).⁷ Multiple myeloma and ehrlichiosis can both have monoclonal electrophoretic patterns and bone marrow plasmacytosis, making differentiation between the two disorders based on the presence of these two abnormalities difficult. In ehrlichiosis or leishmaniasis, however, the electrophoretic pattern is often but not always a monoclonal or oligoclonal pattern superimposed on or arising within a broader-based globulin peak (see Figure 12-7).⁷ In such cases, examination of the stained electrophoretogram bands shows a clearly restricted monoclonal band with sharper edges within a paler, broader background band. In contrast, monoclonal spikes associated with neoplastic disorders are frequently accompanied by normal to decreased nonparaprotein globulin fractions, often reflecting impaired production of other immunoglobulins (Figure 12-8).

A suggested diagnostic approach to hyperglobulinemia is outlined in Figure 12-9. An initial attempt should be made to assess degree of hyperglobulinemia and correlate the significance of change with physical exam findings and suspected clinical diagnosis. Mild to moderate elevations of globulins are commonly associated with

skin disease, parasites, and systemic or localized infectious diseases. Mild hyperglobulinemia is also associated with immune-mediated disease and nonlymphocytic neoplasia but rarely requires extensive evaluation. Specific diagnostic evaluation for these processes is generally performed in lieu of protein electrophoresis. *Important:* More than one cause for hyperglobulinemia is possible in some instances (especially infectious).

In dogs with hyperglobulinemia and severe pruritic dermatitis, diagnostic evaluation may involve only a physical examination to identify fleas and ticks or skin scrapings to detect mites. *Demodex canis* mites are usually detected easily, whereas *Sarcoptes scabiei* are often difficult to find. For canine heartworm disease, ELISA antigen testing is the preferred screening procedure. In areas endemic for ehrlichiosis or RMSF, serologic tests are indicated (see Chapter 15), particularly if the patient has anemia, thrombocytopenia, leukopenia, or a combination thereof. Testing for other infectious disorders (e.g., brucellosis, blastomycosis, histoplasmosis, coccidioidomycosis) is dictated by geographic location and other physical, laboratory, or radiographic abnormalities. If joint pain, stiff gait, or increased joint fluid volume accompanies hyperglobulinemia, then radiographs and joint fluid analysis (see Chapter 10) are indicated. Rheumatoid factor (RF) testing, antinuclear antibody (ANA)

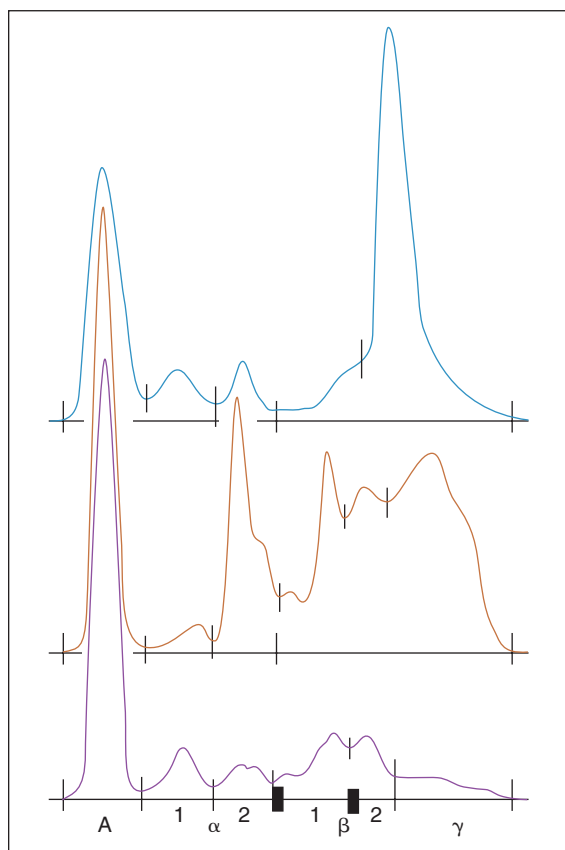


FIGURE 12-5 Electrophoretograms of different canine sera. *Top*, Monoclonal gammopathy (i.e., ehrlichiosis); *middle*, polyclonal gammopathy (i.e., blastomycosis, ehrlichiosis, dirofilariasis); *bottom*, normal. A, Albumin; 1 α 2, α_1 and α_2 globulins; 1 β 2, β_1 and β_2 globulins; γ , gamma globulins.

testing, rickettsial titers, or a borreliosis titer (or a combination of these tests) may be helpful if the joint fluid is a nonseptic exudate.

Hyperglobulinemia in cats can also be attributed to a variety of infectious processes, including skin disease, parasites, and fungal infection (e.g., histoplasmosis, cryptococcosis), but FIP is generally the most likely cause. FIP titers are generally not useful. Body cavity effusions from cats with FIP tend to have high total protein values and variable nucleated cell counts. Some advocate evaluating effusion fluid:serum γ -globulin ratios in cats when FIP is suspected, but caution must be taken to not overestimate the value of this ratio (see Chapter 15). Confirmatory testing for FIP can be performed on affected tissue biopsies, usually liver, using an immunohistochemical stain for FIP virus. Other causes for hyperglobulinemia in cats are dirofilariasis (less common than in dogs) and chronic feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) infections (globulin values vary in the latter two diseases).

In a clinical setting, serum protein electrophoresis is generally reserved for patients with substantial hyperglobulinemia (>5 g/dl) that appear inappropriately ill

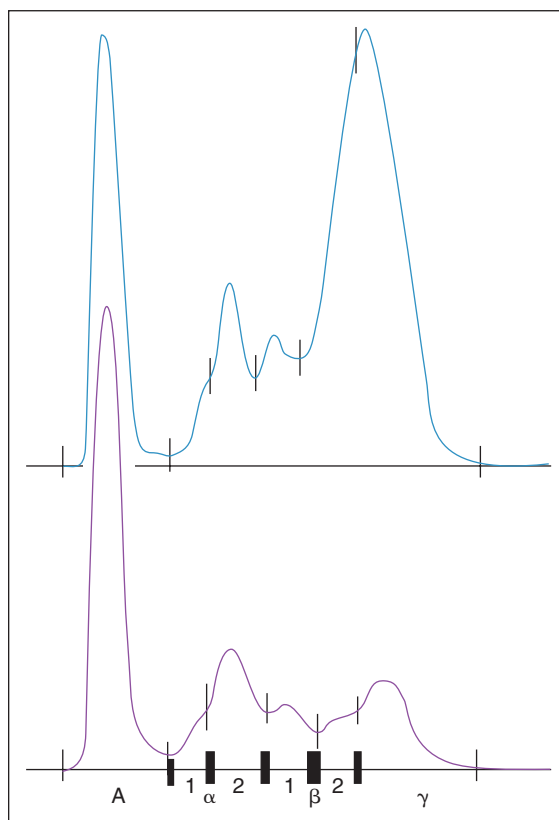


FIGURE 12-6 Electrophoretograms of feline sera. *Top*, Polyclonal gammopathy (i.e., feline infectious peritonitis [FIP]); *bottom*, normal. A, Albumin; 1 α 2, α_1 and α_2 globulins; 1 β 2, β_1 and β_2 globulins; γ , gamma globulins.

and have no confirmed diagnosis. In these patients, serum protein electrophoresis should be performed especially if any signs of hyperviscosity are present (see Serum Viscosity later) to differentiate monoclonal from polyclonal gammopathies. Polyclonal gammopathies have numerous causes, most commonly chronic infectious diseases, nonlymphoid neoplasia, and immune-mediated disorders (see earlier in this section). If the cause of polyclonal gammopathy is unknown, thoracic and abdominal imaging, serologic testing, and/or biopsy of any masses or lesions may be indicated.

Monoclonal gammopathies are usually caused by lymphoproliferative disorders (e.g., plasma cell neoplasia, B-cell lymphosarcoma). Evaluation of patients with monoclonal gammopathy may include skeletal radiographs, serum and urine immunoelectrophoresis, and bone marrow biopsy with cytologic and histologic evaluation. Diagnosis of multiple myeloma in dogs requires finding at least two of the following: lytic skeletal lesions, bone marrow plasmacytosis, Bence Jones proteinuria, or a monoclonal spike on serum protein electrophoresis due to the presence of a paraprotein. Skeletal lesions are uncommon in feline multiple myeloma. In areas endemic for ehrlichiosis, an *Ehrlichia canis* titer should be performed in dogs with a monoclonal gammopathy. Ehrlichiosis commonly causes proteinuria and bone

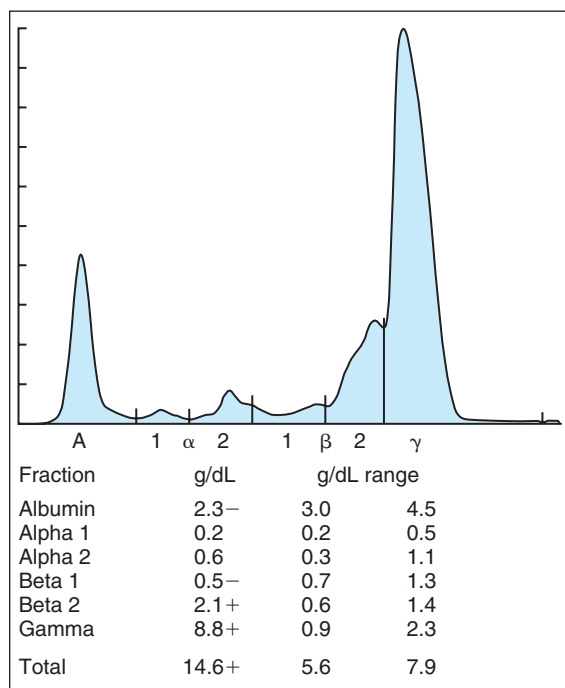


FIGURE 12-7 Electrophoretogram of dog with ehrlichiosis showing a monoclonal gammopathy. Note the high and narrow peak in the γ region arising within a broader peak located in the β_2 region. A, Albumin; 1 α 2, α_1 and α_2 globulins; 1 β 2, β_1 and β_2 globulins; γ , gamma globulins. (Courtesy of Susan Fielder, TVMDL.)

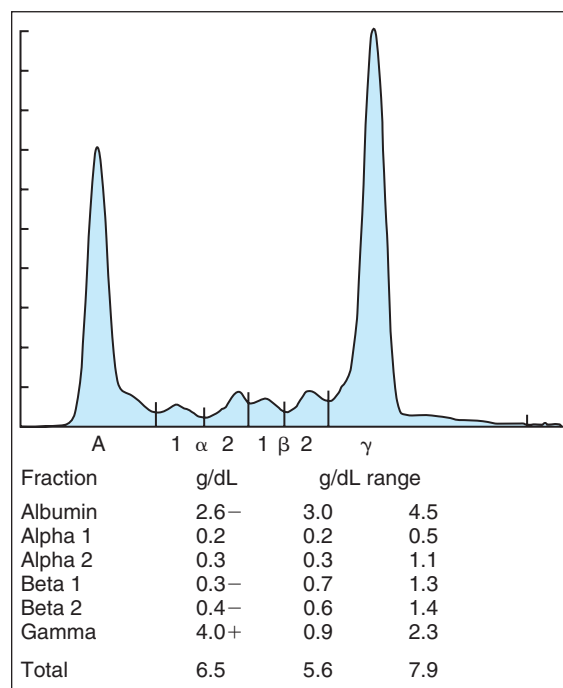


FIGURE 12-8 Electrophoretogram of dog with plasma cell myeloma showing a monoclonal gammopathy. Note the high and narrow peak in the γ region and the normal α and β peaks. A, Albumin; 1 α 2, α_1 and α_2 globulins; 1 β 2, β_1 and β_2 globulins; γ , gamma globulins. (Courtesy of Susan Fielder, TVMDL.)

marrow plasmacytosis, closely resembling multiple myeloma. FIP rarely causes monoclonal spikes. If a monoclonal gammopathy is found in a patient with multiple myeloma or lymphosarcoma, immunoelectrophoresis or quantitation of immunoglobulins by RID (or rocket electrophoresis) can be performed to identify the class of immunoglobulin composing the paraprotein. These tests can also detect nonparaprotein immunoglobulin deficiencies, which are occasionally present in patients with lymphoproliferative disorders or immune deficiencies (see [Causes of Hypoglobulinemia](#) next).

Causes of Hypoglobulinemia • As with hypoalbuminemia, causes for hypoglobulinemia are divided into nonselective and selective disorders. The most common causes are nonselective hypoglobulinemias due to external blood loss and PLE (see [Causes for Hypoalbuminemia](#) earlier). Nonselective hypoglobulinemia should not be seen with PLN unless near-complete loss of glomerular integrity occurs. Selective causes are much less common and are usually attributed to immunodeficiencies (acquired or congenital), neonatal status, and hepatic insufficiency (see Chapters 9). However, hypoglobulinemia due to hepatic insufficiency is often not recognized clinically because it is associated with the reduced production of the α - and β -globulins (e.g., fibrinogen, transferrin, haptoglobin, lipoproteins) and not immunoglobulins, which compose most of the globulin fraction.

Immunoglobulin production may actually increase to offset loss of oncotic pressure from hypoalbuminemia.¹⁵

Acquired immunodeficiencies are often attributed to chemotherapy or radiation therapy or other compounds (e.g., toxins, drugs). These immunodeficiencies can be permanent or transient depending on the cause. Congenital combined or selective immunodeficiencies occur but are rarely diagnosed, probably because immunodeficient puppies or kittens rapidly succumb to infections. In dogs, these infections are usually due to distemper or parvovirus and occur in the postnatal period after maternal immunity wanes. Immunodeficiency should be suspected when more than one pup in a properly cared for litter dies of infection in the first 2 to 6 months of life. It is important to remember that newborn animals are physiologically hypogammaglobulinemic and have serum total protein concentrations 60% to 80% of adult values, and are technically immuno-incompetent without maternal antibodies. Severe combined immunodeficiencies are noted in Jack Russell terriers, basset hounds, and Cardigan Welsh corgis; selective IgM and IgA deficiencies are seen in Doberman pinschers and German shepherds, respectively.¹⁵ Selective IgG and IgA deficiency is described in weimaraners.⁴ CBC, serum chemistry profile, serum protein electrophoresis, and immunoelectrophoresis are typical initial tests performed on puppies with suspected immunodeficiencies. Immunoglobulin quantitation by RID or rocket electrophoresis is recommended for

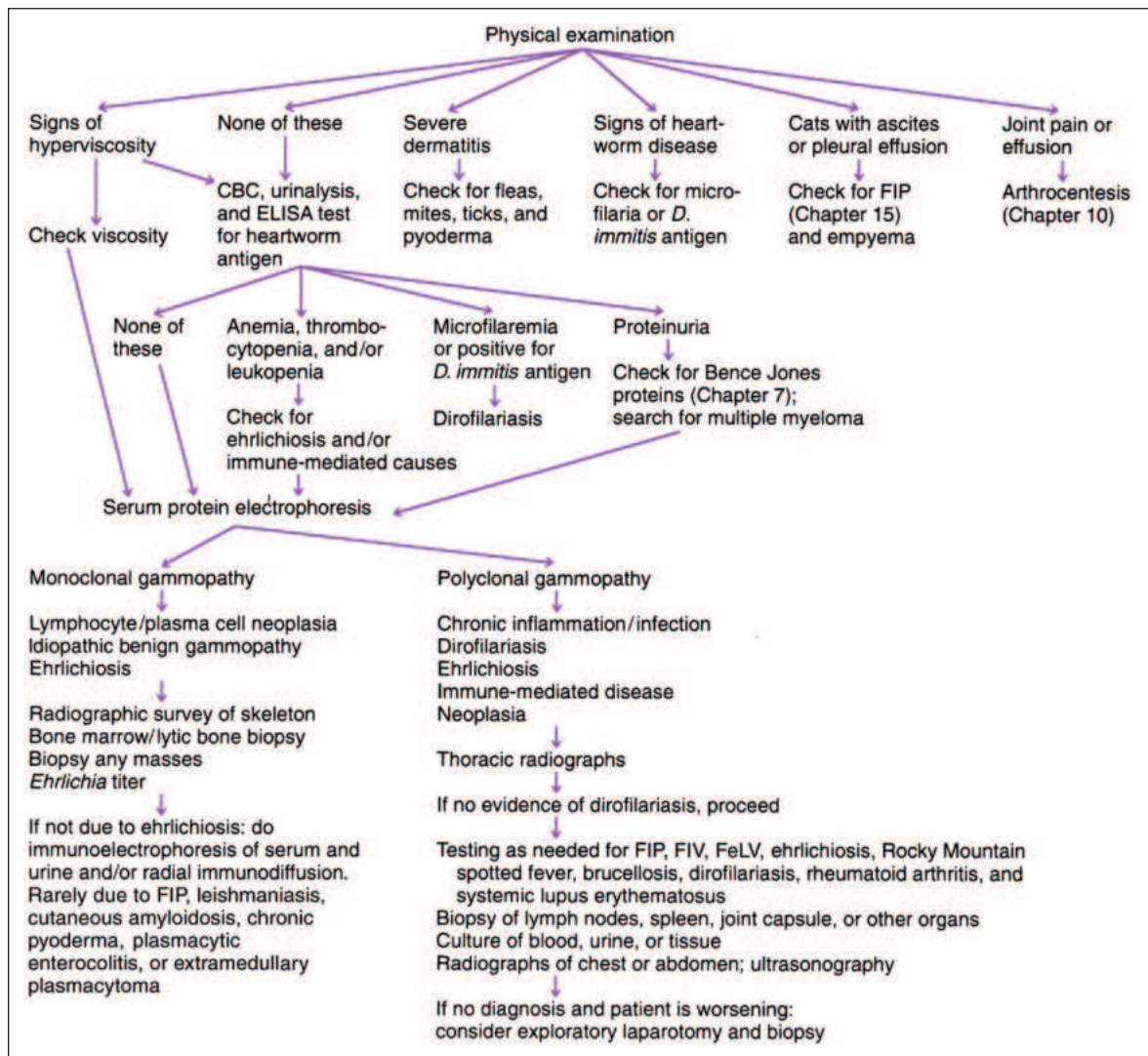


FIGURE 12-9 Diagnostic evaluation of moderate to severe hyperglobulinemia (globulin ≥ 5.0 g/dl) in dogs and cats. CBC, Complete blood count; ELISA, enzyme-linked immunosorbent assay; FeLV, feline leukemia virus; FIP, feline infectious peritonitis; FIV, feline immunodeficiency virus.

confirmation and characterization of the type of immunoglobulin deficiency present.

Immunoglobulin quantitation techniques are more precise than qualitative methods (i.e., immunoelectrophoresis). Selective (single class or subclass) and partial immunoglobulin deficiencies may not be readily detectable via serum electrophoresis and immunoelectrophoresis, and all immunoglobulin classes (and IgG subclasses when available) should be quantitated when a humoral immunodeficiency is suspected. Humoral immunodeficiency is usually detected by finding decreased IgG and IgA and normal to decreased IgM concentrations. Selective IgA deficiency and transient hypogammaglobulinemia occur in dogs. Patients with selective IgA deficiency may show chronic problems involving mucosal immunity (e.g., antibiotic-responsive

enteropathy) or be asymptomatic. Serum electrophoresis in cases of selective or partial immunoglobulin deficiency may yield normal results or even show polyclonal elevations in globulins. Immunoelectrophoresis of normal canine serum may show a low or absent stainable IgA precipitation band because of the relatively low concentration of IgA or as the result of quality assurance problems involving antisera or technique. When selective IgA deficiency is suspected, the best diagnostic test is quantitative RID or rocket electrophoresis. IgA deficiency is sometimes accompanied by elevated concentrations of IgM or IgG. A complement measurement and evaluations of lymphocyte and phagocyte functions may also be indicated for patients with suspected congenital immunodeficiency (see [Miscellaneous Immunologic-Based Tests](#) near end of chapter).

NOTE: In patients with paraproteinemias (e.g., multiple myeloma, macroglobulinemia, B-cell lymphosarcoma) immunoglobulins of non-neoplastic origin are usually depressed.

The relationship between chronic inflammatory enteropathies (e.g., lymphocytic plasmacytic enteritis) and serum IgA deficiency is unclear at this time. It is likely that deficiency of local secretory IgA is not always reflected by serum IgA concentrations. In addition, low concentrations of serum IgA have been associated with canine allergic and parasitic disorders, with return to normal after successful therapy. This finding suggests that those disease processes may lead to down-regulation of IgA as an immunomodulation event, and low IgA probably has no causative role in the underlying chronic inflammatory bowel disease. Breed-specific normal ranges for immunoglobulins may provide a clearer picture of the role of selective IgA deficiency in chronic enteropathies.

SERUM VISCOSITY

Rarely Indicated • Serum viscosity is indicated in patients with monoclonal gammopathies, hyperglobulinemia with signs of hyperviscosity (i.e., poor tissue perfusion, dilated retinal vessels, retinal hemorrhage or retinal detachment, renal disease, central nervous system [CNS] dysfunction, bleeding problems), and established plasma hyperviscosity where continual monitoring is needed. Hyperviscosity may be suspected based on finding high serum protein (usually >10 g/dl) or on physical characteristics of serum (i.e., viscous or syrup-like). Polycythemia (packed cell volume [PCV] > 60%) can also cause increased blood viscosity and should be eliminated as a possibility in patients showing clinical signs of hyperviscosity.

Advantages • The test is simple and diagnostically significant.

Analysis • Viscosity is measured in serum with an Ostwald viscosimeter or a 0.1-ml capillary pipette. Time for a given volume of serum to flow from the pipette is compared with that for the same volume of water.

Normal Values • Relative viscosity, 1.4 to 1.8. (Relative viscosity = flow time of serum [seconds] divided by flow time of water [seconds].)

Artifacts • Volume depletion due to dehydration causing increased serum protein concentration may increase serum viscosity but is unlikely to be clinically significant. Markedly increased blood viscosity can interfere with tests using flow-through devices (e.g., hematology autoanalyzers).

Drug Therapy That May Alter Serum Viscosity • Any drug causing volume depletion can increase serum viscosity.

Causes of Serum Hyperviscosity • A relative viscosity greater than or equal to 4 is abnormal in people and probably abnormal for dogs. Because of the relatively large size of IgM, it has the greatest potential to cause hyperviscosity. IgA (which can exist as a polymer or dimer) and very high concentrations of IgG can also cause hyperviscosity. Clinically significant serum hyperviscosity is almost invariably caused by lymphoproliferative disorders (e.g., multiple myeloma, macroglobulinemia, lymphosarcoma; see Box 12-2). Hyperviscosity syndrome rarely occurs in gammopathies (monoclonal or polyclonal) caused by ehrlichiosis or other insidious diseases.

The diagnostic approach is described in Figure 12-9 under Monoclonal Gammopathy. Because lymphosarcoma and plasma cell myeloma are the major causes of serum hyperviscosity, aspiration of bone marrow, enlarged lymph nodes, splenic or liver lesions, or other masses is often indicated. If results are equivocal, biopsy and histopathologic evaluation of bone marrow or involved tissues are indicated.

CRYOPRECIPITATION

Cryoglobulins are usually monoclonal or complexed immunoglobulins that reversibly precipitate or gel at low temperatures but dissolve when heated. Cryoglobulins are rarely found in canine multiple myeloma and macroglobulinemia.

Rarely Indicated • Testing for cryoglobulins is indicated with paraproteins that precipitate or gel when blood or serum is refrigerated at 4° C.

NOTE: Cryoglobulins are not cold agglutinins (i.e., antibodies binding antigen reversibly at temperatures < 37° C).

Advantage • Detection of cryoglobulins is important, because failing to detect them may cause false-negative tests for hyperglobulinemia or monoclonal paraproteinemia in patients with clinical or laboratory evidence of hyperviscosity.

Analysis • The clinician should contact a reference lab about assaying for cryoglobulins.

Causes of Cryoglobulinemia • Macroglobulinemia or multiple myeloma of IgM and IgA classes may cause canine cryoglobulinemia. Finding cryoglobulinemia indicates diagnostic evaluation for lymphocyte and plasma cell neoplasia as described for monoclonal gammopathies.

ANTINUCLEAR ANTIBODY

Occasionally Indicated • Abnormalities suggestive of systemic lupus erythematosus (SLE), such as symmetric dermatitis principally distributed on the head and mucous membranes, hemolytic anemia, thrombocytopenia,

BOX 12-3. CAUSES OF INCREASED ANTINUCLEAR ANTIBODY TITER IN DOGS AND CATS

Systemic Lupus Erythematosus (SLE) (titer most consistently elevated in this disease)*

Skin Disorders

Pemphigus erythematosus (seldom pemphigus vulgaris)[†]

Discoid lupus

Generalized demodicosis

Flea bite hypersensitivity

Plasma cell pododermatitis

Hematologic Disorders

Immune-mediated hemolytic anemia (IMHA)

Immune-mediated thrombocytopenia (IMT)

Cardiopulmonary Disorders

Bacterial endocarditis

Dirofilariasis

Other Disorders

Cholangiohepatitis

Feline leukemia virus (FeLV)

Feline infectious peritonitis (FIP)

Rheumatoid arthritis

Lymphocytic thyroiditis

Various neoplasms

Ulcerative autoimmune stomatitis[†]

*Moderate to high titers.

[†]Moderate titers.

NOTE: ANA titer, if positive, in disorders without * or † is likely to be low.

nonseptic polyarthritis, myositis, proteinuria, or fever of unknown origin, are indications for measurement of ANA. Less common are neuromuscular, cardiac, or pulmonary abnormalities. The test can be used to monitor patients being treated for SLE.

Advantages • The test is simple and indicative of immune-mediated disease when positive with a relatively high titer in conjunction with compatible clinical abnormalities.

Disadvantages • ANA is not a disease-specific test (i.e., many diseases besides SLE may be associated with a low or occasionally high titer; Box 12-3).

Analysis • ANA is measured in serum by indirect immunofluorescence testing (IIT). The result should be reported as the highest dilution of a patient's serum causing definite staining of nuclei. Several patterns of nuclear fluorescence are recognized: homogeneous (diffuse), rim (peripheral), speckled (fine or large speckles), and nucleolar. Other tests are being developed for the diagnosis of SLE, such as flow cytometry using a microsphere assay to detect antihistone antibody (histone is a nuclear protein cited more commonly as an antigen associated with SLE formation), but thus far have not proven more effective in replacing IIT.¹¹

Normal Values • Values vary among laboratories owing to different substrates, controls, and procedures used. Accuracy requires procedural consistency and experience. Fetal and newborn sera do not stain nuclei. Most veterinary laboratories use tissue culture monolayers of human epithelial-2 (HEp-2) cells as substrate, which allows improved discernment of fluorescent patterns of staining and more standardized procedural consistency. When this test method was used in a comparative study involving 112 canine serum samples, a significant positive titer was established at a screening dilution of 1:25 for greater than 95% specificity, whereas a minimum significant ANA titer of 1:100 was established as the corresponding significant titer using rat liver sections, which identified the identical group of ANA-positive dogs at the same specificity of greater than 95%. A recent study in dogs using the flow cytometric microsphere assay¹¹ showed that ANA titers of less than 1:160 were considered exclusionary for SLE when one or fewer clinical signs or abnormal laboratory results for SLE were present, and titers greater than or equal to 1:160 were considered diagnostic for SLE when more than two clinical signs or abnormal laboratory results (see Causes of Increased Antinuclear Antibody Titer, later) were present. Sixty-one percent of the dogs in this study with SLE had titers greater than or equal to 1:640, indicating a good correlation between high titers and the presence of SLE. However, small numbers of dogs without SLE also had titers of greater than 1:160.¹⁴

Drug Therapy That May Alter Antinuclear Antibody Titer • Anything decreasing antibody synthesis (e.g., cytotoxic drugs, chronic or high-dose corticosteroid therapy) can decrease titers. Positive ANA titers have been attributed to treatment with griseofulvin, hydralazine, procainamide, sulfonamides, and tetracyclines. Positive ANA titers can occur in cats treated with propylthiouracil or methimazole. Some of these cats develop drug-induced immune-mediated hemolytic anemia (IMHA) and immune-mediated thrombocytopenia (IMT).

Artifacts • Improper reagent preparation, storage, or application; inadequate controls.

Causes of Increased Antinuclear Antibody Titer • A positive titer may occur in a number of infectious, inflammatory, and neoplastic disorders. A partial list of diseases (in addition to SLE) is given in Box 12-3. Normal dogs and cats can also have detectable ANA; however, these tend to be low titers. Positive titers obtained in disorders other than SLE are generally not markedly elevated; therefore it is important to consider values established by the laboratory for low, moderate, and high titers. Equally important is consideration of other clinical and clinicopathologic changes consistent with a diagnosis of SLE.

It is imperative in patients with suspected SLE to evaluate clinical presentation and results of laboratory data, including ANA. Clinical signs which indicate testing for SLE include polyarthritis, skin or oral cavity lesions, polymyositis, generalize immune-mediated disease, and (particularly in cats) neurologic disturbances. Abnormal laboratory results include thrombocytopenia, leukopenia, proteinuria, and hemolytic anemia.¹⁴ It is suggested

that at least one of these laboratory abnormalities be present prior to testing for SLE.¹⁴

A positive ANA titer is the most important criterion for diagnosis of SLE, providing established clinical criteria are met and exclusionary diagnoses are not made (e.g., FeLV or FIP infection, cholangiohepatitis, rickettsial or systemic parasitic diseases). No well-established patterns of ANA fluorescence in cats and dogs distinguish SLE from non-SLE immune-mediated diseases or other conditions associated with positive ANA titers (see Box 12-3). Although ANA titers are frequently higher in SLE than in other disorders, there can be overlap, especially when no inclusion criteria are considered.¹⁴ Likewise, magnitude of titer does not parallel severity of disease, high titers are not always indicative of SLE, and elevated but lower titers do not exclude a diagnosis of SLE. Periodic ANA titers may be useful in monitoring a lupus patient's response to therapy.

A positive ANA titer can be an indication for performing additional tests to distinguish SLE from non-SLE disease: dermatitis favoring mucocutaneous junctions is an indication for biopsy, histopathologic examination, and immunohistochemical or direct immunofluorescent testing (see *Miscellaneous Immunologic-Based Tests* later in chapter); hemolytic anemia is an indication for an antiglobulin (i.e., Coombs) test (see Chapter 3) and tests for hemoparasites; and swollen, painful joints are an indication for arthrocentesis, fluid analysis (see Chapter 10), and RF test (see later discussion).

LUPUS ERYTHEMATOSUS TEST

Occasionally Indicated • A lupus erythematosus (LE) test is indicated in cases of suspected SLE (see previous section on Antinuclear Antibody).

Advantages • The test is specific; it does not require species-specific reagents, and therefore is more widely available.

Disadvantages • The test is time-consuming and much less sensitive than the ANA test; it requires a very fresh blood sample.

Analysis • Depending on the laboratory, heparinized or clotted blood is used. Formation of LE cells *in vivo* is rarely demonstrated in routinely stained bone marrow smears or in joint fluid from patients with polyarthritis (Figure 12-10), but it is highly suggestive of SLE when present. A laboratory experienced in performing and interpreting LE cell tests is necessary.

Artifacts • LE cells must be differentiated from “tart” cells, which are neutrophils that have phagocytized intact nuclei. The LE cell test is complement-dependent, and low concentrations of complement, excessive heparin, or failure to use freshly drawn blood may cause false-negative results.

Drug Therapy Causing False-Negative Results • Steroid therapy alters test results. The LE cell test is more sensitive to effects of steroids than is an ANA titer.

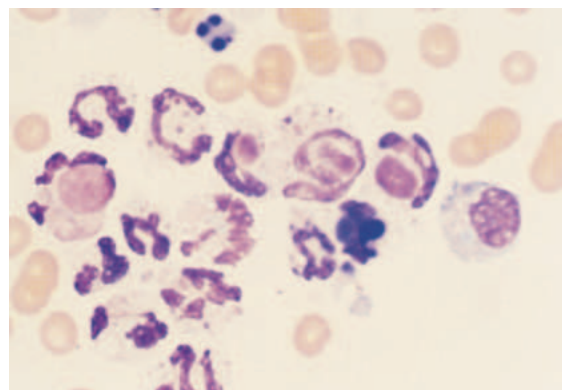


FIGURE 12-10 Canine lupus arthritis. This canine synovial fluid smear has one large lupus erythematosus (LE) cell in the far left, which is a neutrophil containing a large round violet LE body that is composed of nuclear proteins from a dead lysed cell bound to antinuclear antibodies. Many other neutrophils have multiple, smaller inclusions that are probably antibody-antigen complexes, and these white blood cells are called ragocytes. Both are indicative of immune-mediated joint disease.

Causes of Positive Lupus Erythematosus Cell Preparations • Ideally a minimum of three to four LE cells on a slide is necessary for a diagnosis of SLE. The test should be performed at least three times before results are considered negative. The test is specific for SLE but insensitive. Negative results are common in SLE patients that are ANA positive. Such patients may lack the particular auto-antibodies to histone-DNA involved in LE cell formation but have other types of ANA detectable by immunofluorescence. Positive test results may rarely be obtained in other diseases (e.g., osteochondritis dissecans, nonimmunologic joint disease, neoplasia, DIC). If a positive LE cell preparation is obtained, an ANA titer and tests to obtain other evidence of SLE are indicated (as described earlier under Antinuclear Antibody). An ANA titer is the preferred screening test for SLE.

ANTIGLOBULIN (COOMBS) TEST

See Chapter 3.

TESTS FOR IMMUNE-MEDIATED THROMBOCYTOPENIA

Dogs and cats showing marked thrombocytopenia (<50,000/ μ l) may have IMT. This is typically a diagnosis of exclusion because no widely available in-house test allows a definitive diagnosis of IMT. Bone marrow examinations in patients with IMT often exhibit megakaryocytic hyperplasia, which would strongly support a clinical diagnosis of IMT.¹² However, IMT cannot be excluded in a differential diagnosis if bone marrow examination reveals megakaryocytic hypoplasia, because the immune-mediated process can also target megakaryocytes.

Measurements of platelet-associated antibodies by flow cytometry and immunoradiometric assay for detection of platelet-associated immunoglobulins are examples of specific tests used for the potential diagnosis of IMT.^{13,16} However, both IMT and non-immune-mediated diseases (e.g., infectious, neoplastic, inflammatory) can have platelet-associated antibodies present, so detection of antiplatelet antibodies does not definitively make a diagnosis of IMT.⁵ Likewise, these tests generally require blood samples to be handled in a very specific fashion and require specialized laboratories.

RHEUMATOID FACTOR TEST

Rarely Indicated • An RF test is indicated in dogs, particularly small breeds, suspected of having rheumatoid arthritis (RA) because of lameness, heat, swelling, or pain of multiple joints, particularly peripheral joints. Crepitation, joint laxity, and erosive synovial changes may be detected in chronic cases. Nonspecific signs include anorexia, fever, depression, and reluctance to move and may precede clinically or radiographically detectable evidence of joint disease.

Disadvantages • The test is insensitive (i.e., many false-negative results) and not highly specific for RA in dogs because of relatively low titers.

Analysis • Serum is submitted refrigerated, but it is always advisable to contact the reference lab to determine how to ship samples properly. The Rose-Waaler test (adapted from the human test for RF) is traditionally considered the gold standard test for canine RF, although turbidimetric, nephelometric, and ELISA assays are newer tests used by human laboratories for human RFs.¹ Canine RFs are autoantibodies targeting the Fc portion of IgG but may also be mixed complexes of IgG, IgA, and IgM. Factors are detected by incubating rabbit IgG-sensitized sheep red blood cells (RBCs) with serial dilutions of the patient's serum (the rabbit Rose-Waaler test). If RF is present, agglutination occurs. Because canine sera often contain naturally occurring antibodies to sheep RBCs, a control must be used with unsensitized sheep RBCs. If agglutination to an equal or higher dilution appears in the control, natural antibodies must be absorbed from the sera before testing for RF. Latex agglutination tests for canine RF are not recommended because results are poorly reproducible and lack specificity. Latex agglutination titers may differ substantially from Rose-Waaler titers. The ELISA test is able to detect all classes of immunoglobulin and is considered an acceptable alternative to the Rose-Waaler test.¹

Results • For the Rose-Waaler test in normal animals, the differential titer (i.e., difference between titers at which agglutination of unsensitized versus sensitized sheep RBCs occurs) should be less than eight.² A titer against sensitized RBCs not corrected for the actual titer of natural antibodies to sheep RBCs can be misleading because some normal animals have higher titers of naturally occurring antibodies to sheep RBCs. Some

laboratories perform the Rose-Waaler test by preabsorbing all test sera with sheep RBCs. In this case a titer of less than 1:16 is expected in normal dogs.

Artifacts • Serum submitted for RF testing should not be frozen because RF activity (especially IgM) may be destroyed, causing false-negative results. Canine RF tends to self-associate, forming multimeric complexes that significantly lower detectable titers. Wide, sometimes negative, fluctuations in RF titer occur over time; these fluctuations do not appear to correlate with disease severity. False-positive results may occur in the Rose-Waaler test if a patient's serum has antibodies against sheep RBCs and appropriate controls or absorption of these antibodies is not performed.

Causes of an Increased Rheumatoid Factor Titer • Because RF is an antibody against Fc fragments of immunoglobulin molecules that become exposed only after antibody binds to antigen, any disease with long-standing immune complexes can eventually induce RF formation. In the Rose-Waaler test, a differential titer of greater than or equal to 1:8 is positive for RF.² Between 40% and 75% of dogs with RA have a positive RF test result. Hence, a negative result does not eliminate RF. The RF test is rarely positive in normal dogs and occasionally positive in some patients with SLE, because RF may be a part of the SLE complex. Incidence of RF in other arthropathies and systemic diseases has not been adequately studied via the Rose-Waaler method, but other methods have shown RF in titers comparable with those of patients with RA in these arthropathies. Therefore, a positive RF test result should never be the sole criterion for a diagnosis of canine RA (*important*).

RA is a progressive, erosive, immune-mediated polyarthritis that must be differentiated from other types of joint diseases, preferably before joint destruction occurs. Unfortunately no test for canine RF is highly reliable in making this distinction. Other routine tests indicated in making the diagnosis include joint radiographs and synovial fluid analysis. Septic polyarthritis often causes erosive lesions, especially involving larger joints, plus evidence of sepsis on routine blood and synovial fluid examinations (see Chapter 10), including culture. In contrast, RA typically causes erosive lesions of smaller peripheral joints before progressing to larger joints. Radiographic lesions may be lacking or inconclusive early in the course of RA. Furthermore, no distinguishing cytologic features can reliably differentiate among RA, SLE, and other types of immune-mediated joint disease that have similar joint fluid cytology. Histopathologic examination of synovium from affected joints is the most reliable means of diagnosing canine RA.

Histopathologic examination of synovium allows an early diagnosis and therapy in patients lacking classic radiographic changes. An ANA titer helps distinguish SLE from RA; but it is occasionally positive in RA. Finding both types of autoantibodies may represent the rare, combined occurrence of both SLE and RA (i.e., a so-called overlap syndrome) or merely the appearance of multiple autoantibodies in a patient with RA or SLE. In either case, clinical criteria are required for diagnosis.

Miscellaneous Immunologic-Based Tests • Numerous immunologic-based tests are available in select laboratories. These include immunostaining of tissues, indirect immunofluorescent testing, tests for cellular immunity, phagocyte function assays, enumeration of proportions of T and B lymphocytes, and evaluation of lymphocyte functions *in vitro*.

Occasionally Indicated • In general, tests are performed on samples where an immunologic-based disease is suspected or a specific categorization of neoplasia (e.g., T-cell or B-cell lymphosarcoma) is needed for both definitive diagnosis and/or treatment considerations. The clinician should consider performing immunostaining or indirect immunofluorescent testing on skin lesions from mucocutaneous lesions where immune-mediated diseases such as pemphigus vulgaris or foliaceus, discoid lupus erythematosus, bullous pemphigoid, and the like are suspected. Similar testing on tissues with immune-mediated diseases targeting basement membranes (e.g., renal glomerular disease) can be performed. One should also consider performing tests for cellular immunity, phagocyte function assays, proportions of T and B lymphocytes, or lymphocyte function assays on animals with recurrent infections and suspicion of an immunodeficiency or white blood cell disorder (e.g., leukocyte adhesion deficiency). Immunohistochemistry or immunocytochemistry should be considered to differentiate types of tumor (e.g., histiocytic sarcoma versus anaplastic carcinoma) or specifically categorize a tumor (e.g., B-cell lymphosarcoma versus a T-cell lymphosarcoma).

Advantages • Results of tests can give specific causes of diseases where histopathology or routine diagnostic testing may only suggest a cause. In some instances, test results may alter therapeutic regimens to treat a specific type of disease or cancer, such as with a T- or B-cell lymphosarcoma.

Disadvantages • Positive or negative test results may not entirely exclude or include certain diseases due to variability of disease and testing measures. Collecting specimens for these highly specialized tests needs to be performed carefully and with knowledge of how to properly obtain and process the samples. Administration of immunosuppressive drugs or corticosteroids or high levels of endogenous corticosteroids (e.g., hyperadrenocorticism) can lead to false-negative testing for some immune-mediated disorders.

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13

Reproductive Disorders

Cheri A. Johnson

GENERAL DIAGNOSTIC APPROACH

Clinicopathologic testing of the reproductive system is often indicated in normal animals for the purposes of optimizing reproductive performance, and in animals with urogenital disorders. The most important diagnostic procedures are history and physical examination, which will direct the remainder of the evaluation. The history for all females should include questions regarding changes in the mammary conformation and mammary secretions, as well as changes in the appearance of the external genitalia and the presence of vulvar discharge, and whether the changes are cyclic in nature. The history should also investigate changes in behavior, such as maternal behavior, sexual activity, excessive licking of genitalia, or changes in urinary patterns. For breeding females, the history of estrous cycles should include the dates of the onset of each cycle, physical and behavioral characteristics of the female during each cycle, duration of each estrous period, and breeding dates and method of insemination (natural or artificial). The stud's reproductive performance with other bitches before and after breeding the bitch in question is important. Dates and methods of pregnancy examination and any signs of abortion should be noted. If pregnancy occurred, was parturition normal? What were gestation length and litter size?

For all males, history should investigate changes in the appearance of external genitalia, preputial discharge, excessive licking of genitalia, and changes in urinary patterns. For breeding males, history should include libido, mating ability, number of females bred, conception rate, breeding methods (i.e., natural or artificial insemination, frequency of use, how insemination dates are chosen), and reproductive performance of bitches before and after being bred to the male in question. Reproductive history should include methods used to screen for infectious diseases affecting reproduction (e.g., *Brucella canis* in dogs and viral rhinotracheitis in cats) (see Chapter 15), medications known to affect reproductive function (e.g., glucocorticoids), familial association with infertility, and stressful activities such as racing or being shown.

Physical examination of male reproductive tract includes inspection of the penis and preputial and scrotal skin. Both testes, epididymides, and spermatic cords

should be palpated. The prostate is palpated rectally and transabdominally. Physical examination of the female includes inspection and palpation of mammary glands and vulva. The uterus is palpated transabdominally. In bitches of adequate size, the posterior vagina is palpated with a gloved finger; rectal palpation may also be helpful.

VAGINAL CYTOLOGY

Many reproductive disorders cause abnormal vaginal cytologic findings, even when no vulvar discharge is present. When a discharge is present, one must first differentiate normal from abnormal and then identify the cause. The first step is vaginal cytology. The morphologic characteristics and numbers of vaginal epithelial cells, red blood cells (RBCs), white blood cells (WBCs), and bacteria are noted, as is the presence of other elements (e.g., neoplastic cells, mucus, debris, uteroverdin, endometrial cells, macrophages). The effects of estrogen on vaginal epithelial cells are useful indicators in animals suspected of having ovarian remnants or hyperestrogenism, and for breeding animals to monitor the stages of the estrous cycle, to determine breeding and whelping management, to investigate abnormal estrous cycles, and to investigate possible "mismating."

Using a speculum, a saline-moistened cotton-tipped swab is inserted into the vagina beyond the urethral orifice, or saline is flushed into and aspirated from the vagina with a pipette, being careful to avoid the clitoral fossa and skin when obtaining the sample. Next, the swabs are gently rolled onto slides, or drops of the saline aspirate are placed on slides. Slides are then fixed and stained. Modified Wright-Giemsa stain (Diff-Quik), new methylene blue (NMB), Wright-Giemsa stain, Wright stain, and trichrome stain are commonly used, but many others are acceptable. A minimum of 100 epithelial cells are examined and tabulated.

Hemorrhage

RBCs are common in normal and abnormal vulvar discharges (Box 13-1); their significance is determined by accompanying cells. Superficial (cornified) vaginal

BOX 13-1. CAUSES OF HEMORRHAGIC VULVAR DISCHARGE IN THE BITCH AND QUEEN**With Mainly Superficial (Mature) Epithelial Cells (estrogen influence)**

Normal estrus; or late proestrus or early diestrus (most common)

Ovarian remnant (common)

Ovarian pathologic condition (i.e., cystic follicles, functional ovarian tumor)

Exogenous estrogen

Without Superficial (Mature) Epithelial Cells (no estrogen influence)

Normal lochia, early postpartum (most common)

Subinvolved placental sites, late postpartum (young, primiparous bitches)

Vaginal laceration

Neoplasia of vagina or uterus (most common cause in old bitches)

Uterine torsion

Bleeding disorder

epithelial cells with RBCs are expected during normal proestrus and estrus but may also occur because of exogenous estrogens or ovarian pathology such as follicular cysts or ovarian remnants. RBCs mixed with mucus are found in lochia, the normal postpartum vaginal discharge. When cytology reveals peripheral blood with occasional intermediate and parabasal epithelial cells, causes of hemorrhage should be sought. These include subinvolution of placental sites, vaginal laceration, uterine and vaginal neoplasia, uterine torsion, and coagulopathies.

Subinvolution of placental sites is a postpartum disorder characterized by a bloody discharge persisting for longer than 8 to 12 weeks. It usually occurs in otherwise healthy primiparous bitches; blood loss is rarely significant. Diagnosis is based on history, physical, and cytologic findings. Diagnosis can be confirmed by a histopathologic assessment of placental sites, but such is rarely necessary. Vaginal lacerations are relatively uncommon but may occur from breeding trauma, dystocia, obstetric procedures, or vaginoscopy. If vaginal laceration is suspected, endoscopy is indicated.

Leiomyoma is the most common uterine and vaginal tumor in small animals, and hemorrhagic vulvar discharge, with or without a palpable mass, is the most common sign. Leiomyomas do not readily exfoliate; therefore neoplastic cells are rarely found cytologically. Uterine torsion occurs almost exclusively in periparturient females. Abdominal pain and unrelenting straining are the prominent findings, and a hemorrhagic discharge is common. Abdominal ultrasound is indicated. Vulvar bleeding is rarely the only clinical sign of a coagulopathy. If a bleeding disorder is suspected, a hemostatic profile (see Chapter 5) is indicated.

NOTE: Bloody vulvar discharge, even when associated with attraction of male dogs, does not always represent proestrus or estrus (see Box 13-1).

Purulent or Septic Discharge

Bleeding can accompany any inflammatory process. If the WBC:RBC ratio is more suggestive of an exudate than of peripheral blood, an inflammatory disease should be sought. WBCs suggest inflammation, but they are normally found in large numbers during the first 1 to 2 days of diestrus and in lesser numbers in normal lochia. Septic (i.e., bacteria seen) or purulent vulvar discharges may originate from vulva, vestibule, vagina, or uterus. The source of a septic/purulent exudate determines prognosis and must be identified. Vaginoscopy is a reasonable next step. Hyperemia, edema, or mucosal lesions should be obvious with vestibulitis or vaginitis. These would not exclude concurrent uterine pathology (Figure 13-1). If endoscopy suggests a uterine and/or cervical source of discharge or if endometrial cells are found during cytologic evaluation, uterine involvement is probable and abdominal ultrasonography is indicated.

Knowing the stage of the estrous cycle is essential for evaluating uterine pathology. During the luteal phase (diestrus), a septic or purulent discharge can occur because of pyometra, pregnancy with concurrent uterine infection, or impending abortion. If the discharge occurs after parturition, postabortion, or otherwise during anestrus, metritis or uterine stump granuloma/abscess should be considered.

Neoplastic Cells

Transmissible venereal tumors (TVTs) and transitional cell carcinomas (TCCs) exfoliate readily. Animals with TVTs usually are presented because of a mass. When TCC involves the urethral papilla, neoplastic cells may be found on vaginal cytology. Fine-needle aspiration of vaginal and vulvar masses is another way to obtain specimens. If cytology does not allow definitive diagnosis of neoplasia, histopathologic assessment of an incisional or excisional biopsy should. Additional diagnostic tests for staging and treatment planning are dictated by tumor type.

Mucus

This is the predominate component of lochia. Mucoïd discharge may also occur during normal late pregnancy and possibly in scant amounts during the nonpregnant, luteal phase (i.e., diestrus). Further testing of these otherwise healthy animals is usually unnecessary. Cervicitis, mucometra, and androgens can also cause mucoïd discharge. Conversely, a scanty mucoïd vulvar discharge is apparently normal in some otherwise healthy bitches (Box 13-2).

Uteroverdin

This dark-green blood pigment is normal in the canine placenta. Its presence in vulvar discharge indicates separation of at least one placenta. This is normal during stage II of labor when accompanied by delivery of puppies, but at all other times it indicates a placental problem.

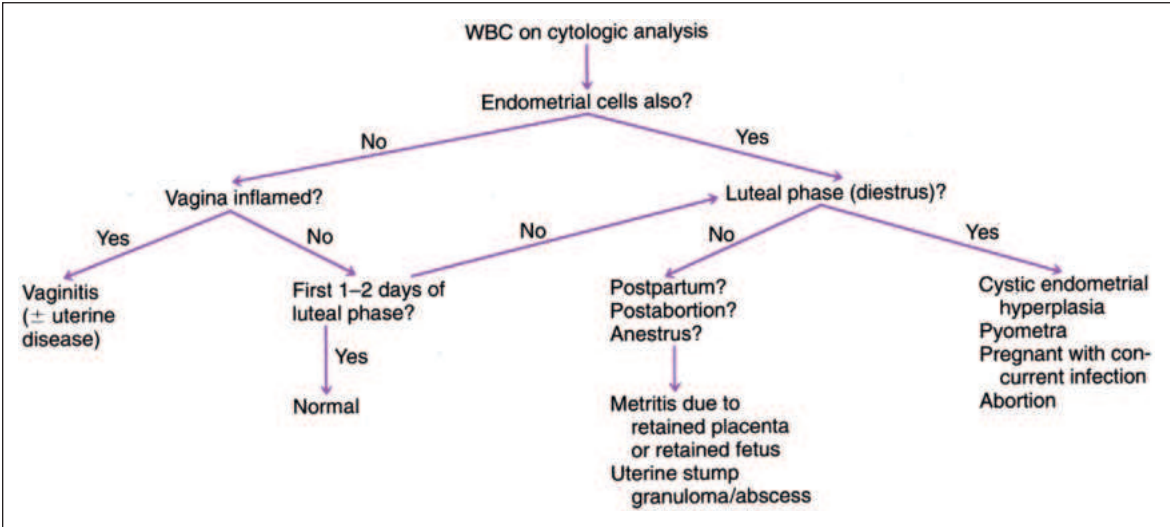


FIGURE 13-1 Diagnostic considerations for purulent or septic vaginal cytology. WBC, White blood cells.

TABLE 13-1. VAGINAL CYTOLOGIC FINDINGS DURING THE ESTROUS CYCLE

	PROESTRUS	ESTRUS	DIESTRUS	ANESTRUS
Parabasal	+	–	–	Occasional
Intermediate	+	Rare	+	Occasional
Superficial- intermediate	Occasional	+	±	Rare
Superficial	Rare	90%	Rare	–
RBCs	–	+	–	–
WBCs	+	±	± first day	–

RBCs, Red blood cells; WBCs, white blood cells.

Vaginal Epithelial Cells

Estrogen causes proliferation and cornification of vaginal epithelium. Therefore vaginal cytology can be used to monitor estrous cycle, especially the follicular phase of proestrus and estrus when ovarian follicles are producing estrogen (Figure 13-2). During proestrus, parabasal, intermediate, and some superficial cells are exfoliated. RBCs, WBCs, and bacteria are present. As estrogen increases,

there is a gradual increase in numbers of cornified epithelial cells and a decrease in WBCs. During estrus, superficial (cornified) cells predominate, eventually accounting for greater than 90% of the exfoliated epithelial cells. RBCs and extracellular bacteria are often present (Table 13-1). WBCs are absent during estrus unless concurrent inflammation exists.

Diestrus (luteal phase) is marked by an abrupt change: parabasal and intermediate cells outnumber the superficial cells. Sheets of epithelial cells are often noted at the onset of diestrus. WBCs almost always return at this time. RBCs and bacteria are often present. Thus it is often impossible to base differentiation of late proestrus from early diestrus on a single vaginal smear. Fewer cells are exfoliated during anestrus; therefore the preparations are relatively acellular. Parabasal and intermediate epithelial cells, with or without a few WBCs and bacteria, are present during anestrus (see Table 13-1). Similar changes occur during the feline estrous cycle, except that RBCs and WBCs are uncommon.

BOX 13-2. CAUSES OF MUCOID VULVAR DISCHARGE

Normal (most common cause in intact bitches)

- Lochia
- Late pregnancy
- Luteal phase (diestrus)

Androgenic stimulation

- Endogenous (intersex)
- Exogenous (mibolerone, testosterone)

Cervicitis

Mucometra

Idiopathic (?) (most common cause in spayed bitches)

Canine Breeding and Whelping Management

Determining stages of the estrous cycle helps determine optimal breeding dates to maximize conception and litter size and to predict whelping dates more accurately. When

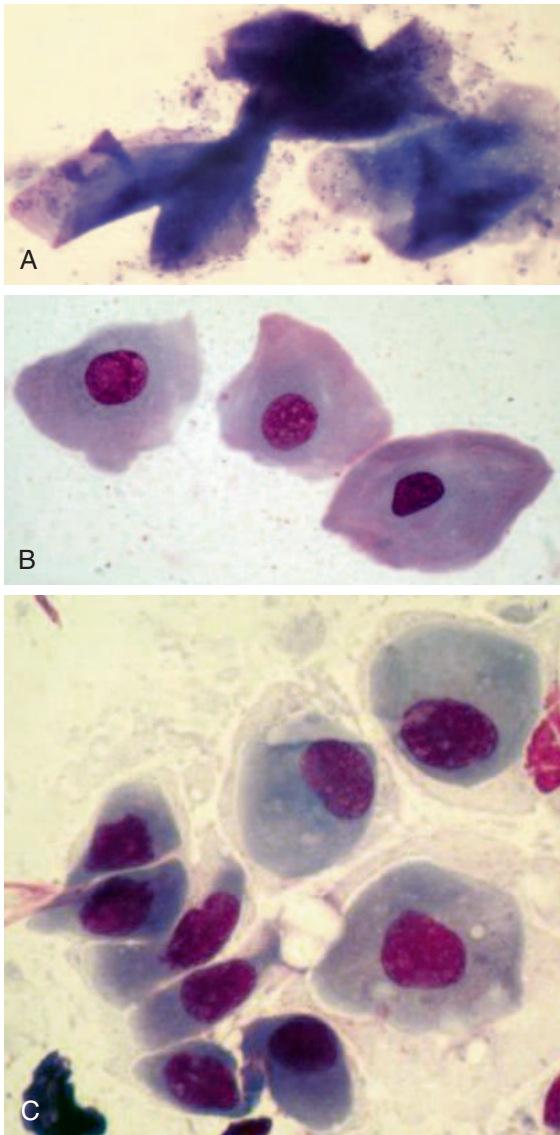


FIGURE 13-2 Typical cells visible in vaginal cytology preparation. **A**, Superficial (cornified) anuclear squamous cells with bacteria. **B**, Intermediate cells. **C**, Six parabasal cells (smaller cells to the bottom and the left) and three intermediate cells (larger cells to the right). (Courtesy of Dr. Harold Tvedten.)

using vaginal cytology as the only laboratory aid to breeding management, maximal conception rates and litter size are obtained when normal bitches are bred on the first day of estrus and again 3 or more days later. Although it may not change conception rates or litter size, breeding every other day is also acceptable (Box 13-3). Normal gestation length varies from 58 to 72 days after the first breeding date. When gestation length is calculated from the first day of cytologic diestrus, 93% of bitches whelp 57 days after day one of diestrus. Calculating gestation length from cytologic diestrus instead of breeding dates is helpful for recognizing prolonged gestation or premature delivery.

BOX 13-3. CANINE BREEDING MANAGEMENT

1. Choose only healthy, *Brucella canis*-negative, normal animals for breeding.
2. Identify the first day of estrus.
 - a. Begin early (day 3) in season (proestrus).
 - b. Check the bitch every other day by behavioral (tease with experienced stud) and/or vaginal cytology examination.
3. And/or use ovulation timing according to serum progesterone or LH (see text).
4. At least 2 breedings, 48 hours apart, are recommended.
5. Pregnancy examination is indicated 20 to 30 days later.
 - a. If pregnant, discuss pregnancy and parturition management.
 - b. If not pregnant, determine serum progesterone to assess ovulation and luteal function.

Mismating

Vaginal cytology may help determine whether a bitch has recently been bred, because sperm heads (not intact sperm) are detected in vaginal smears from 68% and 50% of bitches bred 24 and 48 hours earlier, respectively. Absence of sperm does not rule out recent copulation. Estradiol cypionate (ECP) is NOT recommended to prevent pregnancy following mismating; when vaginal cytology indicates diestrus, 25% of bitches given ECP develop pyometra. ECP may also cause mild to fatal bone marrow suppression.

MAMMARY GLANDS

The most common cause of mammary gland enlargement and secretion is lactation, but mastitis, hyperplasia, and neoplasia are also common.

Mastitis

A bacterial infection of one or more lactating mammary glands, mastitis is most common in postpartum bitches. It is rare in queens and in bitches experiencing false pregnancy. Diagnosis is based on a history of recent parturition and finding fever, lethargy, and warm, swollen, painful glands with purulent milk. If milk is not visibly abnormal, cytology of milk or a mammary aspirate shows purulent inflammation, which is often septic. Galactostasis (i.e., accumulation and stasis of milk) also causes warm, swollen, painful glands, but the dam is healthy, no bacterial infection is seen, and milk is cytologically normal. Galactostasis is most common during weaning.

Mammary Hyperplasia

Mammary hyperplasia (i.e., fibroadenomatous change, fibroadenoma) is characterized by rapid abnormal mammary growth during diestrus or pregnancy in cycling

queens. It has also been reported in neutered queens and toms treated with progestins. If history confirms progesterone stimulation, mammary hyperplasia is suspected. If one is in doubt, biopsy is indicated to distinguish it from neoplasia.

Mammary Neoplasia

Mammary neoplasia is common in middle-aged to older female dogs and cats. It is characterized by mammary mass(es), often with abnormal secretions from the nipple. Fine-needle aspiration of the mass is easy; however, cytologic findings must be interpreted cautiously (see Chapter 16) and are often nondiagnostic. Excisional biopsy and a histopathologic assessment best indicate type and degree of malignancy.

SEMEN EVALUATION

Semen evaluation is indicated whenever there is a possibility of male infertility (Figure 13-3); it is also routine in prebreeding examinations. Cytology of ejaculates is also used to evaluate prostatic, testicular, and epididymal diseases.

Technique

Semen is collected by manual stimulation of the dog’s penis. Care should be taken when handling the semen sample. All equipment should be clean and free of contaminants (e.g., water, excessive lubricant) that may affect sperm viability. Samples should be protected from temperature shock. Dog semen can be handled at room temperature for approximately 15 minutes without adverse effect. Nevertheless, samples should be processed promptly. Slides and coverslips should be maintained at 37° C. The semen sample is evaluated for volume and color, as well as concentration, motility, and morphology of spermatozoa (Table 13-2).

Volume

Canine semen is comprised of three fractions that are ejaculated continuously. The first fraction is clear prostatic fluid; volume is usually several drops. Some

individuals may have up to 2 ml of this fraction. The second fraction is sperm-rich; volume is from 0.5 to 5 ml. The third and largest fraction is prostatic fluid. Normal prostatic fluid is clear and easily differentiated from milky sperm-rich fraction. For routine semen evaluation, clinicians should collect only enough prostatic fluid to ensure that the entire sperm-rich portion has been obtained.

Color

Color is evaluated by direct visualization. Normal canine semen is usually the color of skim milk. An abnormally colored sample should be closely examined for foreign matter; contaminants may decrease sperm viability. A yellow appearance may represent urine contamination. Blood colors the sample pink or red and is usually of prostatic origin or from penile abrasions. The latter is identified by prompt visual inspection of the penis.

Inflammatory cells may cause samples to appear flocculated or yellow-green. These cells can originate anywhere in the urinary or reproductive tracts. Preputial contamination is common. When leukospermia occurs, the source should be sought and the sample cultured for bacteria and *Mycoplasma* spp. Dogs should be tested for *Brucella canis* infection (see Chapter 15).

Concentration

The concentration of the sperm is affected by the relatively large volume of the sperm-free prostatic fluid. Therefore number of spermatozoa is reported as the total sperm per ejaculate, not sperm per milliliter. An easy way to count spermatozoa is using a hemocytometer (see Chapter 2). Either the RBC or the WBC and platelet Unopette may be used for canine semen. The RBC pipette dilutes the sample 1:200. The WBC and platelet pipette dilutes the sample 1:100. Dilution may be unnecessary for oligospermic samples. Values of 250 × 10⁶ to 2000 × 10⁶ sperm per ejaculate are normal. Breed variation is considerable because sperm production is directly related to testicle size.

Motility

A drop of semen is placed on a warm slide (37° C), covered with a warm coverslip, and allowed to sit on the slide warmer for 15 to 30 seconds. It is then examined microscopically for progressive motility. Spermatozoa should move in a rapid, steady, forward manner across the field. One hundred sperm are examined, and the percentage having progressive motility is estimated. Seventy percent to 75% motility is expected, but dogs with good fertility usually have greater than 80%. Wave patterns (e.g., as seen in bull semen) are rare in canine semen because the sperm concentration is too low. Exposure to heat or cold, excessive lubricant, water, urine, inflammatory cells, and the like can diminish percentage and vigor of motility. This commonly occurs with chilled and frozen thawed semen used for artificial insemination.

TABLE 13-2. CHARACTERISTICS OF NORMAL CANINE SEMEN

FRACTION	VOLUME	COLOR
Pre-sperm	Drops (rarely several ml)	Clear
Sperm-rich	0.5–5.0 ml	Cloudy white, opalescent
Prostatic fluid	1–20 ml	Clear
Total sperm/ ejaculate	250 × 10 ⁶ to 2000 × 10 ⁶	
Motility	≥80% progressively motile	
Morphology	<25% abnormal	

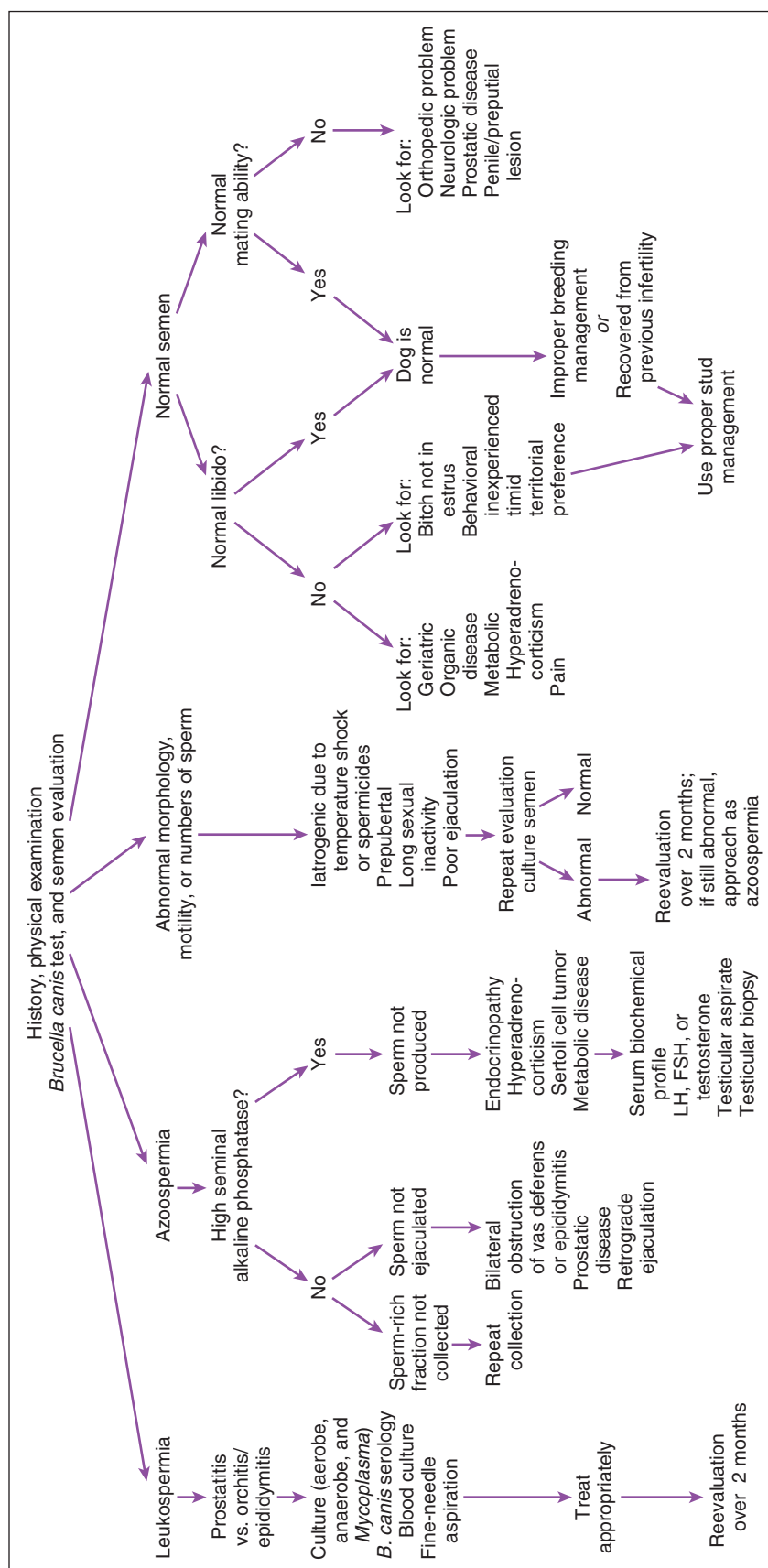


FIGURE 13-3 Diagnostic approach to male infertility. FSH, Follicle-stimulating hormone; LH, luteinizing hormone.

Morphology

Phase-contrast light microscopy is ideal for evaluating sperm morphology but has limited availability. Several stains may be used to evaluate morphologic characteristics of sperm. Unfortunately, some stains cause morphologic defects in canine sperm. These defects may reflect inappropriate osmolality or pH. Common morphology stains contain eosin-nigrosin and typically do not stain cells other than spermatozoa. Samples may also be stained with NMB or Wright stain. At least 100 (preferably 200) spermatozoa are examined and classified as being normal or abnormal. If a spermatozoon has more than one abnormality, it is classified according to the most severe abnormality. Abnormalities affecting size and shape of the sperm head, acrosome, and midpiece and proximal droplets are usually considered most severe. Loose or detached heads that are otherwise normal and bent tails are considered less severe; however, they are often the first abnormalities noticed after testicular insult. Dogs with good fertility usually have less than 20% to 25% abnormal sperm. Abnormalities resulting from improper sample handling should disappear in subsequent, properly handled samples. One of the most common iatrogenic changes in canine semen is bent tails.

During the morphologic examination, presence of other cell types or foreign matter should be recorded. If excessive numbers of cells other than spermatozoa (e.g., WBCs) are found, their source should be investigated. These cells are most easily evaluated when stains such as Wright are used. Crystals are a common type of foreign matter. They may be found in samples contaminated with urine or talc.

Seminal Alkaline Phosphatase

Alkaline phosphatase is produced by the canine epididymis and is found in high concentrations (i.e., >1000 IU/L) in normal ejaculates. Determination of seminal plasma alkaline phosphatase helps evaluate azoospermic semen samples. The sample is centrifuged as for serum (e.g., 3000 rpm for 10 minutes), and the supernatant seminal plasma is harvested. Concentration of alkaline phosphatase in seminal plasma is then determined in the same manner as for serum alkaline phosphatase (see Chapter 9). When an azoospermic sample has low seminal alkaline phosphatase (i.e., <300 IU/L) there is either bilateral obstruction from the epididymides, or the sperm-rich fraction was not actually ejaculated. If the sample has high alkaline phosphatase but no spermatozoa, testicular failure or obstruction from the testes to the epididymides exists. Azoospermic animals with seminal alkaline phosphatase in the midrange require additional testing.

Interpretation of Semen Evaluation

Semen samples do not reflect testicular or epididymal function on the day the sample is collected. Spermatogenesis requires approximately 60 days in dogs. To help establish a prognosis or resolve doubt about causes of an unsatisfactory sample, the dog should be reevaluated

several times over at least 2 months. A dog's age, breed (testicle size), and frequency of use must be considered before pronouncing a sample "satisfactory" or "unsatisfactory." Finding semen of satisfactory quality is not proof of fertility; a male must also have normal libido and mating ability (see Figure 13-3). Likewise, presence of unsatisfactory semen does not necessarily signify sterility, unless azoospermia or complete necrospemia (i.e., dead spermatozoa) is found.

PROSTATE

Prostatic disease (e.g., benign prostatic hyperplasia, bacterial prostatitis, prostatic abscess, prostatic and paraprostatic cysts, prostatic neoplasia) is common in older male dogs. History and physical examination (especially rectal palpation) often localize the problem to the prostate, but additional tests are necessary to determine the cause. Prostatic fluid is the third and largest fraction of canine ejaculates; therefore evaluation of ejaculated prostatic fluid has been advocated for diagnosis of prostatic disease. Prostatic massage, fine-needle aspiration, and biopsy of the prostate can also be performed.

Ejaculate characteristics correlate well with histopathologic examination of biopsy specimens from dogs with benign prostatic hyperplasia and prostatitis. Hemorrhage is the most frequent abnormality in semen from dogs with prostatic hyperplasia, whereas inflammation is the most frequent abnormality from dogs with chronic bacterial prostatitis. Because prostatic fluid normally refluxes into the urinary bladder, urinary tract infection and prostatitis frequently coexist.

Percutaneous fine-needle prostatic aspiration and biopsy, especially with ultrasonographic guidance, are excellent methods of obtaining material for microbiologic and cytologic evaluation. Biochemical evaluation of canine prostatic fluid has not been helpful in differentiating various prostatic diseases. Serum and seminal prostate-specific antigen (PSA) and prostatic acid phosphatase are often unchanged in dogs with prostatic disease. In dogs with benign prostatic hyperplasia, canine prostate-specific esterase (CPSE) is increased relative to normal dogs, but it is increased with other prostatic diseases as well.

TESTES AND EPIDIDYMIDES

The most important diagnostic procedure for evaluation of the testes and epididymides is physical examination, but ultrasound, fine-needle aspiration, and semen evaluation are also useful. Fine-needle testicular aspiration is easily performed using a 25-gauge needle and is especially helpful in evaluating focal lesions. It may also be performed to demonstrate the presence or absence of spermatogenesis, but histopathology is necessary to assess the entire spermatogenic cycle. Aspiration of epididymides causes more discomfort and may result in spermatic granuloma formation.

ENDOCRINE EVALUATION

Luteinizing hormone (LH), estradiol, progesterone, and testosterone assays are offered by several commercial veterinary diagnostic laboratories. Normal ranges vary considerably from laboratory to laboratory. In-house tests for LH, progesterone, and relaxin are also available.

Luteinizing Hormone

Serum concentrations of LH are used to predict ovulation (i.e., “ovulation timing”) and to determine presence or absence (i.e., already been spayed or neutered) of gonads. Serial determination of LH beginning during proestrus can identify preovulatory LH surges in bitches. Because duration of the LH surge averages about 24 hours, daily measurements are made. Ovulation is expected approximately 2 days after the LH surge, and canine oocyte maturation is completed about 2 to 3 days later. Therefore first insemination is timed for about 4 to 6 days after the LH surge to optimize conception and litter size in dogs.

Following gonadectomy, serum LH and follicle-stimulating hormone (FSH) concentrations are chronically elevated because negative feedback from gonadal sex hormones is gone. Conversely, when gonadal tissue is present, LH will be low except during the preovulatory LH surge. Measuring serum LH is useful for determining if a female has already been spayed, for determining if there is ovarian remnant left after spay, and for differentiating a castrated male from one with cryptorchidism. Finding low LH indicates presence of gonadal tissue or, much less likely, exogenous estrogen or androgen. Finding high LH indicates one of two things: either there is no gonadal tissue, or the female is in heat and about to ovulate (i.e., the LH surge). The latter can be confirmed by physical examination for signs of heat, by vaginal cytology for evidence of cornified epithelial cells (i.e., estrogen), by finding serum progesterone greater than 2 ng/ml (6 nmol/L), or by measuring LH days later when an LH surge would have subsided. Interpretation of FSH is easier because serum concentrations are consistently higher in spayed than in intact bitches. At present there is no commercially available FSH assay.

Progesterone

Measurement of serum progesterone concentration is used to predict and confirm ovulation, assess remnant ovarian tissue, monitor luteal function, and predict parturition. Progesterone concentrations can be used for ovulation timing in bitches because transition of progesterone concentration from anestrus levels of less than 1 ng/ml to greater than 2 ng/ml (6 nmol/L) coincides with the LH surge. Unlike the need for daily LH determinations, measuring progesterone every 2 to 3 days is usually adequate. Breeding can be timed using progesterone as it is with LH (i.e., as a preovulatory event) or, breeding can be timed according to postovulation progesterone concentrations. Pregnancy rates have been best when insemination is performed when progesterone concentrations are greater than 8 ng/ml (25 nmol/L) up to 19 to 26 ng/ml (60 to 80 nmol/L). Progesterone

concentrations greater than 8 ng/ml (25 nmol/L) indicate that ovulation has occurred.

In a bitch, luteal function normally lasts longer than 60 days after estrus, whether or not pregnancy occurs. In a queen that ovulated but did not become pregnant, luteal function persists for approximately 40 days. Low concentrations of progesterone during this time (i.e., expected diestrus) indicate failure to ovulate or failure to maintain normal luteal function (Figure 13-4). During expected anestrus phase of the cycle, finding serum concentrations greater than 2 ng/ml may indicate that ovarian tissue is still present in a previously spayed animal, or that luteal cysts or progesterone-producing granulosa cell tumors are present.

Serum progesterone concentrations greater than 2 ng/ml (6 nmol/L) are necessary to maintain pregnancy in bitches. Parturition begins within 24 hours of progesterone dropping below 2 ng/ml. Progesterone concentrations can help predict parturition and manage dystocia. Parturition is expected 65 ± 1 days after the preovulatory rise (i.e., LH surge) and within 24 hours of the parturition decline to less than 2 ng/ml. Monitoring serum progesterone helps determine the efficacy of abortifacients and treatment of luteal cysts with prostaglandins. In both situations, progesterone must be less than 2 ng/ml to ensure treatment success.

NOTE: Serum separator tubes must NOT be used for progesterone assays because results will be significantly lower. Serum or plasma can be used. Clinicians should check with the laboratory.

Testosterone

Serum testosterone can be measured to evaluate suspected cryptorchid or intersex animals and to evaluate the hypothalamic-pituitary-gonadal axis. Serum testosterone concentrations fluctuate greatly within and among individuals. Pulses of testosterone secretion occur about every 30 to 90 minutes, depending on the species. Determination of testosterone on a single, random occasion has limited diagnostic value. For example, normal, fertile male cats may intermittently have undetectable amounts. On the other hand, finding high concentrations would indicate presence of testicular tissue. Typically, testosterone is determined before and after administration of human chorionic gonadotropin (hCG) or gonadotropin-releasing hormone (GnRH); substantial increase in testosterone signifies functional testicular tissue. A widely accepted protocol for hCG or GnRH stimulation testing in dogs and cats has not been established. Therefore, consultation with the laboratory performing the assay for the dose of hCG or GnRH, frequency of sampling, and interpretation of results is recommended (see Appendix I).

Estradiol

Evaluation of serum concentrations of estradiol is usually not necessary in dogs and cats because common conditions associated with pathologic production of estradiol are easily evaluated by other methods. The most common

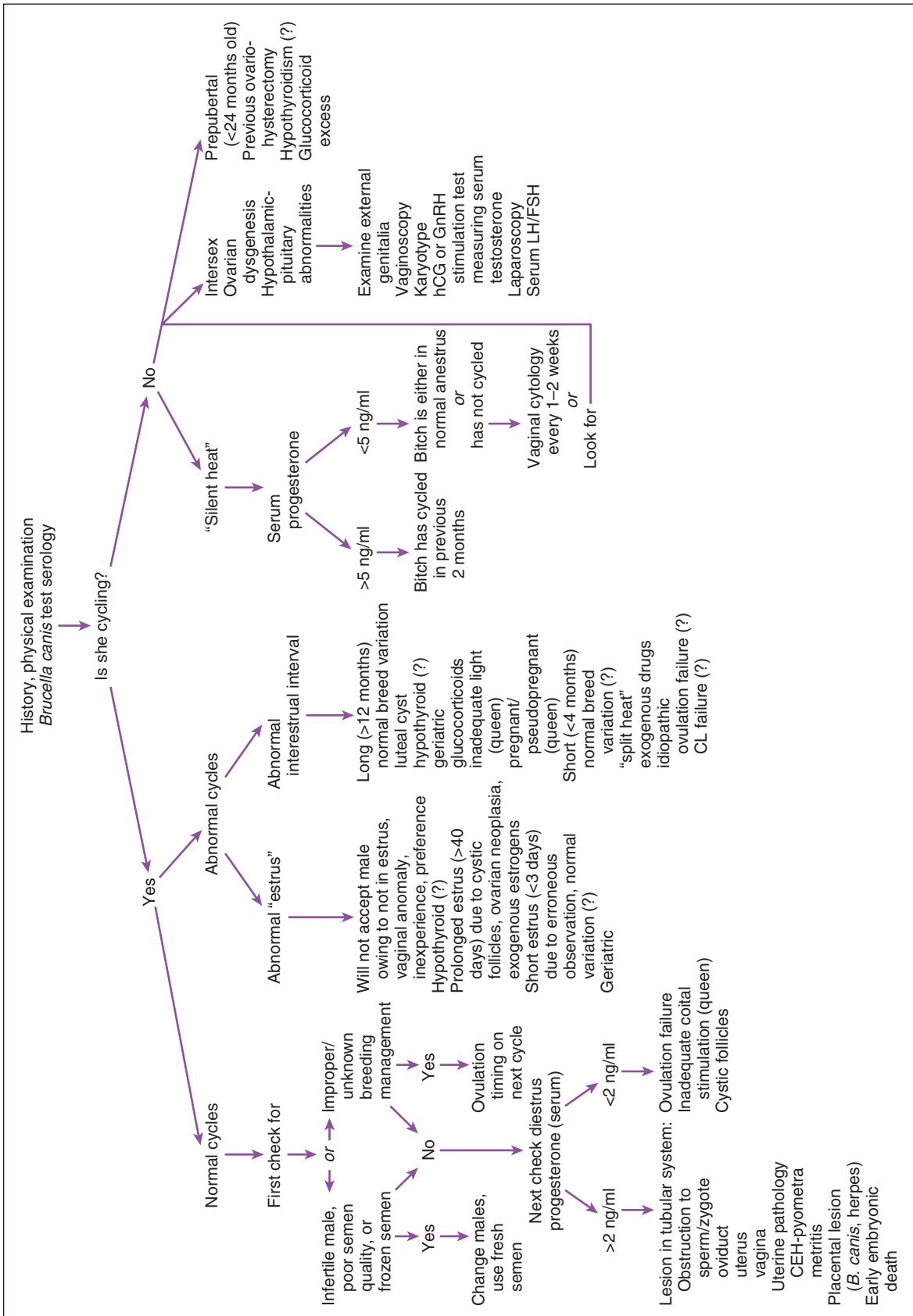


FIGURE 13-4 Diagnostic approach to infertility in a bitch. CEH, Cystic endometrial hyperplasia; CL, corpus luteum; FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone.

causes of hyperestrogenism are estrogen-producing testicular tumors in male dogs, and ovarian remnant syndrome and follicular ovarian cysts in female dogs and cats.

The most common signs of hyperestrogenism in male dogs are alopecia, hyperpigmentation, lichenification, gynecomastia, and pendulous prepuce. Testicular tumors are best identified by palpation, ultrasound examination, or both. Ovarian remnant syndrome is usually characterized by resumption of estrous cycles and estrous behavior after ovariohysterectomy. Some bitches with ovarian remnants do not begin cycling but instead show dermatologic changes similar to those in males with hyperestrogenism. Finding cornification of vaginal epithelial cells coincident with classic behavioral or physical signs of estrus is diagnostic. Cystic follicles can cause persistent estrus or abnormally frequent estrous cycles. Ovarian ultrasonography should be diagnostic. The effects of estrogen on the preputial epithelium are similar to those on the vaginal epithelium.

Relaxin

Measurement of serum relaxin can be used to diagnose pregnancy in bitches and queens. It is usually detectable between 20 and 28 days after breeding but sometimes not until day 31. The test is very specific because relaxin is produced primarily by the placenta. A positive test result indicates pregnancy. False-positive results have not been reported; however, relaxin may remain positive for a few days after parturition or abortion because of remaining placental tissue. A sudden disappearance of relaxin from blood (i.e., suddenly a negative result) would indicate that spontaneous abortion occurred. False-negative results occur when the test is performed too early during gestation. It is also possible that a small litter size could yield a false-negative result.

ANCILLARY TESTS

Karyotyping may be performed on animals suspected of having intersex conditions or abnormal gonadal development. Fine-needle aspirates are easily obtained from testes, epididymides, prostate and vaginal lesions. Biopsy specimens can be obtained from any part of the reproductive tract, although gonadal biopsy might have deleterious effects on remaining normal tissue.

Complete Blood Count

The packed cell volume (PCV) of pregnant bitches and queens declines after about day 20 of gestation and continues to decline until parturition, when PCVs of 30.6% $\pm$ 0.8% can be expected in bitches. Pregnant animals may have mild, mature neutrophilia. Dogs are very sensitive to toxic effects of estrogens on bone marrow stem cells. Thrombocytopenia, leukopenia, anemia, or a combination thereof can occur because of pharmacologic doses of estrogens and estrogen-producing tumors (see Chapter 3).

Neutrophilic leukocytosis with a left shift, variable degrees of neutrophil toxicity, and monocytosis are

commonly associated with mastitis, metritis, and prostatic abscesses. The leukogram of animals with pyometra varies, although leukocytosis with a left shift is expected. Profound leukocytosis (i.e., 100,000 to 200,000/ μ l) with a left shift may occur because of a closed-cervix pyometra. Conversely, leukopenia with a degenerative left shift may occur in animals with severe sepsis as the result of pyometra or prostatic abscess.

Urinalysis

Concomitant urinary tract infection and bacterial prostatitis in males and vestibulovaginitis in females is fairly common. Even in the absence of infection, some bitches with vaginitis have increased frequency of urination (i.e., pollakiuria) that could be mistaken for urinary tract disease. For animals with urinary tract signs, and in those with persistent vaginal or preputial discharge, urinalysis and culture are indicated. Obtaining urine samples by cystocentesis is ideal to minimize contamination from the genital tract. Because of normal reflux of prostatic fluid into the bladder, urine from sexually intact male dogs will contain protein-rich seminal fluid, which can cause positive reactions for protein and false-positive reactions for heme on urine chemistry tests, whether or not spermatozoa are found in the urine sediment.

Suggested Readings

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14

Neurologic Disorders

Jonathan M. Levine and Gwendolyn J. Levine

Diagnosis of neurologic disease has continued to evolve in human and veterinary medicine, in large part due to advances in imaging, molecular medicine, and clinical pathology. Magnetic resonance imaging (MRI) allows clinicians to obtain better data concerning structural nature of central nervous system (CNS) disturbances and has been demonstrated to provide prognostic information independent of physical assessment in canine disk herniation and fibrocartilagenous embolism (FCE). Conditions such as ischemic territorial infarct which were believed to be uncommon in dogs prior to the advent of MRI are increasingly recognized. Molecular testing for exercise-induced collapse and degenerative myelopathy has better defined the etiopathogenesis of these diseases. Biomarkers may be a source of additional prognostic data and targeted therapies for CNS neoplasia or spinal cord injury. Despite these remarkable advances, physical examination-based neuroanatomic localization is still the starting point in the diagnostic approach to veterinary neurologic disease.

NEUROANATOMIC LOCALIZATION

In veterinary medicine, nervous system disturbances (lesions) are localized based on physical assessments. Classic localization regions include: forebrain (prosencephalon), caudal brainstem (midbrain, pons, or medulla), cerebellum, peripheral vestibular apparatus, spinal cord (C1-C5, C6-T2, T3-L3, L4-S3 segments), and general neuromuscular system. In some instances multiple regions can be simultaneously affected (multifocal disease), or clinical signs may reflect involvement of large expanses of the nervous system (diffuse disease). Components of a neurologic examination include assessment of mentation, gait, postural reactions, spinal reflexes, cranial nerves, nociception, and paraspinous structures.

Descriptors of alertness in companion animals include: normal, obtunded, demented, stuporous, or comatose. "Obtunded" implies an animal that interacts normally with the environment, but requires additional stimulation to do so. Animals described as "demented"

do not interact appropriately; those that are "stuporous" only respond to noxious stimuli; and "comatose" animals are unresponsive even to deep nociceptive testing. Abnormal mentation is consistent with prosencephalic or caudal brainstem localization.

Neurologic gait disturbances typically reflect a combination of paresis (weakness) and ataxia (incoordination). Paresis can be characterized as either upper motor neuron (UMN) or lower motor neuron (LMN). Stride length is elongated in domestic species with UMN paresis and shortened in those with LMN paresis. Animals with caudal brainstem or C1-C5 lesions will have UMN paresis in all limbs. Those animals with C6-T2 lesions will have LMN paresis in the thoracic limbs and UMN paresis in the pelvic limbs. Animals with paresis limited to the pelvic limbs may have either T3-L3 (UMN) or L4-S3 (LMN) lesions. Generalized neuromuscular disease results in LMN signs in all limbs.

Ataxia can be categorized as general proprioceptive (GP), vestibular, or cerebellar. Animals with GP ataxia cross over the limbs or scuff the dorsum of the paw. The presence of GP ataxia indicates a disturbance within the caudal brainstem, spinal cord, or generalized neuromuscular system (likely peripheral nerve). Vestibular ataxia is associated with a broad-based gait, often accompanied by leaning and head tilt toward the side of least vestibular tone. Animals with vestibular ataxia may have a caudal brainstem, peripheral vestibular apparatus, or cerebellar localization. Cerebellar ataxia consists of overflexion at the distal limb joints with dysmetria and hypermetria. It is seen with diseases that affect the cortex of the cerebellum.

Postural reactions are evocative tests that assess motor strength and proprioception. Knuckling and hopping are the two most frequently used postural reactions in small animals. Delay or absence does not localize the lesion but simply implies an abnormality in the LMN, UMN, or GP systems. The limbs affected combined with other elements of the neurologic examination allow for localization.

Spinal reflexes are used along with gait analysis to determine whether UMN or LMN paresis is occurring in a particular limb. Animals with LMN paresis often have

depressed or absent reflexes whereas those with UMN paresis will have preserved or increased reflexes. Presence of a crossed extensor reflex implies UMN loss ipsilateral to the limb that extends out. Thoracic limb flexor withdrawal (C6-T2 spinal cord segments), pelvic limb flexor withdrawal (L6-S2 spinal cord segments), patellar reflex (L4-L6 spinal cord segments), and cranial tibial reflex (L6-S1 spinal cord segments) are believed to be most reliable, although objective data to support this contention are lacking.

Cranial nerve assessment is essential to localizing lesions within the cranial vault. Cranial nerve II is associated with prosencephalon and mediates visual pathways. Cranial nerves III and IV are associated with midbrain and are important in coordinating extraocular movement and pupillary constriction. Cranial nerve V is involved in facial sensation and innervates masticatory muscles; nuclei are located in the pons. Cranial nerves VI through XII have cell bodies located in the medulla; these nerves coordinate eye movements, balance, and swallowing.

Paraspinal palpation and nociceptive assessment are reserved for the end of the examination because they can be painful. Assessment of nociception is essential in animals with spinal cord disease or those with severe intracranial signs. Dogs with thoracolumbar disk herniation that have intact deep nociception have an 86% to 96% chance of voluntary ambulation after surgery, whereas dogs with absent deep nociception have approximately a 50% to 60% chance of voluntary ambulation post-surgery. Paraspinal palpation must be performed carefully and is used to detect zones of hyperesthesia. Diseases that affect bones or ligaments of the vertebral column, regional soft tissues, meninges, intervertebral disk, nerve root, or dorsal horn of the spinal cord may result in paraspinal hyperesthesia.

Neurologic examination can be confounded by disease in other body systems. For example, animals with osteoarthritis in a limb may not perform hopping postural reaction appropriately due to pain associated with weight bearing. Orthopedic disease can limit joint range of motion, which can falsely reduce flexor withdrawal reflexes. Finally, systemic illness such as metabolic derangement or cardiac disease can mimic paresis and ataxia seen with structural lesions in the nervous system. Therefore neurologic examination data need to be considered in light of all available clinical findings.

In the coming years, neurologic examination-derived head trauma and spinal cord injury scales are likely to play an increasing role in veterinary medicine. Scales facilitate health-care professional communication, objective accounting of clinical progress, and prediction of recovery. The modified Glasgow Coma Scale has been used in dogs with head trauma and is predictive of 48-hour outcome.²³ In dogs with disk-associated spinal cord injury, the modified Frankel Scale, Texas Spinal Cord Injury Scale, and 14-point pelvic limb motor score are valid means to qualify lesions severity, correlate to MRI markers of injury, and are easily performed.¹³ The authors strongly encourage clinicians to make standard and appropriate use of these systems to facilitate better care delivery.

DIFFERENTIAL DIAGNOSES

Differential diagnosis lists are based on neuroanatomic localization, signalment, as well as onset, progression, and duration of clinical signs (Tables 14-1 and 14-2). Neoplastic diseases, disk herniation, and idiopathic epilepsy all have age and breed predilections. Vascular diseases of the CNS usually have a rapid onset and are nonprogressive, whereas CNS degenerative diseases are insidious in their onset and clinical course. Appropriate neuroanatomic localization coupled with a strong differential list will allow clinicians to formulate a diagnostic plan.

NEUROIMAGING

Vertebral Column Indications • Radiography, myelography, computed tomography (CT), and MRI can be used to assess the vertebral column. Radiography is probably best viewed as a cost-effective means of screening for overt structural diseases (e.g., fracture, luxation, discospondylitis, large lytic bony tumor). Diagnostic accuracy of radiographs for disk herniation, cervical spondylomyelopathy, or intraparenchymal spinal cord disease is poor. Myelography shares many of the disadvantages of radiography in that the spinal cord cannot be directly visualized and images are available for review in a limited number of planes; myelography is also associated with adverse patient events (e.g., seizure, post-myelogram myelopathy, death). CT is the best means to evaluate bony pathology and is the study of choice to define vertebral fracture or luxation. Like myelography, soft tissue detail is not ideal; therefore visualization of intraparenchymal spinal cord lesions or nonmineralized disk herniation may be challenging. MRI has revolutionized diagnosis of vertebral column diseases. Signal patterns may indicate certain pathologic processes (e.g., hemorrhage, edema), can be specific for certain etiologies, and may provide data relating to outcome. For example, each L2 vertebral length of T2-weighted hyperintensity within the spinal cord of dogs with thoracolumbar disk herniation lowers the probability of walking by 1.9-fold.¹⁴

Intracranial Indications • MRI is the study of choice for nontraumatic intracranial disease. Preliminary studies indicate it is reasonably capable of distinguishing neoplastic from inflammatory etiologies. It is believed to have superior sensitivity for detecting ischemic territorial infarcts compared to CT, especially when modern sequences such as diffusion-weighted imaging are used. MRI is the ideal method for diagnosis of congenital diseases (e.g., Chiari-like malformation). In the years to come, diffusion tensor imaging, MRI tractography, MRI texturing, and functional imaging will likely continue to advance MRI-based diagnostics.

ELECTRODIAGNOSTICS

“Electrodiagnostics” refers to a group of techniques that measure spontaneous and evoked activity arising from muscles, nerves, and CNS structures. Electromyography

TABLE 14-1. COMMON NEUROLOGIC DISEASES IN DOGS, LISTED BY NEUROANATOMIC LOCALIZATION AND ETIOLOGIC CATEGORY

	FOREBRAIN	CAUDAL BRAINSTEM	PERIPHERAL VESTIBULAR	CEREBELLUM	SPINAL CORD	GENERALIZED NEUROMUSCULAR DISEASE
Degenerative	Leukodystrophy Storage disease Cognitive dysfunction	Leukodystrophy Storage disease	N/A	Abiotrophy NAD Storage disease	DM Disk herniation LS disease	Muscular dystrophy Breed-specific
Malformative	Hydrocephalus Chiari-like malformation	Quadrigeminal cyst Chiari-like malformation Dandy-Walker malformation	N/A	Quadrigeminal cyst Chiari-like malformation Dandy-Walker malformation	CSM A-A subluxation Arachnoid cyst Syringomyelia Kyphosis Scoliosis Spina bifida	N/A
Metabolic	Hypothyroidism Electrolytes Hypoglycemia	Hypothyroidism	Hypothyroidism	N/A	N/A	Hypothyroidism Diabetes mellitus Hyperthyroidism Cushing syndrome Addison disease
Neoplastic	Meningioma Glioma Lymphoma Ependymoma CPP Metastatic	Meningioma Glioma Lymphoma Ependymoma CPP Metastatic	Adenocarcinoma Squamous cell carcinoma	Meningioma Glioma Lymphoma Ependymoma CPP Medulloblastoma Metastatic	Meningioma NST Lymphoma Ependymoma Glioma Nephroblastoma Metastatic	Paraneoplastic
Nutritional	Thiamine deficiency	Thiamine deficiency	N/A	Thiamine deficiency	Cobalamin deficiency	N/A
Idiopathic	Idiopathic epilepsy		IVD			
Inflammatory	Viral Protozoal Rickettsial Fungal Nematode Bacterial	Viral Protozoal Rickettsial Fungal Nematode Bacterial	Bacterial	Viral Protozoal Rickettsial Fungal Nematode Bacterial	Viral Protozoal Rickettsial Fungal Nematode Bacterial	Viral Protozoal Rickettsial Idiopathic polyradiculopathy Idiopathic polymyositis Myasthenia gravis
	Granulomatous ME Necrotizing ME MUE	Granulomatous ME Necrotizing ME MUE		Granulomatous ME Necrotizing ME MUE	Granulomatous ME SRM MUE	
Trauma						
Toxin	Strychnine Metaldehyde Lead Bromethalin	Metronidazole	Chlorhexidine Loop diuretics	5-Fluorouracil Marijuana		Vincristine Cisplatin Botulism Tick paralysis Coral snake bite
Vascular	Ischemic infarct Hemorrhage	Ischemic infarct Hemorrhage	N/A	Ischemic infarct Hemorrhage	FCE	N/A

A-A, Atlanto-axial; CPP, choroid plexus papilloma; CSM, cervical spondylomyelopathy; DM, degenerative myelopathy; FCE, fibrocartilagenous embolism; IVD, idiopathic vestibular disease; LS, lumbosacral; ME, meningoencephalitis; MUE, meningoencephalitis of unknown etiology; N/A, not applicable; NAD, neuraxonal dystrophy; NST, nerve sheath tumor; SRM, steroid-responsive meningoencephalitis.

TABLE 14-2. COMMON NEUROLOGIC DISEASES IN CATS, LISTED BY NEUROANATOMIC LOCALIZATION AND ETIOLOGIC CATEGORY

	FOREBRAIN	CAUDAL BRAINSTEM	PERIPHERAL VESTIBULAR	CEREBELLUM	SPINAL CORD	GENERALIZED NEUROMUSCULAR DISEASE
Degenerative	Leukodystrophy	Leukodystrophy	N/A	Abiotrophy	Disk herniation	Muscular dystrophy
	Storage disease	Storage disease		NAD		Breed-specific
				Storage disease		
Malformative	Hydrocephalus		N/A	Hypoplasia	Syringomyelia Kyphosis Scoliosis Spina bifida	N/A
Metabolic	Electrolytes Hypoglycemia	N/A	N/A	N/A	N/A	Hypothyroidism Diabetes mellitus Hyperthyroidism Cushing syndrome Addison disease Paraneoplastic
Neoplastic	Meningioma	Meningioma	Adenocarcinoma	Meningioma	Meningioma	
	Glioma	Glioma	Squamous cell carcinoma	Glioma	Lymphoma	
	Lymphoma	Lymphoma		Lymphoma	Ependymoma	
	Ependymoma	Ependymoma		Ependymoma	Glioma	
	Metastatic	Metastatic		Metastatic	Metastatic	
Nutritional	Thiamine deficiency	Thiamine deficiency	N/A	Thiamine deficiency	Cobalamin deficiency	N/A
Idiopathic	Idiopathic epilepsy		IVD			
Inflammatory	FeLV	FIP	Bacterial	FIP	FeLV	FIV
	FIP	Rabies		Rabies	FIP	Rabies
	FIV	<i>Toxoplasma</i>		<i>Toxoplasma</i>	Rabies	<i>Toxoplasma</i>
	Rabies	Fungal		Fungal	<i>Toxoplasma</i>	Idiopathic polyradiculopathy
	<i>Toxoplasma</i>	Nematode		Nematode	Fungal	
	Fungal	Bacterial		Bacterial	Nematode	Idiopathic polymyositis
	Nematode Bacterial				Bacterial	Myasthenia gravis
Trauma						
Toxin	Strychnine	Metronidazole	Chlorhexidine	5-Fluorouracil		Vincristine
	Metaldehyde		Loop diuretics	Marijuana		Cisplatin
	Lead					Botulism
Vascular	Bromethalin					Tick paralysis
	Ischemic infarct	Ischemic infarct	N/A	Ischemic infarct	FCE	Coral snake bite
	Hemorrhage	Hemorrhage		Hemorrhage		N/A

FCE, Fibrocartilagenous embolism; FeLV, feline leukemia virus; FIP, feline infectious peritonitis virus; FIV, feline immunodeficiency virus; IVD, idiopathic vestibular; N/A, not applicable; NAD, neuraxonal dystrophy.

(EMG), motor nerve conduction (MNC), and sensory nerve conduction (SNC) are commonly performed studies to evaluate the peripheral nervous system (PNS). Somatosensory evoked potentials (SSEPs), motor evoked potentials (MEPs), electroencephalograms (EEGs), and brainstem auditory evoked responses (BAERs) are used to

evaluate the CNS. In modern veterinary practices, EEG is typically used to aid in seizure detection and as a means to qualify cerebrocortical activity in animals suspected of brain death. BAER can determine integrity of auditory pathways in animals suspected of deafness or severe caudal brainstem injury.

Electrodiagnostics (i.e., EMG, MNC, SNC) are critical in the evaluation of animals with PNS disease. Presence of abnormal spontaneous activity on EMG is usually associated with axonal or muscle pathology, as opposed to junctional diseases such as myasthenia gravis. Slowing of MNC velocity may suggest a myelin disorder or loss of large-diameter axons, whereas reduction in compound motor unit action potential amplitude may indicate myopathy, axonopathy, or junctional disease. By combining results from EMG, MNC, sensory studies, repetitive nerve stimulation, and other studies, a clinician trained in electrophysiology can identify which regions of the PNS may have abnormalities. Electrophysiology cannot definitively diagnose etiology; further diagnostics such as serology and biopsy are required.

CEREBROSPINAL FLUID ANALYSIS

Common Indications • Cerebrospinal fluid (CSF) is one of the only means to assess cellular responses to nervous system disease. Routine collection is suggested in animals with CNS signs or radiculopathy. In animals with intracranial CNS disease, collection is usually performed after neuroimaging (see “**Contraindications**”). CSF should always be acquired prior to myelography to prevent delivery of intrathecal iodinated contrast in animals with primary CNS inflammation and to avoid post-myelographic pleocytosis from hindering CSF interpretation.

Contraindications • High intracranial pressure may increase risk of brain herniation following CSF acquisition. Animals with intracranial disease should always receive advanced imaging prior to CSF withdrawal. Imaging that supports brain herniation, midline shift, or mass effect serves as a relative or strict contraindication for collection, depending on severity of changes. The site of CSF acquisition has not been clearly shown to alter risk of brain herniation across species. Bleeding diathesis, vertebral instability, and regional soft tissue infection can increase risk of post-CSF acquisition adverse events.

Procedure for CSF Collection • CSF can be acquired from cerebellomedullary cistern (CMC) or lumbar cistern (LC) under general anesthesia. Collection from the CMC is performed by placing the animal in lateral recumbency (the side of recumbency should match the handedness of the clinician performing the procedure) on a level, secure table. The animal's head and neck are brought to the edge of the table, and the neck is flexed approximately 90 degrees. Holding the head parallel to the tabletop is crucial for proper insertion of the collection needle and recognition of landmarks. The most commonly used technique for identifying the site of needle insertion relies on identifying the intersection of two lines, which we will call “x” and “y”. The “x” line runs from the occipital protuberance to the spinous process of the axis and defines the horizon. The “y” line runs between the cranial edges of the right and left wings of the atlas. Prior to needle insertion, the area surrounding the CSF acquisition site is clipped and sterilely prepped. A 22-gauge spinal needle (1.5 inches for dogs <

25 kg or cats; 2.5 inches for dogs > 25 kg) is then inserted through soft tissues underlying the CSF acquisition landmark. Traditionally, the bevel of the needle is directed cranially, as this may increase CSF flow rate. In small dogs or cats, the skin may need to be penetrated discretely from underlying soft tissues. The needle is advanced parallel to the table at an angle to match the mandible (often slightly caudally directed). Typically a decrease in resistance is felt after the needle penetrates the interarcuate ligament and dura, which indicates the CMC has been entered. This decrease in resistance is not always appreciated, and frequent removal of the needle stylet or advancing the needle without the stylet to check for CSF can be helpful.

Collection of CSF at the LC requires positioning the dog in lateral recumbency and flexing the lumbar vertebral column by flexing the pelvic limbs toward the ventral abdomen. The cranial wings of the ilium are located at the level of L6. Typically, a 22-gauge spinal needle (2.5 to 6 inches, dependent on dog size) is inserted parallel to the L6 spinous process to enter the L5-L6 interarcuate space. The needle is usually inserted until it contacts the ventral floor of the vertebral canal.

Most clinicians prefer CMC to LC collection, as it is easier to perform, is less frequently contaminated with blood, allows for larger volumes of CSF to be obtained, and is often easier to interpret in the setting of CNS disease because data concerning expected characteristics are more abundant. Theoretically LC CSF may be preferable for focal disease caudal to the CMC.

CSF Handling • When the CMC or LC is entered, CSF is collected by allowing it to flow freely from the needle hub. Direct aspiration of CSF from the needle hub under pressure is not recommended, and aspiration from the hub using a 25-gauge needle and 3-ml syringe is rarely needed. Usually, 1 ml of CSF (10 to 15 drops) is obtained per 5 kg of animal body weight. If a lab that can process the sample is nearby, it should be collected into a red-top tube. Storage of red-top collected CSF at 4° C for 24 to 48 hours can be attempted without changes in differential count if 1 drop of autologous serum is added per 9 drops of CSF; when serum is added to CSF, protein concentration will be altered so a separate aliquot of CSF should be withheld for this purpose.³ Ethylenediaminetetraacetic acid (EDTA) tubes are requested by some labs to preserve cell architecture; EDTA falsely increases the protein concentration and dilutes cell concentration.

Analysis • **CSF Appearance** • Normal CSF is colorless and clear. CSF turbidity is seen with red blood cell (RBC) counts greater than 400/μl or nucleated cell counts (NCCs) greater than 200/μl.¹⁸ A swirl of blood in the collection needle hub strongly suggests iatrogenic hemorrhage, although in some instances CSF with iatrogenic blood contamination will emerge from the needle with a homogeneous pink color. Hemorrhage in the CSF due to CNS disease is uncommon; when it happens, RBC destruction begins within 1 to 4 hours.¹² Xanthochromia (yellowing of the CSF) is a consequence of hemoglobin breakdown secondary to endogenous RBC destruction. Microscopic evidence of erythrophagocytosis

or hemosiderophages also suggests previous hemorrhage within the CNS or subarachnoid space.

CSF Protein Concentration • Normal CSF has a very low protein concentration, with a reference range that varies based on acquisition site and processing laboratory. At the authors' institution, reference ranges for CMC and LC CSF protein concentration are less than 25 mg/dl and less than 35 mg/dl, respectively, for small animals. Increased CSF protein concentration is a common but nonspecific finding (Table 14-3). Protein may be elevated because of blood-brain barrier disruption, blood contamination, or intrathecal production of immunoglobulins. Elevated protein with normal total NCC is termed albuminocytologic dissociation and is believed to be most common with etiologies that are noninflammatory. The Pandy test is a qualitative assessment for the presence of intrathecally produced globulins.

CSF Red Blood Cell Count • Red blood cells are not found in normal CSF; their presence is usually the result of blood contamination. Endogenous CSF hemorrhage may occur due to inflammatory diseases that allow for RBC diapedesis or from vascular disruption. Erythrophagocytosis, hemosiderophages, and xanthochromia suggest subacute, previous CSF hemorrhage. Disk herniation, FCE, neoplasia, and CNS inflammation are common etiologies associated with this finding.

Significant iatrogenic CSF hemorrhage can impair the interpretation of CSF. However, RBC counts less than 500 cells/ μ l do not alter NCC or total protein.²⁵ In some reports, RBC counts up to 15,000 cells/ μ l were not associated with NCC elevated beyond reference range.³²

CSF Nucleated Cell Count • "Pleocytosis" refers to increased CSF NCC beyond reference range (typically >5 NC/ μ l for CMC and LC CSF). Pleocytosis may be mild (greater than reference value but <25 cells/ μ l), moderate (26 to 100 cells/ μ l), or marked (>100 cells/ μ l).¹⁸ Etiologies that result in CSF pleocytosis are varied. Severity of pleocytosis, predominant cell type, and results of other data (e.g., neuroimaging) must be used together to render a clinical diagnosis. For example, dogs with granulomatous meningoencephalitis have a median NCC of 250 cells/ μ l, usually have lymphocyte or mononuclear cell predominance, and often have signal changes on brain MRI.²⁰ In contrast, dogs with steroid-responsive meningitis have a median NCC of 1775 cells/ μ l, often have neutrophilic predominance, and usually have normal MRI.²

CSF Cytology • Normal CSF has few cells, which are a mixture of large mononuclear cells and lymphocytes. Greater than 25% neutrophils in a CSF sample with normal total NCC should be regarded as abnormal. The cytologic appearance of CSF is usually classified as neutrophil predominant (>70%), large mononuclear predominant (>70%), lymphocyte predominant (>70%), mixed cell (no one cell type >70%), and eosinophilic (>10% to 20%).¹⁸ Neutrophilic CSF is seen with some forms of infectious CNS disease, steroid-responsive meningitis, meningioma, and acute CNS injuries.⁹ Large mononuclear, lymphocytic, and mixed cell pleocytosis

occurs due to a wide variety of inflammatory, neoplastic, and compressive diseases (see Table 14-3).²⁹ Eosinophilic CSF is uncommon and associated with idiopathic inflammation, larval migrans, and mycotic disease.²⁹

CSF Biomarkers • Biomarkers are molecules located within the body that have prognostic, predictive, or diagnostic value. For canine intervertebral disk herniation, elevated NCC, protein concentration, and creatine kinase (CK) activity have been associated with injury severity and ambulatory outcome.³⁴ Matrix metalloproteinase-9 expression has also been associated with injury severity.¹⁶ Dogs with steroid-responsive meningitis frequently have elevated CSF immunoglobulin A (IgA) concentration, although the specificity and sensitivity of this finding remain unclear.² In the coming years, developments in the area of biomarkers seem likely to assist veterinarians in care delivery and prediction of outcome.

ANTIBODY-BASED INFECTIOUS DISEASE TESTING

Occasional Indications • Detection of antibodies to various infectious agents has been a long-standing complement to neuroimaging and CSF analysis. Testing is usually performed in animals with inflammatory CNS or PNS disease. Despite most clinicians' familiarity with antibody-based infectious disease tests, results can be challenging to interpret. In many cases, "positive" and "negative" titers must be viewed cautiously. First, antibodies within CSF or serum may be present due to past exposure to an agent. Rates of exposure and prevalence of disease become essential aids in titer interpretation. For example, when exposure without disease is common (e.g., toxoplasmosis), then titer positivity may not be compelling. Likewise, if the prevalence of a disease is very low in a region, then positive predictive value of a titer decreases. Secondly, antibody isotype may aid in determining whether active infection is present. Immunoglobulin M (IgM) is classically associated with recent infection, whereas time is required to switch to immunoglobulin G (IgG). Third, presence of antibodies in CSF may be the result of blood contamination or non-specific leakage across the blood-brain barrier. Finding a second antibody in the blood and CSF to an agent that uncommonly affects the CNS (e.g., parvovirus), may be a strategy to determine whether antibodies are present in the CSF due to blood contamination or leakage. Fourth, serial antibody titers can be helpful in determining exposure versus active disease. Rising titers and isotype switching are supportive of an ongoing immune response. Finally, numerical titer itself can be informative as exceedingly high values are more likely to be clinically relevant.

Infectious disease titers are typically best used when signalment, history, imaging, and CSF point to infectious CNS disease as a reasonable differential diagnosis.²¹ Knowledge of regionally prevalent infectious CNS diseases must be used to select appropriate tests. Molecular testing, such as polymerase chain reaction (PCR), represents an additional means to gather data concerning infectious etiologies.

TABLE 14-3. CEREBROSPINAL FLUID CHARACTERISTICS IN NERVOUS SYSTEM DISEASES OF DOGS AND CATS

DISEASE	NO. OF ANIMALS	ACQUISITION SITE	% ABNORMAL	PROTEIN, mg/dl (RANGE)	NC/μl (RANGE)	CYTOLOGY	REFERENCE
Infectious Inflammatory							
Bacterial encephalitis*†	14	CM	93% (13/14)	164–777 (mean, 337)	18–10,850	Neutrophilic (9/13) Monocytic (2/13)	Radaelli and Platt ²⁴
Canine distemper*	25	CM	—	4–136 (mean, 37)	0–501 (median, <25)	Lymphocytic	Vandevelde and Spano ³⁰
FLP*	16	CM	25% (4/16 elevated protein)	20–1120 (mean, 97)	—	Neutrophilic (1/2) Lymphocytic (1/2)	Foley et al. ¹⁰
FLP*	10	—	—	6–1090 (median, 6)	1–295 (median, 28)	—	Boettcher et al. ⁴
Protozoa—Dog	19	CM	89% (17/19)	Normal—strongly elevated	0–<1000	Mixed mononuclear	Tipold ²⁹
Noninfectious Inflammation—Dog							
GME*	29	CM	93% (27/29)	9–1848 (mean, 226)	0–11,840 (median, 250)	Lymphocytic (15/29) Mononuclear (6/29)	Munana and Luttgen ²⁰
Idiopathic tremors/“white shaker”†	22	CM	50% (11/22)	12–46 (median, 21)	6–40 (median, 13)	Lymphocytic (4/8) Large mononuclear (3/8)	Wagner et al. ³¹
NME*	14	CM	86% (12/14)	25–203 (mean, 88)	0–540 (mean, 120)	Lymphocytic (8/12) Mononuclear (2/12)	Levine et al. ¹⁵
NME*	12	CM	100% (12/12)	58–228 (mean, 122)	71–630 (mean, 347)	Lymphocytic (12/12)	Cordy and Holliday ⁷
SRM	36	CM	100% (36/36)	7–1333 (median, 66)	6–9720 (median, 1775)	Neutrophilic (36/36)	Bathen-Noethen et al. ²
SRM	20	CM	100% (20/20)	17–480	25–2500	Neutrophilic (12/20) Mononuclear (8/12)	Lowrie et al. ¹⁷

Disk Herniation—Dog							
Cervical*	111	L	82% (85/104) protein increase 23% (26/111) NC increase	10–306 (median, 60)	0–54 (median, 2)	Lymphocytic (11/26) Neutrophilic (8/26)	Windsor et al. ³³
Cervical*	25	CM	44% (11/25)	Median, 21	0–12 (median, 2)	Mononuclear	Thomson et al. ²⁸
Thoracolumbar*	312	L	66% (152/232) protein increase 61% (190/312) NC	3–1920 (median, 57)	0–428 (median, 8)	Lymphocytic (78/190)	Windsor et al. ³³
Thoracolumbar*	57	CM	49% (28/57)	12–110 (median, 26)	0–245 (median, 3)	Neutrophilic (60/190) Neutrophilic (12/17) Mixed cell (4/17)	Witsberger et al. ³⁴
Thoracolumbar*	35	CM	26% (9/35)	10–42 (mean, 19)	0–11 (mean, 2)	—	Levine et al. ¹⁶
Vascular							
FCEM—Dog†	32	L (26), CM(6)	44% (14/32)	50–239	7–84 (median, 6)	Neutrophilic (4/6) Mixed cell (2/6)	De Risio et al. ⁸
FCEM—Cat	5	CM(4), L(1)	80% (4/5)	15–193 (median, 38)	0–495 (median, 6)	Neutrophilic (3/4)	Mikszewski et al. ¹⁹
Ischemic Infarct Brain—Dog‡	33	—	12% (4/33)	—	—	Neutrophilic Mononuclear	Garosi† ¹¹
Neoplasia—Dog							
Meningioma—brain*	56	CM	84% (43/51)	26–210 (mean, 66)	5–580 (median, 17)	Neutrophilic (10/15)	Dickenson et al. ⁹
CNS Neoplasia—any†	24	—	83% (20/24)	26–1953	6–184	Mixed cell (6/9) Lymphocytic (2/9)	Bohn et al. ⁵
Degenerative Myelopathy—Dog*	15	CM	0% (2 blood contaminated) 80% (8/10)	13–31	0–8	—	Coates et al. ⁶
Vertebral Instability/Malformation	10	—		6/10 abnormal	2/10 abnormal	Lymphocytic (1/2) Mononuclear (1/2)	Bohn et al. ⁵

CM, Cerebellomedullary; CNS, central nervous system; FCEM, fibrocartilagenous embolic myelopathy; FIP, feline infectious peritonitis; GME, granulomatous meningoencephalomyelitis; L, lumbar; NC, nucleated cell; NME, necrotizing meningoencephalitis; SRM, steroid-responsive meningitis.

*Necropsy-confirmed or surgically-confirmed diagnoses only.

†Data for protein concentration and nucleated cell count include cases with abnormalities in these parameters only.

‡Personal communication regarding dogs described with ischemic infarction in Garosi.¹¹

Analysis and Interpretation of Specific Tests • See Chapter 15.

INFECTIOUS DISEASE POLYMERASE CHAIN REACTION

Occasional Indications • PCR-based technology allows for detection of genomic RNA or DNA associated with various infectious organisms. Putative advantages of PCR over antibody-based testing are (1) the organismal genome should be specific for infection, and (2) sensitivity may be high as few genomic copies are required for detection.²¹ Despite these advantages, there are concerns that in some instances PCR may have low sensitivity for detecting CNS infection; this may be especially true in cases where blood is the tissue assayed and the organism of interest has a transient blood-borne phase. Cerebrospinal fluid may be the ideal sample for PCR-based techniques to detect infectious encephalitis, although transient infections or those limited to deeply buried CNS parenchyma may be challenging to detect via assessment of CSF. To the authors' knowledge, data are not currently available concerning sensitivity or specificity of CSF PCR for identifying infectious encephalitis in dogs and cats. Also, with the variety of PCR techniques available, including degenerate PCR and multiplexed PCR, comparisons regarding sensitivity and specificity would be difficult. In humans, CSF PCR has dramatically enhanced the detection of infectious encephalitis. In some diseases sensitivity and specificity are both greater than 95%, and early, accurate results have enhanced rapid delivery of therapeutics.²¹

Currently, the authors use CSF PCR when sample size permits. In some cases, use of degenerate PCR techniques, which can screen whole families of infectious organisms, has resulted in diagnosis where traditional techniques (e.g., histopathology, titers) have been misleading. Fluids such as blood and urine can be assessed by PCR if the biology of suspected diseases indicates the presence of organisms to be likely.

Analysis and Interpretation • See Chapter 15.

URINE ORGANIC ACIDS AND GLYCOSAMINOGLYCANS

Rare Indications • Excessive concentrations of organic acids or glycosaminoglycans can be found in urine of animals with inborn errors of metabolism or overload of normal systems by exogenous metabolites. Screening for urinary organic acids is useful in the diagnosis of methylmalonic aciduria, a disease that has been reported to cause seizures and stupor in Maltese dogs. Animals with D-lactic aciduria and various mitochondrial diseases may also have altered urine organic acid profiles.

Analysis • Analysis is performed upon urine. If urine cannot be shipped to the laboratory immediately, it must be frozen at -20°C . When shipped, it must be shipped overnight on ice. One should contact the laboratory for specifics on shipping.

Interpretation • Dermatan sulfate, a glycosaminoglycan, can be present in increased concentration in the urine of dogs with mucopolysaccharidosis.

ACETYLCHOLINE RECEPTOR ANTIBODY

Common Indications • Acquired myasthenia gravis (MG) is an autoimmune disease resulting in neuromuscular dysfunction in dogs and cats. Animals may have generalized neuromuscular weakness or can present with focal disease (e.g., esophageal, ocular, or laryngeal weakness in the absence of limb paresis). Clinical signs result from development of autoantibodies that target nicotinic acetylcholine receptors.

Analysis • Acetylcholine receptor antibodies can be detected in the serum via radioimmunoassay, available at the University of California at San Diego (<http://vetneuromuscular.ucsd.edu/>). There are no special handling requirements.

Interpretation • The test is 98% accurate for diagnosing acquired myasthenia gravis in dogs.²⁷ False-negative results are most common in animals receiving glucocorticoids at the time of sampling.

CREATINE KINASE

Frequent Indications • CK is an enzyme associated with muscle energy metabolism and is released into blood as a consequence of myocyte injury.

Artifacts • Restraint associated with blood acquisition and recumbency can cause mild elevations in CK (usually $<1000\text{ IU/L}$). Activity of CK can rapidly decrease in blood products stored at room temperature and may even decrease in frozen aliquots, so it is imperative to process samples rapidly.

Analysis • The test is performed on serum or plasma.

Interpretation • The half-life of canine CK is 2 to 3 hours.²⁶ Significant elevations in CK activity suggest presence of disease that results in muscle disruption (e.g., immune-mediated polymyositis, infectious polymyositis, muscular dystrophy). Trauma, shock, and seizures can lead to profound myonecrosis and thus may also result in marked increases in CK. Results of CK should be correlated with clinical examination, electrophysiology, and muscle biopsy.

DYNAMIN-1 GENE

Occasional Indications • Exercise-induced collapse (EIC) is a common disease affecting active Labrador retrievers. After 10 to 15 minutes of strenuous exercise that results in hyperthermia, dogs may begin to exhibit ataxia, paresis, leaning, falling, and limb flaccidity. Generalized seizures and spasticity occur less commonly;

death is a rare event following EIC. Typically, dogs will recover normal function within 45 minutes of a collapse event. Recently, a mutation in the dynamin-1 gene that results in amino acid substitution within dynamin protein was found to be highly associated with EIC. The dynamin protein normally functions to regulate endocytic vesicle formation and is temperature sensitive in some species when mutated.

Analysis • The test is performed upon EDTA blood, semen, or dew claws (puppies). Semen and dew claws should be stored frozen until they are ready to ship. Samples should be sent with frozen ice packs to arrive in 1 to 5 days. Testing is commercially available through the University of Minnesota Diagnostic Laboratory (<http://www.cvm.umn.edu/vdl>).

Interpretation • Ninety-seven percent of dogs with EIC were homozygous for the mutation, whereas only 9% of unaffected dogs were homozygous.²²

SUPEROXIDE DISMUTASE (SOD) GENE

Rare Indications • Degenerative myelopathy (DM) is a disease affecting older dogs that are often medium to large breeds; German shepherds, boxers, and Pembroke Welsh corgis are believed to be commonly affected. Dogs usually have slowly progressive signs reflecting involvement of the T3-L3 spinal cord without paraspinal hyperesthesia. Etiopathogenesis of DM is still unknown, but recent data suggest an association between mutation of the superoxide dismutase-1 gene (*SOD1*) and clinical signs.¹ In one report, dogs with DM always had *SOD1* mutations, but mutations were also quite common in many unaffected animals (in some breeds, upward of 70% of animals studied).¹

Analysis • EDTA blood, semen, or tissue (e.g., liver, muscle, kidney, spleen) may be tested. Five to 10 ml of EDTA blood is kept refrigerated until mailed to the lab, but it cannot be kept for more than 1 week before being mailed. It should be sent with a cool pack. Semen (1 straw) and tissue (approximately 1-inch cube) should be frozen and shipped with a frozen cool pack. The Orthopedic Foundation for Animals (www.offa.org) currently processes samples for SOD gene testing.

Interpretation • The high rate of mutation carriage in unaffected dogs makes *SOD1* testing of limited value in confirming DM, although it may have a role as an exclusionary assay. MRI of the vertebral column suggesting an absence of spinal cord compression or intraparenchymal change along with CSF lacking pleocytosis remain the cornerstones of diagnosing DM *in vivo*.

OTHER GENETIC TESTS

Rare Indications • Genetic tests are now available for a multitude of uncommon and rare nervous system disorders affecting small domestic species. These include

(1) ceroid lipofuscinosis, which may result in confusion, behavioral change, and blindness in juvenile or adult dogs; (2) pyruvate dehydrogenase deficiency, a mitochondrial myopathy associated with exercise intolerance; (3) phosphofructokinase deficiency, which results in hemolytic anemia and myopathic weakness; and (4) mucopolysaccharidosis VI, which results in bone malformation and spinal cord compression in neonatal cats.

Analysis • See Appendix I for what samples are required, shipping requirements, and where to send the samples.

LACTIC ACID

Infrequent Indications • Suspected metabolic myopathies, especially in Labrador retrievers; evaluation of metabolic acidosis; evaluating perfusion in patients being treated for shock; helping determine prognosis for critically ill animals (e.g., dogs with gastric dilatation/volvulus).

Disadvantage • Sample handling is very important.

Analysis • Lactate is assayed in lithium heparin plasma or from blood collected in iodoacetate tubes. Fluoride tubes may be similar. Analysis may be performed with standard laboratory analyzers or with point-of-care instruments.

Normal Values • 2 to 13 mg/dl (0.22 to 1.44 mmol/L)

Danger Values • Values greater than 6.0 mmol/L are associated with a poor prognosis (see following).

Artifacts • Venous stasis (e.g., prolonged holding off of the vein), struggling during venipuncture, and a recent meal may significantly increase blood lactate levels. Samples taken from cephalic veins tend to have slightly higher values than samples taken from jugular veins. The plasma should be quickly separated from the RBCs or the blood stored at 4°C for less than 2 hours before plasma is harvested. If the blood sample is allowed to sit at room temperature, the lactate concentration progressively increases. If iodoacetate tubes are used, blood can be held at room temperature for up to 2 hours before harvesting plasma. Harvested plasma should be refrigerated or frozen. Oxaloacetate anticoagulant may cause artifacts. Aspirin, epinephrine, and phenobarbital may alter blood lactate levels.

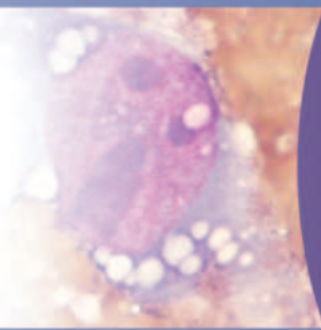
Causes of Increased Lactic Acid • One reason for measuring lactic acid levels in dogs is to look for metabolic myopathies in Labrador retrievers. The first blood sample is taken with the dog at rest. Then the dog is exercised by walking it briskly for 10 to 15 minutes, and another blood sample is taken. A marked increase above the normal range after minor exercise suggests this disorder. Another reason is to evaluate patients (usually critically ill) for lactic acidosis and to help determine their prognosis. Dogs with gastric dilatation/volvulus that have blood lactate concentrations greater than 6.0 mmol/L have a worse prognosis and are more likely to have gastric wall

necrosis. Persistent increased lactic acid concentrations in animals being treated for shock indicates that adequate perfusion is probably not being achieved and appears to be associated with a poorer prognosis.

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Microbiology and Infectious Disease



15

Michael R. Lappin

Infectious agents may be identified directly by cytologic analysis, histopathologic evaluation, culture, viral isolation, antigen detection, or nucleic acid amplification techniques. Polymerase chain reaction (PCR) assays are the most commonly used nucleic acid amplification techniques in small animal infectious diseases. Detection of antibodies against infectious agents provides indirect evidence of prior exposure or current infection. This chapter describes methods for obtaining specimens, outlines currently used testing procedures for some of the more common infectious diseases, and discusses interpretation of results from the various procedures and tests.*

WHEN TO SUSPECT BACTERIAL/FUNGAL/RICKETTSIAL/VIRAL AGENTS

Infectious diseases should be on the differential list for most problems, especially those with fever or other signs of inflammation. History, physical examination findings, and routine clinical pathologic testing are seldom pathognomonic for an infectious cause, but they help the clinician rank differential diagnoses and develop a logical diagnostic plan.

History can increase the degree of suspicion for infectious disease. Exposure to other infected animals or contaminated fomites is important for agents with direct transmission, such as those inducing respiratory disease (e.g., feline herpesvirus 1, canine bordetellosis) or gastroenteritis (e.g., canine and feline giardiasis, canine and feline parvovirus infection). Potential exposure to vectors (e.g., mosquitoes for dirofilariasis; ticks for Lyme borreliosis [*Ixodes* spp.], ehrlichiosis [*Rhipicephalus sanguineus*], *Rickettsia rickettsii* [Rocky Mountain spotted fever; *Dermacentor* spp.], and *Babesia canis* [*R. sanguineus*]) or appropriate travel history (e.g., coccidioidomycosis in the Southwest; blastomycosis in the Mississippi, Missouri, and Ohio River valleys) can also suggest infectious

disease. Vaccination history, deworming history, and determination of whether other animals or people in the environment are affected can aid in ranking infectious diseases on a differential diagnoses list.

Physical examination findings may suggest an infectious cause. Infectious agents can induce fever. Lymphadenomegaly as a result of reactive lymphoid hyperplasia can be infectious in origin. Hepatosplenomegaly can be caused by immunologic stimulation induced by chronic intracellular infections (e.g., ehrlichiosis, brucellosis). Endogenous uveitis commonly occurs after infections by feline immunodeficiency virus (FIV), feline infectious peritonitis (FIP) virus, toxoplasmosis, and systemic mycoses. Mucopurulent discharges can suggest primary or secondary bacterial infections. Certain infectious diseases cause specific abnormalities such as dendritic ulcers (feline herpesvirus 1), chorea myoclonus (canine distemper virus), or testicular swelling plus pain (canine brucellosis).

Finally, clinicopathologic abnormalities can suggest disease caused by infectious agents. Neutrophilic leukocytosis, particularly if a left shift or degenerative neutrophils (see Chapter 4) are also present, is consistent with an infectious cause of disease. Gram-negative sepsis is suggested by leukopenia with a degenerative left shift. Monocytosis or lymphocytosis can be induced by persistent infection with a number of intracellular agents that result in persistent infection. Examples include ehrlichiosis, toxoplasmosis, and bartonellosis. Polyclonal (e.g., multiple infectious causes) or monoclonal (e.g., usually induced by neoplasia, rarely associated with canine ehrlichiosis) gammopathies may suggest chronic immune stimulation. Neutrophils in aqueous humor, cerebrospinal fluid (CSF), synovial fluid, or urine may indicate inflammation induced by infectious agents.

CYTOLOGY

Common Indications • Cytologic examination of exudates, blood film, tissue imprint, aspiration biopsy, or wet mount of hair is indicated when bacterial and fungal diseases (and occasionally rickettsial and viral diseases) are suspected.

*See Table 15-1 at the end of this chapter for product specifications.

Advantages • Cytology is inexpensive and readily available and may allow rapid confirmation and identification of an infectious agent. It assists in establishing normal flora and contaminants versus infection (e.g., interpretation of relative numbers of bacteria and yeasts in the ear canal). Cytologic examination also permits visualization of relative numbers of organisms at the time of collection (culture results may be misleading in terms of fast- or slow-growing bacteria).

Disadvantages • Infectious agents cannot always be found (e.g., ehrlichiosis, haemoplasmosis, infections with numbers of organism that are below sensitivity level of cytology). Sometimes a presumptive cytologic diagnosis must be confirmed by other methods (e.g., histopathology, culture, PCR assay), and cytology is of limited value in detecting viral inclusions except in brief viremic stages of canine distemper.

Specimen Procurement and Analysis

See Chapter 16 for discussion of cytologic techniques and cytologic conclusions.

Bacterial Diseases

Discharges from animals with suspected bacterial disease should be placed on a microscope slide, air dried, fixed, and stained with both Gram and Romanowsky-type stains (see Chapter 16). The examination is started on low power (10× magnification), with oil immersion (100×) used for inspection of bacterial morphologic features (i.e., rods, cocci) and Gram stain characteristics (i.e., Gram-positive [blue] or Gram-negative [pink]). The primary disadvantage of Gram staining is that Gram-negative bacteria may be difficult to find because background material stains pink. It is easier to find bacteria (dark-blue stain) and easier to study morphologic detail of other cells (i.e., inflammatory cells) using Romanowsky-type stains. Gram staining may be variable; organisms in body fluids may stain differently from those grown on a blood agar plate. Gram stain demonstrates the gram-positive, branching filaments of *Actinomyces* spp. and *Nocardia* spp. (see Figure 10-11). Acid-fast stains can be used for *Mycobacterium* spp. and to help differentiate *Nocardia* spp. (acid-fast) from *Actinomyces* spp.

Some bacteria have characteristic morphologic features. Large rod-form bacteria containing spores found on fecal cytology of dogs or cats with diarrhea suggest *Clostridium perfringens* (Figure 15-1). Bipolar-staining, gram-negative coccobacilli found in aspirates of inflamed cervical lymph nodes from cats in the Southwest or West suggest *Yersinia pestis*. Short spirochetes found on fecal cytology of animals with diarrhea suggest but do not prove campylobacteriosis. Spirochetes found on cytology of gastric mucosa of vomiting animals suggest helicobacteriosis.

To demonstrate inclusion bodies in acute feline chlamydial conjunctivitis, conjunctival scrapings are obtained with a flat spatula, smeared on a slide, stained with Romanowsky-type stains, and examined for intracytoplasmic aggregations of *Chlamydophila felis* (previously *Chlamydia*).

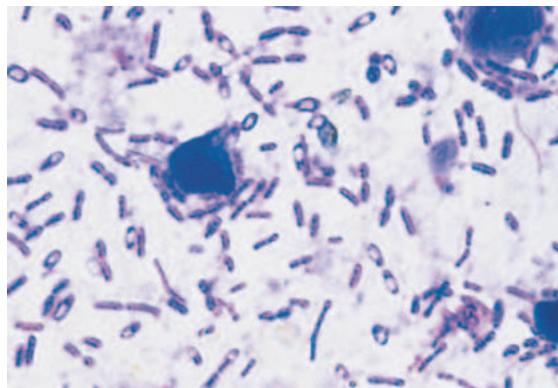


FIGURE 15-1 Smear of diarrheic feces with spore-forming bacteria indicating *C. perfringens*. The spores have clear spaces outlined by a dark wall. (From Nelson RW, Couto CG: *Small Animal Internal Medicine*, ed 4. Elsevier, Inc., St. Louis, 2009.)

Morulae of *Ehrlichia* spp. are rare in the cytoplasm of mononuclear cells (*Ehrlichia canis*), neutrophils (*Ehrlichia ewingii*; *Anaplasma phagocytophila* [previously *E. equi*]), or platelets (*Ehrlichia platys*). *Mycoplasma haemofelis* (cats only), "*Candidatus* *M. haemominutum*" (cats only), "*Candidatus* *M. turicensis*" (cats only), *M. haemocanis* (dogs only), "*Candidatus* *M. haematoparvum*" (dogs only), *Cytauxzoon felis* (cats only), and *Babesia* spp. sometimes will be identified cytologically on the surface or within canine or feline erythrocytes.

Cutaneous Parasitic Diseases

For demonstration of *Cheyletiella* spp., a piece of transparent adhesive tape is gently pressed against areas with crusts or dandruff and then placed on a microscope slide. Next the hair is clipped, mineral oil is placed on the skin and on a microscope slide, and the skin is scraped using a blunt no. 10 scalpel blade. For skin scrapings to look for *Demodex* spp., the skin should be immobilized and mites expressed from follicles by pinching and scraping the extruded material. For scrapings to look for *Sarcoptes* spp. or *Cheyletiella* spp., the scraping is continued more superficially (inducing a mild capillary ooze) over a larger surface area. After transfer of the scraping, the microscope slide field is scanned at 10× for mites.

Fungal Diseases

For identification of dermatophytes, hairs are plucked from the periphery of a lesion, placed on a microscope slide, and covered with 10% to 20% potassium hydroxide to clear debris. The slide is then heated (not boiled) and examined under the 10× or 40× objective to search for hyphae, spores, conidia, budding yeasts, and fungus-induced damage (e.g., swollen or broken hair shafts). The 40× objective is used to identify arthrospores (dense aggregates of spherical structures that may cover the hair shaft [Figure 15-2]). Failure to find arthrospores does not rule out dermatomycosis. Culture is more sensitive for diagnosis of dermatophytosis (see [Fungal Culture](#)).

Romanowsky-type stains (e.g., Wright) are used in preference to wet mount preparations and ink when looking for fungi other than dermatophytes (see Chapter 16). Romanowsky-type stains are also useful in

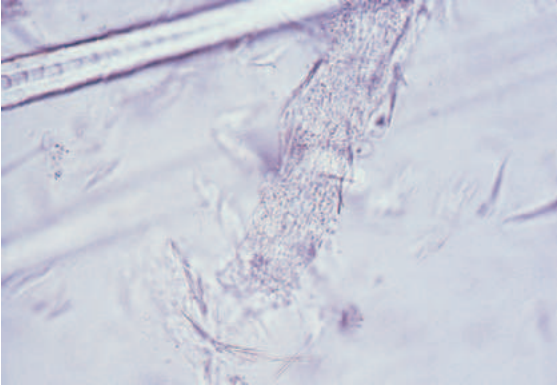


FIGURE 15-2 Canine ringworm skin scraping. One normal hair shaft is at the upper left. The swollen, fragmented hair shaft in the center is full of small, round *Microsporum canis* arthrospores.

identifying yeasts such as *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Sporothrix schenckii*, *Coccidioides immitis*, or *Cryptococcus* spp. (see Figure 11-3) in exudates, CSF, lymph node aspiration cytology, or transtracheal aspiration cytology.

Viral Diseases

Canine distemper virus inclusions in lymphocytes, neutrophils, or erythrocytes (Figure 15-3) are diagnostic of infection but are only present transiently, so false-negative results are common. Feline herpesvirus 1 (FHV-1) infection transiently results in intranuclear inclusion bodies in epithelial cells of the conjunctiva. Rarely, FIP-inducing strains of coronavirus result in transient intracytoplasmic inclusions in circulating neutrophils.

CULTURE AND ANTIMICROBIAL SUSCEPTIBILITY

Common Indications • Culture and antimicrobial susceptibility are indicated in most suspected bacterial diseases (Box 15-1), especially when clinical syndromes have

been resistant to medications. *Remember:* Skin and mucosal surfaces have a resident microflora (Box 15-2); therefore care must be taken to avoid contamination.

Advantages • Culture and antimicrobial susceptibility usually allows the most effective treatment to be administered.

Disadvantages • Culture requires time for agents to grow; also, some organisms are fastidious or have special culture requirements. Other disadvantages are the expense and the ease of contaminating or making inactivate cultures, rendering results worthless.

Bacterial Culture

Specimen Procurement

Body Cavities • The site of skin puncture should be prepared as for blood culture (see discussion in Cardiovascular System). If pyothorax or peritonitis seems likely but fluid cannot be aspirated, lavage (see Chapter 10) is indicated. Because mixed infections are common and pure anaerobic infections may occur, aerobic and anaerobic cultures should be performed.

Cardiovascular System • Blood cultures are indicated in suspected bacterial endocarditis or septicemia. A large vein prepared surgically with sequential iodine and alcohol scrubs is used for three blood culture specimens obtained during a febrile episode over a 24-hour period in dogs with suspected endocarditis. Culture of fewer than three specimens significantly decreases the chance of positive results. At least 5 ml of blood is placed directly into a transport medium that will support the growth of aerobic and anaerobic bacteria, and it is incubated at 20° C for 24 hours. Clotted blood or blood containing ethylenediaminetetraacetic acid (EDTA) or citrate are unacceptable because this decreases isolation of organisms (*Bartonella* spp. are exceptions; they can be cultured from EDTA tubes; see Bartonellosis sections). If a patient is critically ill and sepsis is suspected, three cultures should be obtained over 1 to 3 hours before antimicrobial therapy is instituted. Because the urinary system is a common portal of entry for bacteria into the body, urine

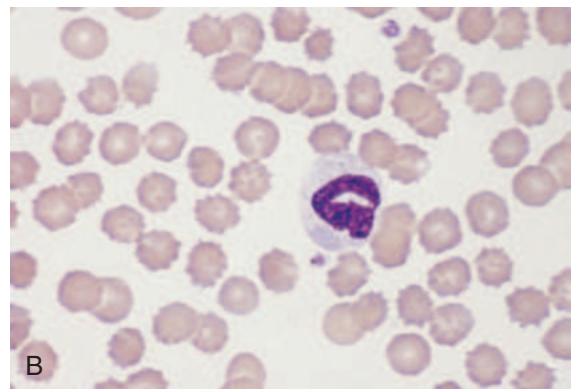
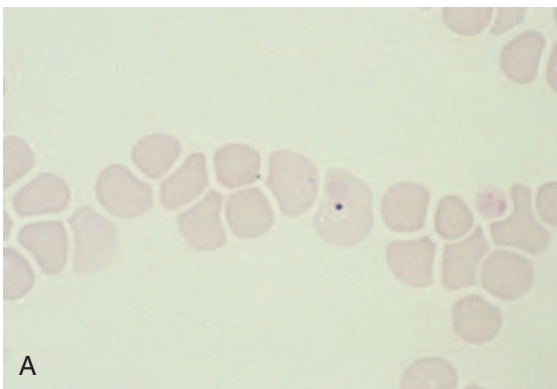


FIGURE 15-3 Canine distemper. **A**, The rounded viral inclusion bodies vary in color from gray to reddish. The most obvious inclusion body is above the dark blue, smaller Howell-Jolly body. **B**, There is a large, gray viral inclusion body in the cytoplasm of the neutrophil at about 7 o'clock at the cell margin.

BOX 15-1. BACTERIA COMMONLY ISOLATED FROM VARIOUS SITES IN INFECTIOUS DISORDERS IN DOGS AND CATS**Integument****Pyoderma**

Staphylococcus pseudintermedius
Proteus spp.
Pseudomonas spp.
Escherichia coli (usually secondary to staphylococci)

Ears

Malassezia spp.
Pseudomonas spp.
S. aureus/intermedius
Proteus spp.

Respiratory System**Pneumonia**

Pseudomonas spp.
E. coli
Klebsiella spp.
Pasteurella spp.
Bordetella spp.
Staphylococcus spp.
Streptococcus spp.
Mycoplasma spp.

Pleural Cavity

Nocardia spp.
Actinomyces spp.
Pasteurella spp.
 Anaerobes

Gastrointestinal Tract**Intestine**

Salmonella spp.
Campylobacter spp.
Clostridium perfringens
E. coli

Genitourinary Tract

E. coli
Proteus spp.
Klebsiella spp.
S. aureus/intermedius

Eyes**Conjunctiva and Cornea**

S. aureus (coagulase positive and negative)
Streptococcus spp.
S. epidermidis
E. coli
Proteus spp.
Bacillus spp.

Cardiovascular System**Aerobes**

S. aureus
 Beta-hemolytic streptococci
E. coli
Klebsiella spp.
Pseudomonas spp.
Proteus spp.
Salmonella spp.

Anaerobes

Bacteroides spp.
Fusobacterium spp.
Clostridium spp.

Data compiled from Greene CE, editor: *Clinical microbiology and infectious diseases of the dog and cat*, Philadelphia, 1998, WB Saunders.

is often cultured in patients when the source of septicemia or bacterial endocarditis is unknown.

Central Nervous System • Bacterial infection of the central nervous system (CNS) is uncommon. Even when infection occurs, low numbers of organisms make cytology and culture low-yield procedures. If increased numbers of neutrophils and increased protein are detected in CSF (see Chapter 14), however, aerobic and anaerobic bacterial culture and antimicrobial susceptibility testing are indicated. CSF samples should be placed in transport media and delivered to the laboratory as soon as possible. Aerobic and anaerobic bacterial culture should be performed when bacterial infection of the CNS is suspected.

Eye • Conjunctival culture should be performed before topical anesthesia or application of fluorescein stain by rolling a moistened sterile swab over the conjunctiva. Ocular paracentesis is necessary for intraocular culture.

Gastrointestinal Tract • Primary bacterial gastroenteritis occasionally occurs. *Salmonella* spp., *Campylobacter* spp., *C. perfringens*, and *Escherichia coli* are agents that can be involved. These organisms can also be isolated from normal animals, however. *Salmonella* spp. and *Campylobacter* spp. can cause small or mixed bowel diarrhea; *C. perfringens* is usually associated with large bowel diarrhea. Approximately 2 to 3 g of fresh feces should be submitted to the laboratory for optimal results. If delayed transport of feces to the laboratory is expected, the clinician should

BOX 15-2. NORMAL BACTERIAL FLORA AT VARIOUS SITES IN DOGS AND CATS**INTEGUMENT****Skin****Aerobes**

Micrococcus spp.
Staphylococcus spp.
Streptococcus spp.
 Gram-negative rods, including *Pasteurella* spp.
 Diphtheroids

Anaerobes

Clostridium spp.

Ears**Aerobes**

Staphylococcus spp.
Corynebacterium spp.
Streptococcus spp.
 Coliforms
Bacillus spp.

Yeast

Malassezia spp.

RESPIRATORY SYSTEM**Nasal Cavity, Pharynx****Aerobes**

Staphylococcus spp.
Streptococcus spp.
Neisseria spp.
Corynebacterium spp.
Escherichia coli
Lactobacillus spp.
Proteus spp.

Anaerobes

Clostridium spp.
Bifidobacterium spp.
Propionibacterium spp.
Fusobacterium spp.
Bacteroides spp.

Trachea

Streptococcus spp.
Staphylococcus spp.
Pasteurella spp.
Klebsiella spp.
Corynebacterium spp.

EYES**Cornea and Conjunctiva****Aerobes**

Staphylococcus spp. (coagulase positive and negative)
 Nonhemolytic, alpha- and beta-hemolytic streptococci
Bacillus spp.
Pseudomonas spp.
E. coli
Corynebacterium spp.
Neisseria spp.
Moraxella spp.

GASTROINTESTINAL TRACT**Oral Cavity and Feces****Aerobic**

Gram-positive
Streptococcus spp.
Staphylococcus spp.
Bacillus spp.
Corynebacterium spp.
 Gram-negative
 Enterobacteriaceae (especially *E. coli*, *Enterobacter* spp., *Proteus* spp., and *Klebsiella* spp.)
Pseudomonas spp.
Neisseria spp.
Moraxella spp.

Anaerobic

Gram-positive
Clostridium spp.
Lactobacillus spp.
Propionibacterium spp.
Bifidobacterium spp.
 Gram-negative
Bacteroides spp.
Fusobacterium spp.
Veillonella spp.
 Other
 Spirochetes
Mycoplasma spp.
 Yeasts

GENITOURINARY TRACT**Distal Urethra and Prepuce****Gram-positive**

S. aureus
S. epidermidis
Streptococcus spp.
Mycoplasma spp.
Bacillus spp.
Corynebacterium spp.

Gram-negative

Flavobacterium spp.
Haemophilus spp.
Moraxella spp.
Pasteurella spp.
Klebsiella spp.

consult the laboratory for appropriate transport media. Because these organisms have special culture requirements, the laboratory must be notified of the suspected pathogen. A positive culture for *C. perfringens* does not prove it was the cause of disease because not all *C. perfringens* produce enterotoxin. Conversely, not all enterotoxin-positive animals have diarrhea. Thus culture or PCR for *C. perfringens* should be combined with enterotoxin measurement (See Chapter 9).

Genitourinary Tract • Urine obtained by cystocentesis is preferred for urine culture. If a patient is severely thrombocytopenic ($<50,000/\mu\text{l}$), or if cystocentesis cannot be performed, catheterization or a midstream-voided sample is acceptable (quantitative culture is needed). Isolation of bacteria should always be assessed concurrently with the urine sediment. Rarely, difficult-to-diagnose urinary tract infections require maceration and culture of a bladder wall biopsy specimen. Calculi should be crushed with a sterile mortar and pestle and cultured. Culture for *Mycoplasma* spp. or *Candida* spp. should be considered if pyuria is identified in the absence of calculi, masses, and aerobic bacteria. *Leptospire* spp. infection should also be considered in these canine patients.

Culture of the third fraction of an ejaculate (preferred) or prostatic massage is recommended for prostatic culture. Culture of the second fraction of an ejaculate is recommended for testicular culture. Culture of prostatic or testicular material retrieved by aspiration or biopsy can also be performed. Prostatic massage and closed prostatic aspiration or biopsy should be avoided in dogs with suspected prostatic abscesses. Obtaining distal urethral specimens for quantitative culture before and after ejaculation may help avoid confusion caused by urethral contamination. Anaerobic culture of urine or prostatic fluid is rarely useful.

Integument and Ear • In superficial pyoderma, hair is clipped from the surrounding area, but disinfection is not attempted. A pustule is ruptured with a sterile fine-gauge needle, and a swab of pus is cultured. In deep pyoderma, hair surrounding the lesion is clipped and the area is disinfected with an antiseptic. The lesion is squeezed to express exudate, which is collected on a swab. Gloves should be worn.

For culture of ears, a sterile otoscope cone is inserted to the level of the horizontal canal and the ear is swabbed through the cone. When middle-ear infection is suspected, the animal is anesthetized and material for culture is retrieved by myringotomy by penetration of the tympanum with a sterile CSF needle placed through a sterile otoscope cone.

Musculoskeletal System • No normal flora exists in musculoskeletal tissues. Dogs with radiographic evidence of discospondylitis should be evaluated for *Brucella canis* and *Bartonella* spp. infections (see [Diagnostic Tests for Select Bacterial Infections](#)). Intervertebral joints can be cultured after fluoroscopically guided aspiration or when decompressive spinal surgery is required. Most cases of discospondylitis develop after hematogenous spread of bacteria from an extravertebral source. Blood and urine are commonly cultured from patients with discospondylitis; *Staphylococcus* spp. are commonly involved.

Dogs or cats with suppurative arthritis (with or without cytologic visualization of bacteria) should have the synovial fluid cultured for aerobes and *Mycoplasma* spp. (see Chapter 10). Likelihood of positive culture results increases if the synovial fluid contains degenerative neutrophils. L-form bacteria usually cannot be grown from joint fluid via routine culture techniques. Synovial biopsy for culture plus histopathologic evaluation for L-form bacteria is more sensitive than only culture of fluid. *Borrelia burgdorferi* is almost never isolated by routine culture from joints of dogs with Lyme disease. Use of PCR assays on synovial fluid to amplify DNA of *Mycoplasma* spp., *B. burgdorferi*, *A. phagocytophilum*, and *Ehrlichia* spp. may prove to be an effective way of documenting the presence of the organisms in the joints of affected animals, but objective data are lacking.

In osteomyelitis, culture of fistulous tracts is less sensitive than culture of affected bone. Culture for infectious myositis is seldom performed unless suspicion for an anaerobic infection (e.g., *Clostridium* spp.) is based on foul odor, subcutaneous (SC) emphysema, or empyema. The clinician can better evaluate for other infectious myopathies (e.g., toxoplasmosis, leptospirosis) using serologic testing or PCR assays.

Respiratory System • Lower airway specimens are best obtained by transtracheal aspiration or bronchoalveolar lavage during bronchoscopy. Fine-needle pulmonary aspiration biopsy can be used but carries more risk (see Chapter 11). Bacteria can be isolated from the trachea in some clinically healthy dogs. These bacteria are probably transient; common isolates are listed in [Box 15-2](#). Because many organisms isolated from normal dogs have also been associated with lower respiratory tract inflammation, all transtracheal aspiration samples should be evaluated by culture, antimicrobial susceptibility, and cytology. With cytology, the clinician should look for squamous cells coated with bacteria (which indicate oropharyngeal contamination) (see Figure 11-12). Bacteria should not be considered significant unless accompanied by neutrophilic inflammation. *Mycoplasma* spp. have been isolated in pure culture from lower airways of patients with clinical signs of respiratory disease.^{18,71} Culture for *Mycoplasma* spp. should be performed on all transtracheal aspiration samples; these samples need to be transported to the laboratory in Amies medium or modified Stuart bacterial transport medium. *Mycoplasma* spp. culture should be specifically requested. Amplification of *Mycoplasma* spp. DNA from airway secretions by PCR assay can also be used to document infection.

Nasal specimens are best obtained from nasal lavage or core biopsy, or by passing a swab through a sterile otoscope cone (see Chapter 11). The clinician can best obtain pharyngeal specimens using a guarded swab taken during pharyngoscopy. Nasal and pharyngeal cultures can be difficult to interpret because of extensive normal flora in the nasal cavity and nasopharynx (see [Box 15-2](#)).

Specimen Transport

For aerobic culture, no special transport medium is required if the swab remains moist and can be inoculated onto the culture medium within 3 hours. Swabs containing liquid or gel transport media are frequently used,

however. Routine cultures can be safely stored in transport media at room temperature for up to 4 hours. After this time, overgrowth is a potential problem because of various growth rates of different organisms. Refrigerated, routine specimens can be stored in transport media for at least 2 days. Tissue samples can be refrigerated for up to 2 days. Fluids (e.g., urine) can be safely stored at room temperature for 1 to 2 hours, refrigerated for 24 hours, and refrigerated in transport media for 72 hours.⁴⁴ Quantitative culture is not accurate for fluids stored in transport media because of artifactual dilution.

For anaerobic culture, fluid should be aspirated into a syringe, the needle capped with a rubber stopper, and the sample inoculated onto culture medium within 10 minutes of collection. Transport media that support the growth of anaerobic bacteria are available but are not ideal for all fastidious organisms. With these limitations, samples can be refrigerated for 2 days in an appropriate transport medium.

Analysis • Blood agar plates grow most routine bacterial pathogens. A biplate containing blood agar and MacConkey agar is frequently used. The common anaerobic culture medium is thioglycolate. The decision to perform in-office testing instead of using a commercial laboratory is based on case load, available equipment, and expertise.

Sensitivity Testing • Sensitivity testing gives an *in vitro* estimation of suitability of a given concentration of an antimicrobial agent. Two techniques are used: (1) the dilution test and (2) the disk diffusion test.

Dilution Test • This test is quantitative and determines the least amount of antimicrobial agent needed to prevent growth of a microorganism (minimum inhibitory concentration [MIC]). Quantitative susceptibility testing is indicated when antimicrobial dosing schedules need to be monitored closely (e.g., gentamicin) or when disk test results are inapplicable, equivocal, or unreliable (e.g., slow-growing organisms, confirmation of susceptibility to polymyxins, confirmation of susceptibility or resistance to given doses of aminoglycosides). Other indications include anaerobes and testing for synergism or antagonism between antimicrobials.

Advantages • The dilution test may be effective even though disk diffusion techniques suggest otherwise (e.g., antibiotics concentrated in urine).

Disadvantages • Disadvantages of the dilution test include expense, inability to perform the test in the office, and need to determine if required concentrations of a certain antibiotic are feasible. Ideally, blood concentrations of drugs should be more than 4 times the MIC and urine concentrations 10 to 20 times the MIC. MIC sensitivity for topically administered antimicrobials is seldom determined because these methods are based on blood or urine concentrations.

Disk Diffusion Test • This is the most widely used method in clinical practice (i.e., Kirby-Bauer technique). A zone of inhibition of bacterial growth is noted around

a disk containing a fixed amount of antibiotic. The procedure is qualitative and allocates organisms to the sensitive (susceptible), intermediate (indeterminate), or resistant category.

Advantages • Advantages of the disk diffusion test are its simplicity and suitability for most routine cultures, that it can be performed in the office, and its applicability for rapidly growing organisms (e.g., Enterobacteriaceae, *Staphylococcus aureus*).

Disadvantages • This test is not suitable for slow-growing organisms and anaerobes. In addition, there is inaccuracy in predicting susceptibility of poorly diffusing antibiotics (e.g., polymyxins), and factors that influence the test (e.g., pH and thickness of the medium, concentration of organisms, incubation time) must be standardized. It is imperative that proper procedures be followed to avoid errors in diagnosis.

Artifacts • Artifacts result from improper sample collection (i.e., wrong sample, contamination), improper sample transport, failure to notify the laboratory of suspected pathogens (e.g., *Salmonella* spp., anaerobic bacteria, *Campylobacter* spp., *Mycoplasma* spp.), recent antibiotic treatment, and culture for a secondary rather than a slow-growing primary pathogen (i.e., insufficient duration of culture). Failure to grow fastidious anaerobes may be caused by short, seemingly insignificant exposure to oxygen or failure to use prerduced culture media.

Interpretation • Recognizing normal flora (see Box 15-1) is necessary for correct interpretation. Preliminary identification is expected in 18 to 24 hours, and antibiotic sensitivity is reported in 36 to 48 hours. Most aerobic and facultative organisms are identified within 5 days; identification of anaerobic organisms or *Mycoplasma* spp. may require an additional 2 to 3 days.

Bacterial pathogens commonly isolated from various body systems are listed in Box 15-2. The overlap between resident and pathogenic organisms should be noted.

Staphylococcus pseudintermedius is the major pathogen isolated from the skin of dogs with pyoderma. Gram-negative organisms are likely to be contaminants in superficial pyoderma and secondary to *S. pseudintermedius* in deep pyoderma.

Primary bacterial rhinitis is rare in dogs and cats but can result from infection with *Bordetella bronchiseptica*, *Mycoplasma* spp., and *Chlamydomydia felis* (cats). Primary bacterial pneumonia can result from *B. bronchiseptica* or *Mycoplasma* spp., whereas other organisms are usually secondary to viral infections or aspiration.

Bacterial growth from urine obtained by cystocentesis is significant because the bladder is normally sterile. Urine cultures, however, are best interpreted in conjunction with a urinalysis. If growth occurs despite absence of significant pyuria (see Chapter 7), sample contamination, improper sample transport, or diseases causing immune suppression (e.g., hyperadrenocorticism, diabetes mellitus, FIV infection) must be considered. In quantitative culture of urine obtained by catheterization or midstream voiding, greater than or equal to 100,000 colonies/ml is significant. Lower concentrations may

be significant in chronic infections or in females. In samples of prostatic fluid obtained by ejaculation, infection is diagnosed if the specimen contains greater than or equal to 100 times more bacteria than the urethral sample.³⁴ Culture of prostatic aspirates may be more accurate.

Blood cultures can be difficult to interpret. False-positive results are caused by contamination with normal cutaneous microflora, including *Corynebacterium* spp., *Bacillus* spp., coagulase-negative staphylococci, anaerobic diphtheroids, streptococci, and *Clostridium* spp. Isolation of the same organism from two or more cultures strongly suggests that it is pathogenic, whereas growth in only one culture is less certain unless it is a pathogenic bacterium unlikely to be a contaminant.

CSF and synovial fluid are normally sterile; any growth in an aseptically obtained sample is significant.

Fungal Culture

Specimen Procurement

For dermatophyte culture, hair is clipped from the lesion periphery; hair shafts are plucked with forceps and cultured on dermatophyte test medium (DTM) or Derm Duet.

SC and deep fungal infections are best diagnosed by cytologic or histopathologic evaluation, with or without serology. If organisms cannot be identified, cutaneous lesions can be cultured, but these are rarely useful owing to overgrowth by resident bacteria and fungi. The lesion is prepared as for dermatophytes, and a swab is cultured onto Sabouraud and Mycose medium.

Systemic and SC fungi may require 2 weeks' cultivation on Sabouraud medium for growth to occur.

DIAGNOSTIC TESTS FOR SELECT BACTERIAL INFECTIONS

Bartonellosis, Feline (*Bartonella henselae*, *B. clarridgeae*, *B. koehlerae*)

Occasional Indications • To date, most cats with clinical bartonellosis have been infected with *Bartonella henselae*. However, *B. clarridgeae*, *B. koehlerae*, and *B. quintana* have been identified in some clinically ill cats.^{13,14} *Bartonella henselae*, *B. clarridgeae*, and *B. koehlerae* are transmitted among cats by *Ctenocephalides felis* and so cats with a history of flea infestation are more likely to be infected.²⁰ Most cats exposed to *Bartonella* spp. maintain subclinical infections; however, fever, uveitis, lymphadenopathy, endocarditis, myocarditis, hematuria, and hyperglobulinemia have been documented convincingly in experimentally infected or naturally exposed cats. Results have been equivocal for a link between *Bartonella* spp. infection and gingivitis or stomatitis.²³ *Bartonella* testing may be indicated in cats with one or more of these clinical syndromes for which another explanation is not readily apparent.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected by immunofluorescent antibody assay (IFA), enzyme-linked immunosorbent assay (ELISA), and Western blot immunoassay.^{13,49} Results of

these assays prove previous exposure to a *Bartonella* spp., but as serologic cross-reaction occurs between *B. henselae*, *B. clarridgeae*, and *B. koehlerae*, assays based on *B. henselae* antigens cannot differentiate between the agents. Current *Bartonella* spp. infection is proven by culture or PCR assay results. Results of some PCR assays or genetic sequencing can be used to identify the species of *Bartonella* involved with the infection.⁴³ Cats can be infected with more than one *Bartonella* spp. concurrently. Detection of local antibody production by the eye and documentation of *Bartonella* spp. DNA in aqueous humor has been used to document uveitis as a result of bartonellosis.⁵¹ (See Appendix I for laboratories for infectious diseases.)

Up to 93% of healthy cats exposed to *C. felis* can be seropositive, and so antibody tests results alone cannot be used to prove clinical bartonellosis.⁶⁷ Between 3% and 15% of seronegative cats are bacteremic, and so antibody test results cannot be used to exclude *Bartonella* spp. infection from the differential list. Positive blood culture or PCR results prove current infection but do not document clinical illness. Repeated bacteremia has been detected in experimentally inoculated and naturally infected cats; therefore a single negative blood culture or PCR result does not exclude infection.⁴⁷ Because of these findings, it is currently recommended to combine serologic test results with those of blood culture or PCR assay results when evaluating clinically ill cats for bartonellosis. (See Appendix I for laboratories for infectious diseases.) If test results are positive and there is no better explanation for the cause of illness, treatment may be indicated. To date, there has been no proven clinical benefit to following *Bartonella* spp. assay results after positive response to therapy.

Because serologic test results do not accurately correlate with presence of bacteremia and individual culture or PCR assay results can be falsely negative, there is no indication for testing healthy cats for *Bartonella* spp. infection.^{14,46} The Centers for Disease Control and Prevention recommends maintaining flea control and avoiding bites and scratches to avoid bartonellosis.⁴⁵ Healthy cats used for blood donors should be seronegative and culture or PCR negative and should be maintained on flea control products.⁸¹

Bartonellosis, Canine (*Bartonella vinsonii* and *Bartonella henselae*)

Occasional Indications • Dogs from endemic areas or with an appropriate travel history with unexplained myocarditis, endocarditis, granulomatous lymphadenitis, cutaneous vascular disease, hemolytic anemia, polyarthrititis, idiopathic effusions, granulomatous meningoencephalitis, granulomatous rhinitis, or thrombocytopenia should be considered for *Bartonella vinsonii* testing.^{12,13} The full spectrum of *B. henselae*-associated illnesses in dogs has not been determined but appears to be similar to that for *B. vinsonii*.^{13,30} Based on seroprevalence studies, rural dogs with fleas or ticks are most likely to be exposed to *Bartonella* spp.

Analysis, Artifacts, and Interpretation • Circulating antibodies against *B. vinsonii* and *B. henselae* are most commonly detected by IFA. Infection can be documented

by culture or PCR assay result. (See Appendix I for laboratories for infectious diseases.)

Serologic cross-reactivity between *B. vinsonii* and *B. henselae* is variable; therefore individual assays for both agents are indicated. Antibodies can be detected in dogs with and without clinical signs. Seronegative test results make clinical illness caused by *B. vinsonii* or *B. henselae* less likely. However, many infected dogs have been falsely negative for *Bartonella* spp. antibodies. Thus the current recommendation is to combine serologic test results with PCR assay or culture. *Bartonella* spp. are more difficult to amplify or culture from dog blood than cat blood. The gold standard test for documentation of *Bartonella* spp. infection is the combination of culture on BAPGM media with PCR assay (Galaxy Diagnostic, Inc).^{25,59} (See Appendix I: Select Laboratories for Infectious Disease.)

As in cats, no test result proves clinical bartonellosis in dogs, but if test results are positive and there is no better explanation for the cause of illness, treatment may be indicated.

Borreliosis (Lyme Disease) (*Borrelia burgdorferi*)

Occasional Indications • Dogs from areas endemic for *Ixodes* ticks or with an appropriate travel history and fever, lameness, glomerulonephritis, or nonseptic, suppurative polyarthritis should be suspected of having Lyme disease (borreliosis) and screened for antibodies against *B. burgdorferi*.^{10,22,58}

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by IFA, ELISA, and Western immunoblot. The organism can be documented in tissues by culture, histologic techniques, or PCR assay result. (See Appendix I: Select Laboratories for Infectious Disease.)

Serum antibodies are generally used to screen for exposure to *B. burgdorferi* in dogs. Immunoglobulin M (IgM) and immunoglobulin G (IgG) antibodies against *B. burgdorferi* can be detected in canine serum. Titers considered significant vary by laboratory and assay. Both antibody classes can persist in serum for months after exposure. Depending on the assay used, cross-reactivity with *B. burgdorferi* antigens used in IFA and ELISA can occur with other spirochetes; thus a positive titer does not always document exposure to *B. burgdorferi*. *B. burgdorferi* vaccines induce antibodies that are detected by some IFA and ELISA. Western immunoblot can be used to differentiate vaccine-induced antibodies from antibodies resulting from natural infection. Antibodies against the C6 peptide of *B. burgdorferi* are rarely induced by vaccination; point-of-care assays using this peptide are commercially available.⁵⁵ Because *B. burgdorferi* migrates through the tissues, most dogs with borreliosis are positive for antibodies by the time illness is detected. Healthy dogs develop the same antibody responses as clinically ill dogs, however. Because of these factors, interpretation of positive serum antibody titers is difficult. Serum antibodies against *B. burgdorferi* only document exposure to *B. burgdorferi* (or a similar antigen), not clinical disease. An assay to quantify antibodies against the C6 peptide is commercially available (see Appendix I: Select

Laboratories for Infectious Disease); however, clinical utility of this assay has not been documented.⁵⁶

Definitive diagnosis requires demonstration of the organism by culture, histopathologic evaluation of tissue, or PCR assay. Presumptive diagnoses of clinical Lyme disease in dogs can be based on appropriate clinical, historic, and laboratory evidence of disease combined with positive serologic testing and response to therapy.

Brucellosis (*Brucella canis*)

Occasional Indications • Dogs with reproductive tract abnormalities, lymphadenomegaly, hyperglobulinemia, discospondylitis, or uveitis should be suspected of having brucellosis and be screened for antibodies against *Brucella canis*.

Analysis, Artifacts, and Interpretation • Circulating antibodies are most commonly detected in serum by rapid slide agglutination test (RSAT), tube agglutination test (TAT), agar gel immunodiffusion (AGID), and ELISA.^{16,79}

The RSAT and TAT are screening procedures; an RSAT for point-of-care use is commercially available. Both assays should be performed with 2-mercaptoethanol (2-ME) to eliminate heterologous IgM agglutinins responsible for most false-positive reactions. False-positive reactions in the 2-ME TAT may be the result of auto-agglutination in hemolyzed samples. AGID can be performed using cell wall antigens or cytoplasmic antigens. AGID performed with cytoplasmic antigens is the most specific antibody assay; AGID performed with cell wall antigens is the most sensitive. Because of nonspecific precipitin reactions, positive results in AGID with cell wall antigens are difficult to interpret.

Minimal time between infection and a positive test result varies with the test, but most infected dogs are seropositive in the 2-ME TAT and AGID by week 8 to 12 after infection. 2-ME TAT titers from different laboratories cannot be meaningfully compared; however, a titer of 1:50 to 1:100 is generally suspicious, whereas a titer greater than or equal to 1:200 usually correlates with isolation of *B. canis* from blood culture.¹⁶ After cessation of bacteremia, 2-ME TAT titers rapidly decrease to less than 1:200 within a few weeks and remain low (1:25 to 1:50) for 6 months or longer. In AGID, antibodies to external antigens persist for a few weeks, whereas antibodies to internal (i.e., cytoplasmic) antigens persist up to 12 months after cessation of bacteremia. Although these animals are abacteremic, *B. canis* can be isolated from selected organs (e.g., epididymis, prostate).

When the 2-ME RSAT or TAT is used as a screening test and results are positive, a tentative diagnosis of brucellosis is made; positive blood culture or AGID should be used to confirm results. If blood culture or AGID is negative, brucellosis is unlikely. If 2-ME RSAT or TAT results are negative in a dog strongly suspected of having brucellosis, the test should be repeated in 4 weeks to preclude the possibility of early infection.

Definitive diagnosis requires isolation of *B. canis*, although this is not always achieved. Although blood culture is ideal, it is inconvenient and expensive. Culture of urine or an ejaculate may also be performed in males.

Growth usually occurs within 7 days, but cultures should be held for 3 to 4 weeks before being discarded. At least three cultures from specimens obtained several days apart are recommended.

Leptospirosis (*Leptospira* spp.)

Occasional Indications • Serologic testing for antibodies against *Leptospira* spp. should be considered in dogs with undiagnosed fever, ecchymoses, vomiting, diarrhea, muscle pain, uveitis, coughing, dyspnea, renal pain, thrombocytopenia, renal failure (particularly acute), or increased activities of hepatic enzymes.^{77,80} The most common pathogenic serovars in dogs include *Leptospira canicola*, *L. icterohaemorrhagiae*, *L. grippityphosa*, *L. bratislava*, *L. autumnalis*, and *L. pomona*.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by the microscopic agglutination test (MAT), ELISA (IgM, IgG), and microscopic microcapsular agglutination test (MCAT). Most diagnostic laboratories use MAT. The primary disadvantage of serologic testing is that it is difficult to determine whether positive titers are caused by active infection, previous infection, or vaccination. In addition, when results from different laboratories are compared, results commonly vary.

A laboratory that assesses for antibodies against multiple serovars and participates in The International Leptospirosis Society proficiency program should be used.⁷⁶ Antibodies are detected by MAT days to weeks after infection. Acutely infected dogs are often MAT negative; dogs with suggestive clinical signs of disease but negative MAT results should be retested in 7 to 14 days; development of a positive titer confirms recent infection. A fourfold increase in antibody titer also can confirm recent infection. Vaccination can induce positive MAT titers. Because of the presence of cross-reactive antibodies, one cannot assume that the serovar inducing the highest titer during acute infection is the serovar causing infection. The combination of increasing antibody titers with appropriate clinical pathologic abnormalities and clinical findings suggests clinical leptospirosis.

Definitive diagnosis requires demonstration of the organism by urine dark-field microscopy, phase-contrast microscopy, culture, or PCR assay. Examination of urine for leptospire is a low-yield procedure. Demonstration of spirochetes by histopathologic evaluation of renal tissue leads to a presumptive diagnosis, which may be confirmed by tissue culture. Culture or PCR assay can be of most benefit early in the course of infection when MAT results are negative. The organism is in high levels in blood for the first 10 days of infection and then is highest in urine.³⁶ Repeated culture may be needed because of intermittent shedding. Administration of antimicrobial therapy can result in false-negative results.

Tularemia (Rabbit Fever) (*Francisella tularensis*)

Rare Indications • Testing for tularemia (i.e., rabbit fever) should be considered in animals from endemic areas developing fever, lymphadenomegaly, weight loss,

or oral ulceration, particularly if tick exposure, rabbit ingestion, or potential for human infection is confirmed. Tularemia is a direct zoonosis from clinically ill cats to people.

Analysis, Artifacts, and Interpretation • Clinicians measure antibodies in serum using a microscopic agglutination (MA) assay.^{32,60} (See Appendix I: Select Laboratories for Infectious Disease.)

Time between acquisition of infection and a positive titer is not known. In dogs, titers of 1:140 to 1:160 are commonly detected in acute infections. In cats with tularemia, MA titers are generally greater than 1:20. Development of a positive titer or a fourfold increase in titer between acute and convalescent sera (2 weeks later) is presumptive evidence of infection. Definitive diagnosis is obtained by isolation of the bacterium in a culture of a blood specimen or by identification in tissue by immunofluorescence.

DIAGNOSTIC TESTS FOR FUNGAL INFECTIONS

Aspergillosis (*Aspergillus fumigatus*)

Occasional Indications • Dogs and cats with nasal or pulmonary disease can be serologically screened for antibodies against *Aspergillus fumigatus*; cats are affected less frequently than dogs. Results are generally interpreted in conjunction with cytology, radiology, histopathology, and culture.

Analysis, Artifacts, and Interpretation • AGID, counterimmunoelectrophoresis (CIEP), and ELISA have been used to detect circulating antibodies against *A. fumigatus* in serum.⁶² Presence of serum antibodies can represent either exposure or infection, and some dogs with nasal aspergillosis are falsely negative for serum antibodies. In one recent study of dogs with and without nasal aspergillosis, the sensitivity, specificity, and positive and negative predictive values for serum anti-*Aspergillus* antibody results were 67%, 98%, 93%, and 84%, respectively.⁷⁰ Owing to persistence of titers in some treated dogs (i.e., 12 months), monitoring titers to assess therapeutic response is not recommended. Dogs or cats infected with *Penicillium* spp. will be seronegative if assessed only in assays using *A. fumigatus* antigens.

Radiographic demonstration of nasal turbinate destruction suggests aspergillosis or nasal neoplasia. Cytologic analysis (see Figure 16-18) and culture of canine nasal exudate alone are not diagnostic because fungal elements may be nondetectable in affected dogs whereas they may be found in noninfected dogs (including dogs with nasal tumors). The organism is sometimes difficult to culture from an aspergilloma (fungal ball). Nasal lavage is a low-yield procedure for demonstration of the organism; nasal biopsy is suggested (see Chapter 11). Definitive diagnosis should be based on three factors: (1) histopathologic evidence of tissue invasion, (2) an aspergilloma combined with serologic and culture evidence of infection, or (3) serologic and radiographic evidence of infection (i.e., bone lysis). In rare cases with

disseminated disease, cytologic evaluation of aspirates of affected tissue may be useful. If the organism cannot be demonstrated by biopsy samples obtained through the nares, positive serologic test results may support exploratory surgery.

Blastomycosis (*Blastomyces dermatitidis*)

Occasional Indications • Dogs from endemic areas with fever, weight loss, pulmonary interstitial disease, lymphadenomegaly, uveitis and blindness, ulcerative or draining skin lesions, undiagnosed prostatic or testicular disease, intracranial disease, osteomyelitis, or (rarely) renal disease can be serologically screened for antibodies against *Blastomyces dermatitidis* and *B. dermatitidis* antigens if the organism is not demonstrated by cytology (see Figure 11-13), histopathology, or culture.^{52,75} In endemic areas, screening for antibodies against *B. dermatitidis* should be considered in cats with pulmonary interstitial disease, intracranial disease, lymphadenomegaly, ulcerative or draining skin lesions, or uveitis and blindness.

Analysis, Artifacts, and Interpretation • Circulating antibodies are most commonly detected in serum by AGID. *Blastomyces* antigens in urine and blood of dogs have been measured using the MVista Blastomyces antigen EIA (miravistalabs.com).⁷⁴ Because subclinical canine infections are unusual, positive serum antibody test results are considered significant. False-negative results occur in animals with peracute infection or with advanced cases overwhelming the immune system. In dogs, the sensitivities of antigen testing of urine, antigen testing of serum, and AGID serum antibody testing were 93.5%, 87.0%, and 17.4%, respectively.⁷² Thus dogs with a high index of suspicion for blastomycosis that are negative for serum antibodies should be screened for urine or serum antigens. Many cats with blastomycosis are serum antibody negative. Whether serum or urine antigen testing will aid in the diagnosis of blastomycosis in cats remains to be determined.

Definitive diagnosis requires identification of the yeast by cytology, histopathology, or fungal culture. Impression smears from skin lesions and aspirates from enlarged lymph nodes frequently reveal organisms; recovery of organisms from transtracheal aspiration, pulmonary aspiration biopsy samples, or urine is less consistent. Culture requires 10 to 14 days and is of lower yield than cytology or biopsy. Diffuse nodular interstitial pulmonary disease and hilar lymphadenomegaly are common radiographic findings. Positive serologic results combined with appropriate clinical signs and radiographic abnormalities allow presumptive diagnosis.

Coccidioidomycosis (*Coccidioides immitis*)

Occasional Indications • Dogs from endemic areas with pulmonary interstitial disease, fever of undetermined origin, hilar lymphadenomegaly, osteomyelitis, uveitis, pericarditis, and nodular or ulcerative skin lesions can be screened for antibodies against *C. immitis* if the

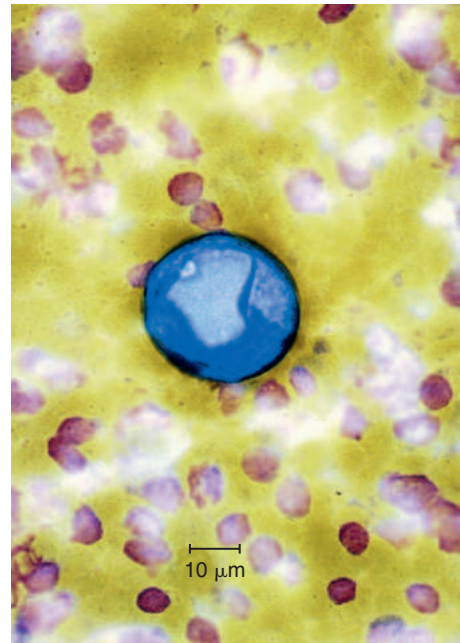


FIGURE 15-4 Cytology of lymph node with a *C. immitis* spherule. Note that the larger spherule contains numerous endospores.

organism is not demonstrated by cytology (Figure 15-4), histopathology, or culture.³⁴ Feline disease is rare but has been associated with nodular or ulcerative skin lesions, pulmonary interstitial disease, osteomyelitis, uveitis, and CNS disease.³⁵ An antigen test has been evaluated for use with samples from humans but has not been validated for use with samples from dogs or cats.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by complement fixation (CF), AGID, ELISA, latex agglutination (LA), and tube precipitin (TP) tests. TP detects IgM antibodies; CF and AGID detect IgG antibodies.³⁴ False-negative results in TP occur in early infections (<2 weeks), chronic infection, rapidly progressive acute infection, and primary cutaneous coccidioidomycosis. False-positive results in the CF test are caused by anticomplementary serum, which may be caused by bacterial contaminants or immune complexes. Finally, cross-reactions in patients with histoplasmosis and blastomycosis may occur with all tests. After resolution of disease, CF titers decrease over weeks but remain positive at a low titer (e.g., 1:32) for months.

Definitive diagnosis requires demonstration of the organism on smears, aspirates, histopathologic evaluation, or culture. The organism is often difficult to demonstrate. Wet mount examination of unstained or stained (periodic acid–Schiff) smears or aspirates is more suitable than dry mounts, which may distort the spherules. Common thoracic radiographic findings are mixed interstitial, bronchial, and alveolar pulmonary patterns and hilar lymphadenomegaly. Positive serologic test results and characteristic radiographic changes allow tentative diagnosis.

Cryptococcosis (*Cryptococcus neoformans*)

Occasional Indications • Cats and rarely dogs with undiagnosed respiratory (especially nasal), CNS, eye (especially uveal tract), and skin (especially nodular or ulcerative lesions) infections can be screened for *Cryptococcus neoformans* and *C. gattii* antigens if the organism is not demonstrated by cytology, histopathology, or culture.^{26,61}

Analysis, Artifacts, and Interpretation • Measurement of antibodies against *C. neoformans* or *C. gattii* is not clinically useful. Cryptococcal antigen is detected in serum, aqueous humor, or CSF using latex agglutination.

Negative serum LA titers may occur in early disease or uncommonly in chronic low-grade infections, in chemotherapy-induced remission, or in nondisseminated disease. Specificity of the serum LA is high. A titer of greater than 1:1 in serum or CSF is positive; very high titers are commonly detected. In some animals, decreases in serum titer parallel response to therapy.⁶¹ Positive titers occur in some animals after apparently successful clinical responses, suggesting persistent low-grade infection or false-positive results.^{27,42} Cryptococcal encephalitis may cause a positive CSF LA titer despite a negative serum LA.

Definitive diagnosis is based on cytologic, histopathologic, or culture demonstration of the organism or a positive LA test result. Cytology is commonly positive (see Figure 11-3) because there are usually numerous yeasts found in affected tissues (i.e., nasal and cutaneous lesions, aqueous and vitreous humor).

NOTE: The organism can occasionally be recovered from nasal washings of normal animals.

CSF may contain the yeast, but concentration techniques (i.e., cytocentrifugation) should be used. Routine cytology stains (e.g., Wright) are adequate to demonstrate the organism. Large numbers of organisms are usually visible despite little or no inflammation. Culture is seldom necessary. Serologic testing is used if the yeast cannot be demonstrated cytologically or to monitor response to treatment. A PCR assay has been used to amplify the organism DNA from tissue but has not been assessed extensively to date.⁴⁵

Histoplasmosis (*Histoplasma capsulatum*)

Rare Indications • Animals with weight loss, pulmonary interstitial disease, uveal disease, diarrhea, or lymphadenomegaly can be serologically screened for antibodies against *Histoplasma capsulatum* if the organism is not demonstrated by cytology, histopathology, or culture.³¹

Analysis, Artifacts, and Interpretation • Primarily, AGID is used to detect circulating antibodies in serum. Presence of serum antibodies confirms exposure but not clinical illness because of infection. AGID has

questionable clinical usefulness because titers persist longer than 1 year after resolution of disease in some animals, and both false-positive and false-negative results occur. Antibody testing is even less rewarding in cats.

Definitive diagnosis requires demonstration of the organism by cytology (see Figure 9-3A), biopsy, culture, or PCR assay. An enzyme immunoassay test used to detect histoplasma antigen in the urine of people has not been validated for use with samples from dogs and cats. The organism is more difficult to demonstrate than *B. dermatitidis*; however, cytologic examination of rectal scrapings in dogs with colonic histoplasmosis is often diagnostic. Fine-needle aspiration of other organs may demonstrate the organism. In most cats with systemic histoplasmosis, the organism is identified on bone marrow cytology. Thoracic radiographs are indicated if pulmonary histoplasmosis is suspected; a nodular interstitial pattern is expected. Culture of *H. capsulatum* is of lower yield than biopsy.

DIAGNOSTIC TESTS FOR SELECT PROTOZOAL INFECTIONS

Babesiosis (*B. canis vogeli* and *B. gibsoni* in Dogs in the United States)

Rare Indications • *Babesia canis vogeli* and *B. gibsoni* infect dogs in the United States, and diagnostic tests can be performed in dogs from endemic areas or in those with an appropriate travel history that have fever, anemia, icterus, splenomegaly (i.e., acute babesiosis), or intermittent fever and weight loss (i.e., chronic babesiosis). Although babesiosis can cause anemia in cats, species infecting cats are not found in the United States. Exposure to *R. sanguineus* ticks (*B. canis*) or pit bull terriers (*B. gibsoni*) are risk factors for exposure to the two agents in dogs.⁶

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by IFA. (See Appendix I: Select Laboratories for Infectious Disease.) In most laboratories, titers greater than 1:40 are considered positive. Experimentally infected dogs develop detectable IgG titers approximately 3 weeks after infection. False-negative results can occur in immature dogs, in peracute cases, or in dogs with concurrent immunosuppression. Antibodies against *B. gibsoni* and *B. canis* may or may not cross-react, depending on the antigen source used by a particular laboratory, and so specific IFA should be used for both organisms. DNA of *B. canis* and *B. gibsoni* can be amplified by PCR assay, and positive results indicate current infection.^{6,17} However, both antibodies and DNA can be detected in dogs that are healthy and those that are clinically ill. Antibody titer magnitude does not correlate to the presence or absence of disease. A titer of greater than 1:320 was suggested for *B. gibsoni*, but not all infected dogs achieve a titer of this magnitude.⁷ It is important to determine which species are involved in a case because response to treatment varies. Duration of positive titers after resolution of disease is unknown. In untreated experimentally infected dogs, titers remained high for at least 6 months. Untreated, seropositive dogs should be

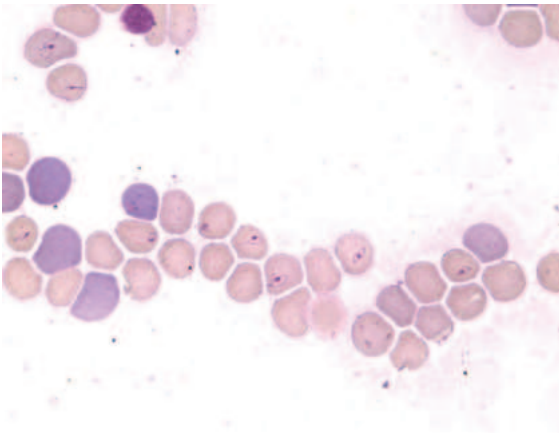


FIGURE 15-5 Blood smear of a dog with *B. gibsoni*. *B. gibsoni* appear as small dots as opposed to the classic “tear drop” shape characteristic of *B. canis*.

considered carriers of the infection. Treatment is indicated only for seropositive, clinically ill dogs.

Definitive diagnosis requires demonstration of the organism in blood smears stained with Romanowsky-type preparations (e.g., Wright and Giemsa) (Figure 15-5) or PCR assay. Organisms are best found in blood (particularly in acute disease) from a microcapillary system (e.g., ventral surface of ear or toenail).

NOTE: Shape of the organism may be distorted in old blood.

In chronic disease or asymptomatic carriers, demonstration of organisms is unreliable, and a tentative diagnosis is based on clinical signs and a positive titer. Dogs with babesiosis are often Coombs positive (see Chapter 3).

Neosporosis (*Neospora caninum*)

Rare Indications • Serology for *Neospora caninum* can be performed in dogs with clinical evidence of polyradiculomyositis, including progressive ascending rigid paralysis, dysphagia, muscle atrophy, and (rarely) myocardial dysfunction or pneumonia.²⁴

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by IFA. (See Appendix I: Select Laboratories for Infectious Disease.) A presumptive diagnosis of neosporosis can be made by combining appropriate clinical signs of disease and positive serology or presence of antibodies in CSF with the exclusion of other causes inducing similar clinical syndromes, in particular, *Toxoplasma gondii*. IgG antibody titers greater than or equal to 1:200 have been detected in most dogs with clinical neosporosis; minimal serologic cross-reactivity exists with *T. gondii* at titers greater than or equal to 1:50. Because the organism is a tissue protozoan, seropositivity may correlate with permanent infection. Circulating antibodies against *Neospora caninum* only document

infection, not clinical disease. However, if antibodies are detected in an animal with appropriate clinical signs of disease, treatment may be indicated in an attempt to slow progression.

Definitive diagnosis is based on demonstration of the organism in tissues. The organism can be differentiated from *T. gondii* structurally, by immunohistochemistry, and by PCR assay. *N. caninum* oocysts are found in the feces of some dogs.⁶⁴

Toxoplasmosis (*Toxoplasma gondii*)

Occasional Indications • **Healthy Cats** • *T. gondii*-specific antibodies form in serum, aqueous humor, and CSF of healthy and diseased cats or dogs. Antibodies do not directly correlate with clinical toxoplasmosis. No serologic test is currently available that accurately predicts when a seropositive cat previously shed oocysts. A seropositive cat is less likely than a seronegative cat to shed the organism if re-exposed.

Clinically Ill Dogs and Cats • Serologic tests for toxoplasmosis should be considered in cats with uveitis, fever, muscle disease, icterus, pancreatitis, apparent inflammatory bowel disease failing to respond to immunosuppressive therapy, CNS disease, and respiratory disease. Serologic tests for toxoplasmosis should be considered in dogs with fever, muscle disease, CNS disease, and respiratory disease. Dogs develop clinical toxoplasmosis less commonly than cats.

Analysis • **Serum Antibody Testing** • Antibodies against *T. gondii* can be detected with multiple techniques, including ELISA, IFA, Western blot immunoassay, Sabin-Feldman dye test, and various agglutination tests.^{24,48} (Table 15-1; See Appendix I: Select Laboratories for Infectious Disease.)

ELISA, IFA, and Western blot immunoassay can be adapted to detect various antibody classes; IgM and IgG are those usually assessed. *T. gondii*-specific IgM is detectable in serum by ELISA in approximately 80% of subclinically ill cats 2 to 4 weeks after experimental induction of toxoplasmosis; these titers generally are negative less than 16 weeks after infection. Detectable IgM titers were present in the serum of 93.3% of cats in a study of clinical toxoplasmosis; IgG titers were detected in 60%.⁴⁸ IgM titers persist in some clinically ill cats for greater than 16 weeks; these cats are frequently co-infected with FIV or have ocular toxoplasmosis. After repeat inoculation with *T. gondii*, primary inoculation with the Petaluma isolate of FIV, and administration of glucocorticoids, some cats with chronic toxoplasmosis experience short-term recurrence of detectable IgM titers.⁴⁸ Healthy and clinically ill dogs occasionally develop detectable IgM titers. Kinetics of postinfection IgM titers in dogs is unknown.

After experimental induction of infection in subclinically ill cats, *T. gondii*-specific IgG can be detected by ELISA in serum from most cats by 4 weeks. Positive IgG antibody titers generally persist for years after infection. Single high IgG titers have been suggested to indicate recent or active infection. The author, however, has demonstrated IgG antibody titers greater than 1:16,384 in subclinically ill cats 5 years after experimental induction

of toxoplasmosis. A positive IgG antibody titer in a single serum sample only documents exposure, not recent or active disease. Demonstration of an increasing IgG titer can document recent or active disease. Unfortunately, the time span from the first detectable positive IgG titer to the maximal IgG titer is approximately 2 to 3 weeks, leaving a very narrow window for documenting an increasing titer. Many cats with clinical toxoplasmosis have chronic low-grade signs, and they are not tested until their IgG antibody titers have reached maximal values. In humans with reactivation of chronic toxoplasmosis, IgG titers only rarely increase; cats appear to be the same.

Several agglutination tests have been evaluated using cat serum. An LA and an indirect hemagglutination assay (IHA) are commercially available. These assays are not species specific and potentially detect all classes of serum immunoglobulins directed against *T. gondii*. Unfortunately, LA and IHA rarely detect antibody in feline sera when positive for only IgM by ELISA. Modified agglutination using formalin-fixed tachyzoites is the most sensitive procedure for detection of *T. gondii* antibodies in cat sera, but it is generally unavailable commercially.

Aqueous Humor and CSF Antibody Measurement • Local production of *T. gondii*-specific IgG in CSF and aqueous humor occurs in experimentally inoculated, subclinically ill cats and in cats and dogs with clinical disease because of toxoplasmosis.

Local IgM production has only been detected in CSF and aqueous humor of animals with clinical disease. Most cats with uveitis and production of *T. gondii*-specific antibodies in aqueous humor have responded to administration of anti-*Toxoplasma* drugs, suggesting that aqueous humor antibody testing aids in diagnosis of clinical ocular feline toxoplasmosis. (See Appendix I: Select Laboratories for Infectious Disease.)

Fecal Examination • Fecal oocysts can be demonstrated using flotation techniques with various solutions with specific gravities from 1.15 to 1.18. Sugar solution centrifugation is probably the optimal technique. Oocysts of *T. gondii* are 10 to 12 μm in diameter, approximately one-eighth the size of *Toxocara cati* eggs. Focusing on only one plane of the microscope slide or coverslip can result in oocysts being overlooked. The oocysts cannot be distinguished grossly from *Hammondia hammondi* or *Besnoitia darlingi* (nonpathogenic coccidians infecting cats). Sporulated oocysts isolated from feces can be inoculated into mice or tissue cultures for definitive identification. Because oocyst shedding has rarely been documented in cats with subfatal, clinical toxoplasmosis, the diagnostic usefulness of fecal examination is limited. Cats with clinical signs referable to *T. gondii* should undergo fecal evaluation, however, because of potential zoonotic risk.

Interpretation • Exposure to *T. gondii* is suggested by finding antibodies in serum, aqueous humor, or CSF. Recent or active toxoplasmosis is suggested by finding an IgM titer greater than 1:64 or a fourfold or greater increase in IgG titer, or documenting local antibody production in aqueous humor or CSF. Because *T. gondii*-specific antibodies can also be detected in the serum, CSF, and aqueous humor of healthy, infected animals, one cannot

base an antemortem diagnosis of clinical toxoplasmosis on these tests alone. Antemortem diagnosis of clinical toxoplasmosis can be tentatively based on the combination of the following:

- Demonstration of serologic evidence of infection
- Clinical signs of disease referable to toxoplasmosis
- Exclusion of other common causes
- Positive response to appropriate treatment

T. gondii was detected by PCR in aqueous humor of 18.6% of cats with uveitis. The organism also can be detected transiently in aqueous humor and blood of healthy, experimentally inoculated cats, however, making the positive predictive value of the PCR for clinical disease less than 100%.⁴⁸

Trypanosomiasis (Chagas Disease) (*Trypanosoma cruzi*)

Rare Indications • Serologic testing for antibodies against *Trypanosoma cruzi* should be considered in dogs from endemic areas and those with generalized lymphadenomegaly, neurologic signs, or myocardial dysfunction (especially second- or third-degree heart block or ventricular tachycardia).

Analysis, Artifacts, and Interpretation • IFA, direct hemagglutination, and CF usually detect circulating antibodies in canine sera.⁴ (See Appendix I: Select Laboratories for Infectious Disease.)

Dogs are generally seropositive 3 weeks after infection. A positive titer documents exposure to the organism, not clinical disease. Positive titers vary by assay. Definitive diagnosis requires demonstration of the organism on blood smear, lymph node impression, or buffy coat and plasma interface smear. *T. cruzi* is occasionally found in peripheral blood without demonstrable organisms in tissue. A standard workup for myocardial disease, including chest radiographs, electrocardiogram, electrolytes, and echocardiography (if available), is indicated. Alternatively, *T. cruzi* amastigotes can be demonstrated in tissues. PCR can be used to amplify organism DNA.

DIAGNOSTIC TESTS FOR SELECT RICKETTSIAL INFECTIONS

Canine Granulocytotropic Anaplasmosis (*Anaplasma phagocytophilum*)

Indications • Dogs living in *Ixodes* spp. endemic areas with acute fever or polyarthritides should be screened for antibodies against *Anaplasma phagocytophilum* (previously *Ehrlichia equi*) or for *A. phagocytophilum* DNA in blood by PCR assay.^{63,66,72} The role this organism plays in chronic disease syndromes in dogs is unknown.

Analysis, Artifacts, and Interpretation • Antibodies against *A. phagocytophilum* in serum can be measured by IFA or commercially available ELISA (SNAP 4Dx, Table 15-1, IDEXX Corporation, Westbrook, ME). The antibodies have only variable cross-reactivity with other *Anaplasma* spp. *Ehrlichia* spp., or *Neorickettsia* spp. and so positive test results likely indicate exposure to *A. phagocytophilum*. Infected dogs can be seronegative when clinical signs of

disease first occur and can be immediately assessed by PCR assay or have repeat serology performed in approximately 2 weeks to evaluate for seroconversion. Alternately, both assays can be performed at the same time. Antibiotic therapy can lead to falsely negative PCR assay results. *A. phagocytophilum* DNA and antibodies can be detected in both healthy and clinically ill dogs, and so positive test results do not document clinical illness. Morulae are only rarely documented cytologically in clinical specimens.

Feline Granulocytotropic Anaplasmosis (*Anaplasma phagocytophilum*)

Indications • Cats living in *Ixodes* spp. endemic areas with fever, mild thrombocytopenia or clinical evidence of polyarthritis should be evaluated for *A. phagocytophilum* DNA in blood by PCR assay.^{8,50} Antibodies can be detected in serum by IFA, but a standardized test is not available. (See Appendix I: Select Laboratories for Infectious Disease.)

Analysis, Artifacts, and Interpretation • DNA of *A. phagocytophilum* has been amplified from several cats with clinical signs of anaplasmosis.⁵⁰ Some of the cats were seronegative when first assayed but had seroconverted when assayed at a later date. Whether the point-of-care assay used to detect *A. phagocytophilum* antibodies in canine serum is valid for use with cat serum is unknown. Antibiotic therapy can lead to falsely negative PCR assay results. *A. phagocytophilum* DNA and antibodies can be detected in both healthy and clinically ill cats, and so positive test results do not document clinical illness. Morulae are only rarely documented cytologically in clinical specimens. Untreated healthy cats can be PCR positive for weeks after tick exposure.

Canine Granulocytotropic Ehrlichiosis (*Ehrlichia ewingii*)

Indications • Dogs living in the Midwest United States with evidence of fever or clinical evidence of polyarthritis should be evaluated for *Ehrlichia ewingii* DNA in blood by PCR assay.^{57,66} Specific antibodies can be detected in serum, but a standardized test is not currently available.⁶⁸

Analysis, Artifacts, and Interpretation • There is variable cross-reactivity between *E. canis* antigens and *E. ewingii* antigens, and so serologic tests for *E. canis* will not always be positive when the infecting agent is *E. ewingii*. PCR assays that amplify the DNA of *E. ewingii* are available and should be performed on blood from dogs with suspected acute infection. Antibiotic therapy can lead to falsely negative PCR assay results. Healthy dogs can be PCR positive for *E. ewingii* DNA in blood.

Canine Monocytotropic Ehrlichiosis (*Ehrlichia canis*, *E. chaffeensis*, *Neorickettsia risticii*)

Common Indications • *Ehrlichia canis*, *E. chaffeensis*, and *Neorickettsia risticii* all infect monocytes of dogs and can be associated with clinical illness.⁶⁶ Based on PCR

assay results in clinically ill dogs, *E. canis* appears to be the organism in this group that is most commonly associated with clinical illness. Serologic testing or PCR assay for *E. canis* is indicated for dogs from endemic areas or with an appropriate travel history and thrombocytopenia, anemia, leukopenia, hyperglobulinemia, proteinuria, polyarthritis, fever, uveitis, lymphadenomegaly, hepatosplenomegaly, or inflammatory CNS disease, particularly if the animal has a history of exposure to *Rhipicephalus* ticks.

Analysis, Artifacts, and Interpretation • Circulating antibodies against *E. canis* are detected in serum by IFA or ELISA; they do not cross-react with *Rickettsia rickettsii* or *Anaplasma platys* (see [Canine Thrombocytotropic Anaplasmosis](#) later). Serologic cross-reactivity between *E. canis* antibodies and those against *A. phagocytophilum* (previously *Ehrlichia equi*; see [Canine Granulocytotropic Anaplasmosis](#) earlier), *E. chaffeensis*, *E. ewingii* (see [Canine Granulocytotropic Ehrlichiosis](#) earlier), and *Neorickettsia risticii* (previously *E. risticii*) is variable. Multiple serologic tests are needed to exclude all of the *Ehrlichia* spp., *Anaplasma* spp., and *Neorickettsia* spp. from the differential list. Thus PCR assays are often combined with serologic tests. In addition, PCR assay results may be positive before seroconversion in some dogs.

In experimentally infected dogs, antibodies against *E. canis* can be detected as early as 7 days and are almost always present by 28 days after inoculation.⁶⁶ Antibody titers continue to increase for weeks to months after inoculation in untreated, experimentally infected dogs. *E. canis* titers of less than 1:80 are suspect and should be rechecked in approximately 14 to 21 days; a titer of 1:80 or higher is diagnostic. Initial positive results in a recently marketed point-of-care test occur at approximately 1:100. Positive titers revert to negative 3 to 9 months after resolution of infection; persistence of titers for greater than or equal to 9 months suggests a carrier state. However, positive antibody titers have been detected for months after apparently successful therapy in some naturally infected dogs.⁵ Clinically ill, seropositive dogs should be treated a minimum of 28 days and until clinical and laboratory abnormalities have resolved.⁶⁶ Whether to treat healthy, seropositive dogs is controversial; the issues involved in this decision were recently reviewed.^{40,66}

The clinician can make a definitive diagnosis of *E. canis* infection by demonstrating morulae (i.e., clusters of the organism) in mononuclear cells, culture, or PCR assay. Morulae are rarely found on routine blood smear or bone marrow aspiration cytology unless the dog has been immunosuppressed. Cytology and PCR assay results can be falsely negative in dogs that have been treated. *Ehrlichia* spp. can be isolated by tissue culture of heparinized infected canine blood or bone marrow aspiration samples, but culture is of limited availability, expensive, and of low yield. *Ehrlichia* spp. can be detected in whole blood by PCR,⁶⁵ which has potential benefit for use in monitoring treatment. (See Appendix I: Select Laboratories for Infectious Disease.) The Consensus Statement on Ehrlichial Disease of Small Animals from the Infectious Disease Study Group of the American College of Veterinary Internal Medicine (ACVIM)⁶⁶ states the following:

If PCR is used to monitor treatment, the PCR assay should be repeated after antimicrobial therapy has been discontinued for 2 weeks. If PCR results are positive, an additional 4 weeks of treatment should be given with the PCR assay repeated after antimicrobial therapy has been discontinued for 2 weeks. If PCR results are positive after 2 treatment cycles, use of an alternate antiehrlichial drug should be considered. If PCR results are negative the test should be rechecked in 2 months; if still negative therapeutic elimination is likely. However, the organism may be sequestered in other tissues like the spleen.

Feline Monocytotropic Ehrlichiosis (*Ehrlichia* spp.)

Rare Indications • Cats with thrombocytopenia, anemia, leukopenia, hyperglobulinemia, proteinuria, polyarthritis, fever, or lymphadenomegaly should be evaluated for *Ehrlichia* spp. DNA in blood by PCR assay if no other obvious cause exists.^{9,11,69} To date, *E. canis* is the monocytotropic strain amplified from naturally exposed cats.¹¹ Antibodies can be detected in serum by IFA, but a standardized test is not available. (See Appendix I: Select Laboratories for Infectious Disease; Protatek Reference Laboratories.)

Analysis, Artifacts, and Interpretation • While serum antibodies against *E. canis* have been detected in the serum of some cats, a number of cats with *E. canis* DNA amplified from blood were seronegative.^{11,69} Thus while IFA testing is available for *E. canis* antibodies in feline serum, it should be combined with PCR assay. Antibodies against *E. canis* can be detected in serum from healthy cats and therefore cannot be used alone to make a definitive diagnosis of ehrlichiosis.⁷⁶ A tentative diagnosis of feline ehrlichiosis is based on the combination of clinical signs, positive serologic or PCR assay results, exclusion of other known causes, and response to tetracyclines.

Canine Thrombocytotropic Anaplasmosis (*Anaplasma platys*)

Occasional Indications • Testing for *Anaplasma platys* infection is indicated for dogs from endemic areas or with appropriate travel history and thrombocytopenia or endogenous uveitis.^{57,66}

Analysis, Artifacts, and Interpretation • Circulating IgG antibodies against *A. platys* are detected in serum by IFA. Antibodies against *A. platys* react with *A. phagocytophilum* antigen used in a commercially available kit.¹⁹ Experimentally infected dogs become antibody positive 13 to 19 days after infection.²⁸ Infected dogs can be seronegative when clinical signs of disease first occur and can be immediately assessed by PCR assay or have repeat serology performed in approximately 2 weeks to evaluate for seroconversion. Alternately, both assays can be performed at the same time. Antibiotic therapy can lead to falsely negative PCR assay results. *A. platys* DNA and antibodies can be detected in both healthy and clinically ill dogs, and so positive test results do not document clinical illness. Morulae are only rarely documented cytologically in clinical specimens.

Rocky Mountain Spotted Fever (*Rickettsia rickettsii*)

Occasional Indications • Serologic testing for *Rickettsia rickettsii* antibodies (Rocky Mountain spotted fever [RMSF]) is indicated for dogs from endemic areas or with an appropriate travel history and acute onset of fever, lymphadenomegaly, petechiae, neurologic signs, stiff gait, peripheral edema, dyspnea, or scleral congestion. History of tick exposure is inconsistent. Exposed dogs either develop acute disease with approximately a 14-day clinical course or are subclinically infected. The primary tick vectors are active from spring to fall in most of the United States; therefore RMSF should only be considered a principal differential diagnosis for clinically ill dogs during this time span. The majority of cases are diagnosed in Southeastern states.

Analysis, Artifacts, and Interpretation • Antibodies against *R. rickettsii* in canine serum can be measured by IFA, ELISA, and LA. ELISA or IFA can detect IgM and IgG antibodies against RMSF. LA is not antibody class-specific. Cutoffs for positive antibody titers, as well as specificity and sensitivity, vary by assay.³³ Antibodies against the nonpathogenic spotted fever group *Rickettsia* (*R. belli*, *R. montana*, *R. rhipicephali*) cross-react with *R. rickettsii* antigens. In dogs with clinical illness because of RMSF, IgM antibody titers are generally positive. Because IgM has short duration in serum, false-negative results may occur with IgM testing. False-positive results are most common in the IgM ELISA. Positive IgG titers are detectable 20 to 25 days after infection. Serum samples with IgG titers greater than or equal to 1:64 are generally considered positive. If IgG or IgM antibodies are not detected in a patient with clinical and laboratory evidence of RMSF, a convalescent IgG titer 2 to 3 weeks later is recommended. Timing of the second titer is not critical because IgG antibody titers do not decrease for at least 3 to 5 months after infection. Documentation of seroconversion or a fourfold increase in IgG titer is consistent with recent infection.

A presumptive diagnosis of canine RMSF can be based on the combination of appropriate clinical, historic, and clinicopathologic evidence of disease; serologic test results; exclusion of other causes of the clinical abnormalities; and response to anti-rickettsial drugs. Documentation of seroconversion or an increasing titer 2 to 3 weeks after initial serologic testing suggests recent infection. Diagnostic criteria used in one recent study included a fourfold rise in antibody titer or a single titer of greater than or equal to 1:1024 if the initial titer was submitted 1 week or more after initial onset of clinical abnormalities.²⁹ Positive serum antibody test results alone do not prove RMSF because subclinical infection is common. In addition, positive serum antibody tests do not document infection by *R. rickettsii* because infection with nonpathogenic spotted fever group agents induce cross-reacting antibodies.

Demonstration of *R. rickettsii* by inoculating affected tissues or blood into susceptible laboratory animals or by documenting the organism in endothelial cells using direct fluorescent antibody staining leads to a definitive diagnosis of RMSF, but these techniques are not clinically practical. PCR can be used to document the

presence of rickettsial agents in blood, other fluids, or tissues and will likely be clinically useful in the future.

DIAGNOSTIC TESTS FOR SELECT VIRAL INFECTIONS

Canine Distemper

Rare Indications • Dogs with appropriate signs of CNS disease can have antibodies in CSF and serum against canine distemper virus.

Analysis, Artifacts, and Interpretation • The clinician can measure CSF and serum IgG antibodies against canine distemper virus by serum virus neutralization, IFA, or ELISA. ELISA can be used to measure serum IgM antibodies. CSF antibodies to distemper virus are increased in some dogs subsequently diagnosed histopathologically as having distemper encephalitis. False-positive results can occur in CSF samples contaminated with blood. Concurrent measurement of serum antibody concentrations can be helpful; if CSF concentrations are greater than serum concentrations, the antibody in CSF had to be produced locally and suggests CNS distemper. Detection of serum IgG antibodies is of minimal diagnostic value because a positive titer could develop secondary to vaccination or previous exposure. A fourfold increase in serum IgG titer over a 3- to 4-week period suggests recent infection. Detection of circulating IgM antibodies is consistent with recent infection but not clinical disease. A point-of-care assay for detection of canine distemper antibodies is available. Vaccinated dogs that are seropositive in this assay probably do not need to be boosted. A presumptive diagnosis of distemper encephalitis can be made with increased CSF protein and leukocytes (lymphocytes predominating) plus a positive CSF antibody titer in a sample not contaminated with peripheral blood. Definitive diagnosis of canine distemper infection requires demonstration of viral inclusions by cytologic examination (see [Figure 15-3A and B](#)), direct fluorescent antibody staining of cytologic or histopathologic specimens, histopathologic evaluation, or reverse transcriptase-PCR (RT-PCR) documentation of distemper viral RNA in peripheral blood, CSF, or conjunctival scrapings. ([Table 15-1](#); See Appendix I: Select Laboratories for Infectious Disease.) Positive RT-PCR test results can be induced by modified live vaccination. Viral inclusions can rarely be found in erythrocytes, leukocytes, and leukocyte precursors of infected dogs. Inclusions are generally present for only 2 to 9 days after infection and therefore often are not present when clinical signs occur. Inclusions may be easier to find in smears made from buffy coats or bone marrow aspirates than those made from peripheral blood. Viral particles can be detected by immunofluorescence in cells from the tonsils, respiratory tree, urinary tract, conjunctival scrapings, and CSF for 5 to 21 days after infection.

Enteric Viruses

Indications • Viral enteritis induced by parvoviruses, coronaviruses, and other viruses should be suspected in young animals with fever and diarrhea, particularly if neutropenia is present (i.e., parvoviruses).

Analysis, Artifacts, and Interpretation • Determining serum antibodies to feline or canine parvoviruses or coronaviruses is rarely performed clinically because positive results do not correlate with clinical disease. A point-of-care assay for detection of canine parvovirus antibodies is available. Vaccinated dogs seropositive in this assay probably do not need to be boosted.

Detecting fecal shedding of canine parvovirus viral antigen by electron microscopy, virus isolation, fecal hemagglutination, fecal LA, or ELISA can be used to confirm current infection. In-office ELISA for canine parvovirus in feces seems to accurately detect fecal shedding of parvovirus in acute cases (see [Chapter 9](#)). The specificity of the assays is good, but they cannot differentiate vaccine strains of parvovirus and virulent strains. False-negative reactions can occur. These assays also detect feline parvovirus.¹ Virus isolation, electron microscopy, or molecular assays can be used to document coronaviruses in feces, but results do not correlate with the presence of illness.

Feline Infectious Peritonitis (FIP)

Rare Indications • FIP is an appropriate differential diagnosis in cats with fever; uveitis; retinal hemorrhage; nonseptic abdominal or pleural exudates or modified transudates; anemia; hyperglobulinemia; and renal, hepatic, or neurologic abnormalities. Results of currently available serum or blood tests cannot be used alone to definitively diagnose FIP.

Analysis, Artifacts, and Interpretation • Circulating antibodies against coronaviruses can be detected by IFA and ELISA in feline serum. Antibody to coronavirus indicates prior exposure to either enteric coronaviruses or FIP-inducing coronaviruses. A positive titer does not diagnose FIP or protect against disease.³ Feline vaccines containing bovine serum occasionally cause false-positive results. Cats with FIP can rarely have negative results because of rapidly progressive disease with a delayed rise in titer, disappearance of antibody in terminal stages of the disease, or immune complex formation. A positive coronavirus antibody titer does not predict whether a cat will ever develop FIP.

Titer magnitude cannot distinguish between exposure to enteric coronaviruses or FIP-inducing strains. Rarely, positive titers can be induced by vaccination for coronavirus. Kittens can be seropositive because of colostrum-derived antibodies until 9 weeks of age. If adult cats in the environment infect kittens, antibodies can be detected again 8 to 14 weeks later.

Current coronavirus infections can be detected by fecal virus isolation, electron microscopy of feces, or RT-PCR of feces. However, positive test results do not indicate FIP because antibody-positive, healthy cats can pass coronaviruses.² Definitive diagnosis of FIP requires histopathologic evaluation of tissues. Lesions visible by light microscopy are generally pathognomonic, but immunohistochemistry can be used to confirm coronavirus particles. RT-PCR can amplify coronavirus RNA from effusions, tissues, and blood and usually correlates with the presence of FIP. Amplification of coronavirus by PCR in effusions and tissues predicts FIP, but detection in

blood does not.^{15,37} Hyperproteinemia and polyclonal gammopathy (detected by electrophoresis; see Chapter 12) can occur, particularly in the noneffusive form. Monoclonal gammopathy rarely occurs. Classic nonseptic pyogranulomatous exudate or modified transudate with high protein and relatively low cell count (see Chapter 10) is commonly used for presumptive diagnosis. Electrophoresis can also be performed on body fluids. A gamma globulin fraction greater than or equal to 32% is highly suggestive of FIP, whereas an albumin:globulin ratio in body fluid greater than 0.81 probably rules out FIP.⁷³ In another study, an albumin:globulin ratio of 0.5 had a positive predictive value of 89% and an albumin:globulin ratio of 1.0 had a negative predictive value of 91%.³⁸

Feline Immunodeficiency Virus (FIV)

Common Indications • Cats with chronic weight loss, fever, rhinitis, conjunctivitis, gingivitis, dermatitis, diarrhea, uveitis, recurrent abscessation, clinical toxoplasmosis, any chronic infectious disease, chronic renal failure, or lymphadenomegaly should be evaluated for FIV infection.

Analysis, Artifacts, and Interpretation • IgG antibodies are detected in serum by ELISA, IFA, and Western blot immunoassay.^{39,53} There are many different in-clinic kits available depending on the country.³⁹ Western blot immunoassay is performed in some commercial laboratories. In the United States, one in-office ELISA is available to detect FIV antibodies and feline leukemia virus (FeLV) antigen combined. This assay is available with and without *Dirofilaria immitis* antigen assay. Seroconversion occurs 5 to 9 weeks after inoculation in experimentally infected cats. Seropositive cats are probably infected with FIV for life. False-positive reactions can occur in the ELISA but are thought to be rare. Positive ELISA results should be confirmed via Western blot immunoassay or IFA, particularly in healthy cats unlikely to have been exposed to FIV. Detection of circulating antibodies only confirms infection, not clinical illness. Kittens can have detectable colostrum-derived antibodies until 12 to 14 weeks. Because many clinical syndromes associated with FIV infection are caused by opportunistic infections, further diagnostic procedures may determine treatable causes. For example, many FIV-seropositive cats with endogenous uveitis are co-infected by *T. gondii*.

Virus isolation and RT-PCR are available in some laboratories and can be used to confirm infection. A recently marketed FIV vaccine induces serum antibodies that are indistinguishable from antibodies induced by natural exposure, at least by use of currently available antibody tests.⁵⁴ The ability of virus isolation or RT-PCR to accurately differentiate naturally exposed and vaccinated cats is currently unknown and varies between laboratories.²¹

Feline Leukemia Virus (FeLV)

Common Indications • Because of diverse manifestations of FeLV infection, testing is indicated in all clinically ill cats, especially those with evidence of infectious,

neoplastic, reproductive, immunologic, or hematologic disease, as well as in clinically normal cats exposed to FeLV-positive cats.⁵³

Analysis, Artifacts, and Interpretation • Viral antigen (p27) is detected by IFA in neutrophils and platelets from blood or bone marrow, or in blood, plasma, serum, saliva, or tears by ELISA. Nucleic amplification assays can also be used to assess the stage of infection.^{41,78} When evaluating for antigen, testing of serum or blood gives the best results; tears and saliva should not be tested. Several point-of-care ELISA tests are available in the United States. Other assays are also available in other countries.³⁹ Antibody titers to FeLV envelope antigens (neutralizing antibody) and against virus-transformed tumor cells (feline oncogenic cell membrane antigen, or FOCMA, antibody) are available in some laboratories, but the prognostic significance of the results is currently unknown; therefore the tests are not used clinically.

FeLV infection has four major outcomes.⁵³ Cats with inappropriate immune responses develop progressive infection and usually develop FeLV-associated diseases. Cats with regressive infection can be transiently positive for p27 antigen in blood or serum but ultimately become negative. Abortive exposure occurs in cats with good immune responses and infection never occurs. Rarely, focal infection of tissues such as the spleen, lymph nodes, small intestine, or mammary glands can occur. Cats with regressive infection, abortive exposure, or focal infection rarely become ill.

ELISA test results generally become positive within 30 days of exposure to FeLV and can revert to negative in cats that develop regressive infection. Cats suspected to have regressive infection can be isolated from other cats and retested by ELISA in several weeks or be tested by IFA or PCR assay. Positive IFA test results prove the bone marrow has been infected and has 99% correlation with virus isolation. These cats generally develop progressive infection. False-negative IFA reactions may occur when leukopenia or thrombocytopenia prevents evaluation of an adequate number of cells. False-positive reactions rarely occur from nonspecific staining of eosinophils. A positive IFA indicates that the cat is viremic and contagious.

Virtually all IFA-positive cats are ELISA-positive. Finding an IFA-positive but ELISA-negative cat suggests technique-related artifact. A negative ELISA result is approximately 100% correlated with negative IFA and an inability to isolate FeLV. Cats that are ELISA-positive but IFA-negative are called *discordant*. Discordant results are usually caused by false-positive ELISA results, false-negative IFA results, or early stages of regressive infections. Use of PCR assays to detect viral RNA or cell-associated DNA (provirus) can be performed on blood, bone marrow, or tissues and be used to evaluate cases with discordant ELISA and IFA results.

Some cats with focal infection localized to bone marrow have positive bone marrow IFA results. The most reliable means of identifying focal FeLV infections is virus isolation or PCR assay. A cat with focal infection may become viremic after extreme stress or administration of glucocorticoids.

DIAGNOSIS OF DIROFILARIASIS (*DIROFILARIA IMMITIS*)

Cytology (Knott's Test or Filter Test)

Common Indications • Cytologic evaluation for microfilariasis is indicated in dogs with signs consistent with heartworm disease (right-sided heart disease, coughing, dyspnea, eosinophilia, polyclonal hyperglobulinemia, protein-losing nephropathy [PLN]), in dogs about to begin prophylactic therapy (with diethylcarbamazine, ivermectin, or milbemycin), and rarely in cats with signs consistent with heartworm disease (i.e., dyspnea, cardiomegaly, unexplained vomiting).

Analysis, Artifacts, and Interpretation • Cytologic testing for *Dirofilaria immitis* is very specific (microfilaria morphology differentiates *D. immitis* microfilaria from those of *Dipetalonema reconditum*), quick, and inexpensive; all concentration techniques (Knott's and filter tests) are much more sensitive than examination of fresh blood smears and are reasonably sensitive in dogs that have not been treated with filaricidal drugs. Up to 40% of dogs have spontaneous occult dirofilariasis and must be diagnosed by serologic testing and radiographic examination. All cytology tests have poor sensitivity in cats. Thus, microfilaria techniques should no longer be used as stand-alone diagnostic tests and should only be used concurrently with antigen tests.^{*,†} A positive test result diagnoses heartworm disease, except in juveniles less than 4 months of age that could have received the microfilaria by transplacental transfer.

Heartworm Adult Antigen Titer

Common Indications • A heartworm antigen titer should be included in annual screening of dogs to evaluate for exposure to *D. immitis*, and should be performed

in dogs or cats with clinical signs, laboratory abnormalities, or thoracic radiographic changes consistent with dirofilariasis. The test can also be used to assess efficacy of adulticide treatment.

Analysis, Artifacts, and Interpretation • ELISA can detect circulating heartworm antigen in serum; several kits are commercially available. There is greater sensitivity when compared with microfilaria detection. In dogs, *D. immitis* antigen tests may be positive as early as 5 to 6 months and are usually positive 6 to 7 months after infection. False-negative results usually occur in early stages of infection and may occur in single-sex infections (male only) or in dogs or cats with low worm burdens (<3 to 5 worms). Retesting in 2 to 3 months should be performed to detect dogs in which results were negative in early stages of infection. After successful adulticide treatment, test results become negative in approximately 12 weeks. In experimental infections, cats testing positive did so about 8 months after infection. However, single-sex or low worm burden infections can lead to false-negative results. Therefore a positive antigen test result is specific for infection, but a negative result does not rule out dirofilariasis. In cats, the combination of serum antigen test results with serum antibody test results is more sensitive than performing either test alone (see [Heartworm Antibody Titer](#), next).⁷⁴

Heartworm Antibody Titer (Feline)

Rare Indications • A heartworm antibody titer should be obtained in cats with coughing, unexplained vomiting, syncope, or radiographic evidence of heartworm disease.

Analysis, Artifacts, and Interpretation • Several ELISAs detect antibodies to *D. immitis* in feline sera. The assays are more sensitive than microfilaria demonstration techniques. The assays are very specific; no cross-reactivity exists with *D. reconditum*. The positive predictive value for heartworm disease is less than 100%, however, because circulating antibodies can be present in cats that have cleared the infection naturally. False-negative antibody test results also occur; therefore serum antibody and antigen tests should be performed in concert in cats with suspected dirofilariasis.⁷⁴

*www.heartwormsociety.org/veterinary-resources/canine-guidelines.html#3

†www.heartwormsociety.org/veterinary-resources/feline-guidelines.html#Diagnostic%20Testing

TABLE 15-1. SELECT DIAGNOSTIC TESTS FOR USE IN THE VETERINARY PRACTICE

PRODUCT	COMPANY	LOCATION
HEARTWORM TESTS		
Witness FHW feline heartworm antibody test	Synbiotics Corporation	San Diego, CA
ASSURE/FH feline heartworm antibody test	Synbiotics Corporation	San Diego, CA
Solo Step FH test cassettes heartworm antibody test	Heska Corporation	Loveland, CO
Witness HW heartworm antigen test	Synbiotics Corporation	San Diego, CA
DiroCHEK HW heartworm antigen test	Synbiotics Corporation	San Diego, CA

Continued

TABLE 15-1. SELECT DIAGNOSTIC TESTS FOR USE IN THE VETERINARY PRACTICE—CONT'D

PRODUCT	COMPANY	LOCATION
SNAP heartworm antigen test	IDEXX Corporation	Westbrook, ME
PetChek heartworm PF antigen test	IDEXX Corporation	Westbrook, ME
Solo Step CH test cassettes heartworm antigen test	Heska Corporation	Loveland, CO
Solo Step CH test strips heartworm antigen test	Heska Corporation	Loveland, CO
FeLV/FIV TESTS		
SNAP FIV antibody/FeLV antigen combo	IDEXX Corporation	Westbrook, ME
SNAP FeLV antigen test	IDEXX Corporation	Westbrook, ME
ASSURE/FeLV feline leukemia virus antigen test	Synbiotics Corporation	San Diego, CA
ViraCHEK/FeLV feline leukemia virus antigen test	Synbiotics Corporation	San Diego, CA
Witness FeLV feline leukemia virus antigen test	Synbiotics Corporation	San Diego, CA
DISTEMPER VIRUS/PARVOVIRUS TESTS		
TiterCHEK CDV/CPV	Synbiotics Corporation	San Diego, CA
ASSURE/Parvo canine parvovirus antigen test	Synbiotics Corporation	San Diego, CA
Witness CPV canine parvovirus antigen test	Synbiotics Corporation	San Diego, CA
SNAP Parvo antigen test	IDEXX Corporation	Westbrook, ME
TiterCHEK CDV/CPV	Synbiotics Corporation	San Diego, CA
OTHER TESTS		
SNAP 3Dx and SNAP 4Dx	IDEXX Laboratories	Westbrook, ME
Toxotest-MT	Tanabe USA, Inc.	San Diego, CA
TPM-Test (for Toxoplasmosis)	Wampole Laboratories	Princeton, NJ
CALAS Cryptococcal Antigen Latex Agglutination test	Meridian BioScience Inc.	Cincinnati, OH
D-Tech CB (for Brucellosis)	Synbiotics Corporation	San Diego, CA
SPECIMEN HANDLING MATERIALS		
DTM Plate	Hardy Diagnostics	Santa Maria, CA
Derm Duet	Bacti-Lab	Mountain View, CA
BDL Culture Swab, Collection and Transport System	Becton Dickinson Microbiology Systems	Franklin Lakes, NJ
BBL CultureSwab Plus	Becton Dickinson Microbiology Systems	Franklin Lakes, NJ
Trypticase Soy Broth	Becton Dickinson Microbiology Systems	Franklin Lakes, NJ

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Cytology of Inflammatory or Neoplastic Masses

16

Harold Tvedten

ROLE OF CYTOLOGY IN DIAGNOSIS

Cytologic evaluation of most lesions has become a very common part of diagnosis. Minimal equipment is needed, making cytology possible in any practice. A nearly immediate cytologic conclusion eliminates waiting one or more days for a histologic diagnosis. Cytologic diagnosis gives a cellular or morphologic interpretation to abnormal structures identified by ultrasound or other diagnostic imaging. Fine-needle aspirates are minimally invasive and minimally stressful for patients. Cytology may save the expense of anesthesia and surgery. This chapter discusses cytologic evaluation of masses. Cytologic analysis of fluid, vaginal secretions, semen, urine sediment, respiratory tract material, and specific organs is described in other appropriate chapters.

Cytology can alter or eliminate surgery. Obviously benign masses, such as lipomas and epidermal inclusion cysts, need not be removed immediately. When they are removed, a close resection (i.e., “shelling it out”) is adequate, whereas wide excision is indicated for potentially malignant neoplasms. Any cytologic evidence of malignancy (e.g., possible carcinoma or sarcoma) dictates a search for metastasis before surgical removal of the mass. Evidence of metastasis is a contraindication for surgical removal. Diagnosis of a systemic neoplasm such as lymphoma usually indicates chemotherapy rather than surgery. Neoplasms that are too vascular or invasive for surgery may require cytology for diagnosis instead of histopathology. If a specific tumor diagnosis is made, specific treatment may be started early. Cytologic diagnosis should be considered a tentative diagnosis of neoplasms and histologic diagnosis should be used for more specific classification of the type of neoplasm and degree of malignancy. Some lesions, such as granulomas and granulation tissue, often look malignant on cytology.

Cytology has limitations. Histopathology is usually more diagnostic and definitive, because more information (i.e., tissue architecture) is available from a histologic section than from a variable number of cells on a cytologic smear. Malignancy of certain types of neoplasms (e.g., canine mammary tumor, perianal gland tumor) is best identified by histologic evidence of invasion of

tumor cells into normal tissue, and this is seldom documented with cytology. Although cytology gives a definitive diagnosis in many cases (e.g., infectious diseases, mast cell tumor, lymphoma, lipoma), cytology often gives only a general diagnosis in other cases (e.g., epithelial mass with prominent cytologic evidence of malignancy). Most neoplasms require histologic evaluation for a definitive diagnosis and best estimate of malignancy. Radical action (e.g., euthanasia) should await confirmed diagnosis.

Of 147 skin tumors, only 105 (71%) cytologic diagnoses agreed with histologic diagnoses.⁷ Exceptions exist in which cytology is as diagnostic as (or more diagnostic than) histology (e.g., individual cell detail of leukemias is more diagnostic than tissue patterns). Cytology was correct in 60 of 64 round cell tumors and occasionally more diagnostic than histology.⁶ Cytologic and histologic diagnoses agreed on all of the following tumors: 37 mast cell tumors, 11 melanomas, 2 histiocytomas, and 1 cutaneous lymphoma.⁷ Accurate cytologic diagnoses were also made for squamous cell carcinomas, lipomas, and metastasis to lymph nodes.

CYTOLOGIC TECHNIQUES

Sample Collection and Slide Preparation

Proper sample collection and slide preparation are absolutely necessary and are a common limiting factor in cytologic diagnosis. Cytologic diagnosis requires an adequate number of cells with good morphology that represent the mass. A fine-needle aspirate (FNA) should be obtained from an area of the mass that reflects the primary problem. The site being sampled determines what material is collected (Figure 16-1). An impression smear from the surface over a mass often has only exudate, bacteria, and necrotic or reactive cells that do not reflect the primary mass. Similarly, a wash over the mass (e.g., nasal flush, bronchoalveolar lavage [BAL]) often collects only exudates and reactive cells on the surface. Pus (septic exudate) may be misleading and suggest the mass is an abscess. FNA samples of deeper tissue are more likely to be diagnostic.

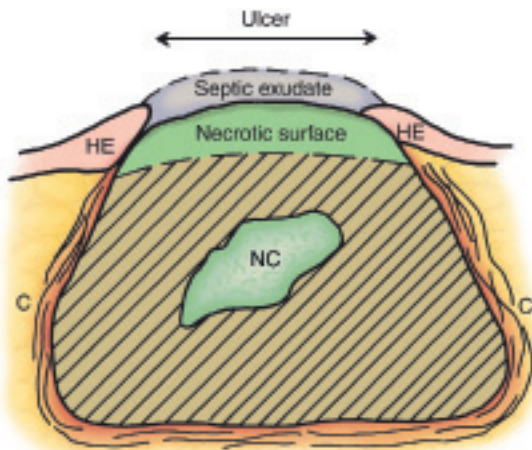


FIGURE 16-1 Sampling sites from a mass. Different cell populations are retrieved from different sites. Cells from the proliferative mass (hatched area) are representative and diagnostic. Samples from the surface may be only necrotic material or septic exudate. Immature-appearing hyperplastic epithelial cells (HE) from the edge of an ulcer may mimic a carcinoma. Material from a necrotic center (NC) may resemble only debris. Cells from the boundary (C) may be fibroblasts from the capsule or a local inflammatory response. Mature fat or blood may be collected from adjacent normal tissue.

NOTE: Impression smears of ulcerated surfaces of masses are usually diagnostically worthless and only reflect secondary inflammation and infection.

Epithelial cells at the edge of ulcers appear anaplastic, because they are actively proliferating to cover the ulcer (e.g., corneal ulcers can cytologically resemble a squamous cell carcinoma). Ulcerated surfaces often have secondary inflammatory and septic changes. Malignant-appearing cells from deep under a surface are much more likely to truly indicate that the mass is malignant. Soft centers of a mass may be necrotic or hemorrhagic, so a smaller firm mass or a more viable-appearing area at the edge of a mass may be more diagnostic.

An FNA need obtain only one or a few small drops of fluid to streak out similar to a blood smear. Too much aspiration can cause bleeding and hemodilution of the sample. FNA means using a 22- to 20-gauge needle. Vacuum may be applied to a 5- to 10-ml syringe after the needle has penetrated the mass, and the vacuum should be maintained while one passes the needle tip back and forth through the mass; then the vacuum should be released and the needle withdrawn. Cells in the needle then need to be gently placed on a smear and not shot out of the needle as a spray onto the slide. Small droplets of fluid dry too quickly to streak out into a monolayer. They remain as thick drops with poor cell morphology. The needle should be removed from the syringe and the syringe filled with air. The needle is reapplied, and a drop of fluid is gently expressed on a glass slide. The drop is then quickly drawn out into a smear with a coverslip or another slide. The needle can also be used to draw out

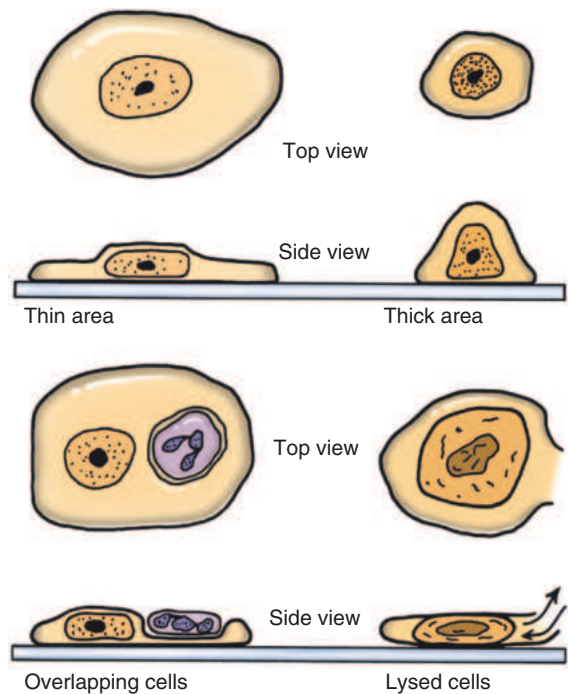


FIGURE 16-2 Cell morphology in three dimensions. The cell on the upper right is in the thick part of a smear, so it fails to spread out over a large surface area. It appears smaller (shorter diameter) and darker from the top compared with the cell on the upper left, which has spread out in a thin area of a smear, allowing proper evaluation. The epithelial cell (lower left) has a neutrophil dimpled into its surface. The neutrophil appears to be in the cell when viewed from above. The partially lysed cell (lower right) has a swollen, enlarged nucleus and nucleolus, which may appear malignant instead of only damaged.

the drop into thin monolayer extensions (so-called “starfish design”).

Cells in a thin area (monolayer of cells) spread out flatly and expose a large surface area to view (Figure 16-2). If the smear is thick, cells are supported more upright on the slide and have a smaller diameter. Cells in thick areas are taller (thicker) and thus stain darker, often too dark to see any detail. Protein-rich fluid, necrotic debris, or ultrasound gel surrounding cells interferes with staining. If fluid is viscous, a squash preparation may help to get a thin smear. A drop of fluid is placed between two slides; while the drop spreads to its maximum diameter, the slides are slid apart while the two surfaces are in contact with each other. This creates a smear on each slide. Lymphoid cells and cells from necrotic centers are very fragile and easily lysed beyond recognition. Use of a coverslip for a squash preparation or blood smear-type smear often causes less damage to cells.

A nonaspirate (capillary) technique is easier to perform and yields less bloody samples of equal or greater cellularity, especially from highly vascular tissues (e.g., liver aspirates).⁹ The technique described here is a modification of the nonaspiration technique used by Dr. Rick Cowell while at Oklahoma State University. Air (i.e., 5 ml) is aspirated into a 10-ml syringe, and a 22-gauge

needle is attached. The syringe is held at the base of the needle to allow for better control, and the mass is stabilized with the operator's free hand. The needle is introduced into the mass and rapidly moved back and forth along the same tract five or six times. Negative pressure (i.e., aspiration) is not applied. The cells are collected by shearing and capillary action. The needle is withdrawn from the mass, and collected cells are quickly but gently expelled onto a clean glass slide by depressing the plunger. The collected material is then spread out as discussed earlier. A common error is the "shotgun spray" in which the small volume of fluid in the needle is sprayed on to the glass as small droplets (like the pattern of shotgun shot hitting a target). These small thick drops dry too quickly to be drawn out into thin smears. Instead, the operator should touch the end of the needle to the glass and gently express the contents out as one drop that is then quickly and gently spread it out as a thin smear. Generally, only one smear can be made from each collection attempt. Therefore three or four collections from different sites should be taken. An alternative is a "packing" technique where only a needle (without syringe attached) is passed through a mass to pack the needle with cells with no aspiration.

If part of a mass is surgically removed, impression smears of representative areas of the mass for cytology should be made before placing the biopsy in formalin. One should use a freshly cut surface and blot off excessive blood and protein-rich fluids. The operator should touch the surface to a glass slide without twisting or rubbing motion. But if no cells are found on those smears or the mass feels hard, then cells should be aggressively scraped off from the surface with a blade and those cells streaked onto a slide. Fibrous masses usually do not release cells easily and may need to be scraped with a scalpel to obtain enough cells for diagnosis. The moist material on the blade is streaked on glass slides.

Patient identification and other information must be noted on the slide. An adequate description is needed for a cytologist, who has not seen the animal or lesion, to interpret the results and provide a useful answer to the veterinarian submitting the sample. The description should include the site and a description of the lesion (e.g., "packing technique from an enlarged liver" or "FNA of a skin tumor near the anus"). Such description is preferable to a common request of "What kind of cells are present?" without describing what lesion in what part of the animal was sampled. A specific question allows a specific answer. If one or more sites were sampled, one should note how each slide relates to the sites sampled. Dates are needed if samples were taken at different times. Slides should be labeled with lead pencil on a frosted end-type slide. Printer-generated paper labels for marking test tubes cause problems on cytology slides. When the slide is stained, the paper label may also be so darkly stained that no word is legible. Computer labels are too large for the slide to fit on the microscope stage and have glue on them. When the excessive paper is removed, glue remains so the slides stick to the microscope stage, preventing that slide or subsequent slides from being movable. Thin two-slide cardboard boxes mailed to a cytologist often arrive with crushed glass slides, because the envelope is machine cancelled even when they are

marked "Hand Cancel." Therefore, slide containers that are too large (e.g., rectangular plastic box) to fit through the post office's automatic stamp canceling machine should be used for mailing.

NOTE: Postal office automated machines often crush slides mailed in thin, flat containers. Use containers too large to fit in such machines.

Smears are routinely air-dried for Wright-type stains and new methylene blue (NMB) staining. Alcohol fixation is required for Papanicolaou's (Pap's) or Sano's stain, which few cytologists use. Air-drying smears slowly in a moist environment may cause cell distortion. A hair dryer may be used to speed drying if the problem persists. The dryer should be held far enough away from the smears to avoid "cooking" cells. There is no need to "flame" smears for cytology of neoplasia, which requires optimal morphology. However, flaming slides from waxy ear swabs or colonies from blood agar plates for Gram staining is helpful. Smears should not be stored in refrigerators or with exposure to dust, molds, pollen, and flies. Flies eat unstained cells. One should handle slides only on the sides of the glass, because squamous cells from fingerprints can contaminate smears and interfere with interpretation.

Smears exposed to formalin often have excessive blue staining with Wright stain. Tissue samples used for impression smears should not have been placed in formalin before the slides were made. Formalin should not be stored near the stains or smears. Formalin should not be submitted in the same package with cytologic smears, where the fumes may act on unstained cells. Heparin anticoagulant also causes a blue discoloration of Wright-stained cells.

NOTE: Formalin causes poor cell staining, so cytology slides and formalin-fixed histopathology samples should be mailed in separate packages.

Stains

A modified Wright-type stain and NMB are usually adequate for diagnosis. The "quick" stains used today may be called "Wright stains" but are really not Wright or Giemsa (i.e., true Romanowsky's) stains. Quick stains such as Diff-Quik and Hemacolor have blue and red dyes to give staining characteristics similar to classic Romanowsky's stains. The blue dyes stain acidic structures such as nucleic acids (deoxyribonucleic acid [DNA] and ribonucleic acid [RNA]) in nuclei blue to purple. Red dyes stain basic structures such as proteins (e.g., hemoglobin in erythrocytes) red to orange. Changes in pH in rinse water or the sample can affect staining characteristics. A quick stain such as Diff-Quik can be adjusted to color cells on the smears more blue or red or darker. The blue and red dyes are in separate jars; by increasing or decreasing the number of times one dips the slide in a color, the intensity of blue or red is increased or decreased. Diff-Quik stains distemper inclusion bodies better on blood

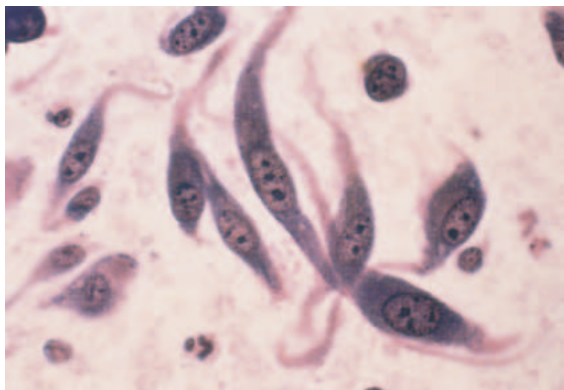


FIGURE 16-3 Nuclear detail, including size and shape of nucleoli and chromatin, are well illustrated by new methylene blue staining. Note the prominent nucleoli in this sarcoma cytology. The cells also demonstrate the classic spindle form of mesenchymal cells.

smears than some “Wright stains.” Water-based “Wright stains” may fail to stain granules in mast cells and basophils. Ear swab smears should be stained in separate staining jars for only ear swabs because the stains can quickly be contaminated with yeast, which are transferred to smears from other patients.

NMB is a monochrome stain with variably intense blue staining. A “wet mount” is made by placing a drop of NMB on an air-dried smear and applying a coverslip. To prevent retention of an air bubble over part of the smear (usually the most diagnostic area), the coverslip should be used to gently pull the drop of NMB over the cells to moisten them before slowly applying the coverslip. Staining is immediate. NMB stains nuclear material well and demonstrates distinct chromatin patterns (Figure 16-3). This nuclear detail is very useful in evaluating malignant criteria. The transparent nature of NMB is a major advantage, because one can see through thicker tissue fragments that would be too darkly stained with a Wright stain. The microscope can be focused through different depths of tissue fragments to judge individual cell detail and architectural patterns in three dimensions. Tissue fragments are often the most diagnostic material on smears from neoplasms but stain too darkly to evaluate with a Wright stain. The fragments are like tiny biopsy sections that allow evaluation of how cells were oriented in the mass. These architectural patterns help identify the tissue type. One can evaluate adjacent cells for true variability suggesting malignancy, compared with the variability of isolated cells on a smear that may have come from different areas or cell types in the mass.

In summary, NMB is excellent for nuclear detail, thick tissue fragments, and most fungi. Wright stain is excellent for inflammatory lesions, because the stained appearance of leukocytes (white blood cells [WBCs]) is similar to that in blood smears and bacteria consistently have a characteristic dull blue color (Figure 16-4). Although a Wright stain is not as good as NMB for nuclear detail, it is acceptable for evaluating tissue cells for criteria of malignancy and excellent for bacteria and most fungi (Figure 16-5).

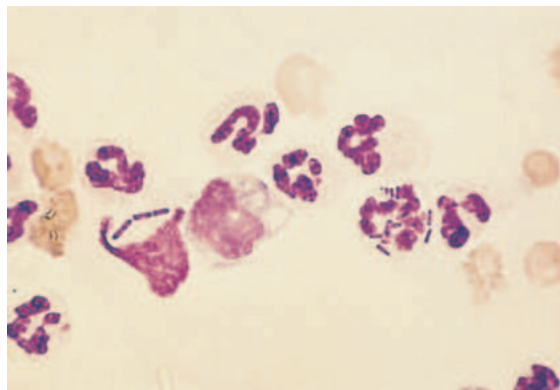


FIGURE 16-4 In this septic exudate there are two neutrophils with distinctly blue intracellular bacteria. Wright (Giemsa)-type stains are optimal for finding bacteria in cytologic samples and are preferred for routine use. These neutrophils appear nondegenerate, though the one with bacteria on the left is damaged. One should interpret whether neutrophils are degenerative or not, based on how the intact neutrophils appear. Ignore damaged cells.

Use of both Wright and NMB stains for the same lesion works well, because different characteristics of the cells are illustrated by different stains.

Other stains may be used. Sudan stain is useful for diagnosis of fatty liver, chylothorax (Figure 16-6), aspiration pneumonia (Figure 16-7), or lipid granulomas. A drop of Sudan stain (or other neutral fat stain) is drawn over a smear to stain the cells and background selectively for lipid. Excess stain is poured off and then the smear is counter-stained with a drop of NMB to show adequate cell detail. NMB stains the nucleus and other cell structures blue, and the Sudan stains neutral fat red.

Veterinarians sometimes request that their cytologic samples are Gram stained because different antibiotics are used for gram-positive versus gram-negative bacteria. However, Gram staining is absolutely not recommended for cytologic smears. Gram staining is inconsistent for bacteria in exudate. In thick smears the bacteria may not

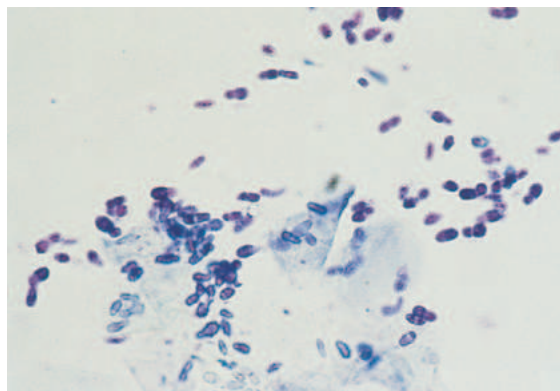


FIGURE 16-5 A Wright-type stain is excellent for identifying yeast and bacteria in ear swabs. This photo has many budding yeast (*Malassezia*).

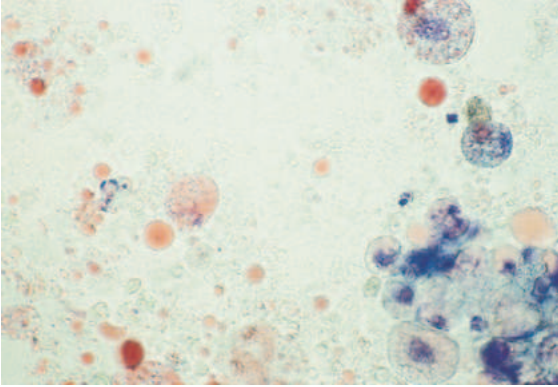


FIGURE 16-6 The presence of neutral fat droplets in vacuoles, in macrophages, and free in the background is well demonstrated by a combination of Sudan stain counter-stained with new methylene blue. This is a simple and cheap test for chylothorax.

decolorize, creating a false impression that the bacteria are gram positive. In thin smears, bacteria may decolorize too much, suggesting they are gram negative. Gram staining is not sensitive for screening cytologic smears for gram-negative (i.e., red) bacteria in low numbers in a red, proteinaceous background (Figure 16-8). Wright-Giemsa-type stains consistently stain bacteria blue and are greatly

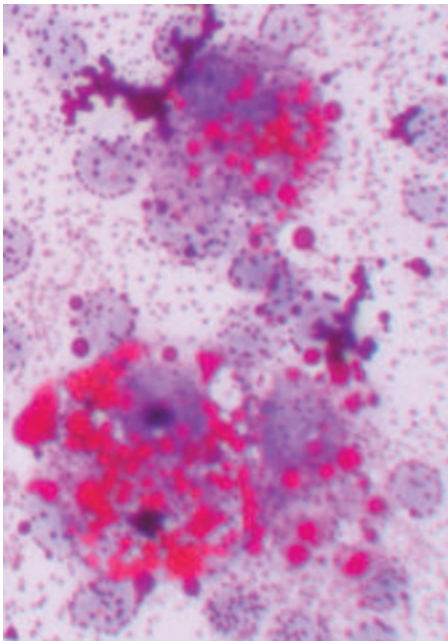


FIGURE 16-7 Three macrophages from an impression smear of lung with aspiration pneumonia contain several vacuoles of neutral fat, which is stained bright red with Sudan stain. The smear was counter-stained with new methylene blue to show nuclear and cell details. The vacuoles were clear and distinct on Giemsa-stained smears. Aspirated lipids are difficult to remove from the lungs and are evidence of aspiration-type pneumonia.

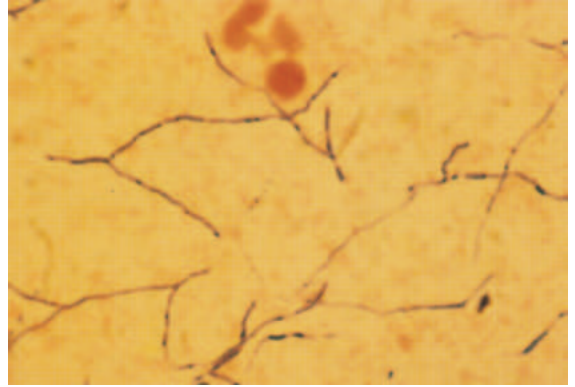


FIGURE 16-8 This Gram stain of a septic exudates shows the gram-positive, filamentous, beaded and branching *Actinomyces* well, but the hundreds of gram-negative rods are not well seen. The frequency of bacteria in cytologic samples is often quite low, so finding an occasional gram-negative bacterium in a red background is difficult.

preferred for finding bacteria on cytologic smears. Wright-Giemsa stains allow easier detection and definition of size and shape. In general, coccoid bacteria are likely gram-positive *Staphylococcus* and *Streptococcus*. Gram staining is best restricted to bacteriology laboratories where smears are consistent in thickness and staining is interpreted daily on bacteria where the final classification is determined daily. Gram stain differentiates gram-negative from gram-positive bacteria well on uniformly thin smears from cultures on blood agar plates. Acid-fast stain is rarely needed, because mycobacterial infections are uncommon (see Figure 16-8). Mycobacteria are unique in that they do not stain at all with Wright-Giemsa stains because of their waxy coat (Figure 16-9).

Pap's stain is often used in human medicine (cervical swabs) and rarely in veterinary practice. Pap's stain has

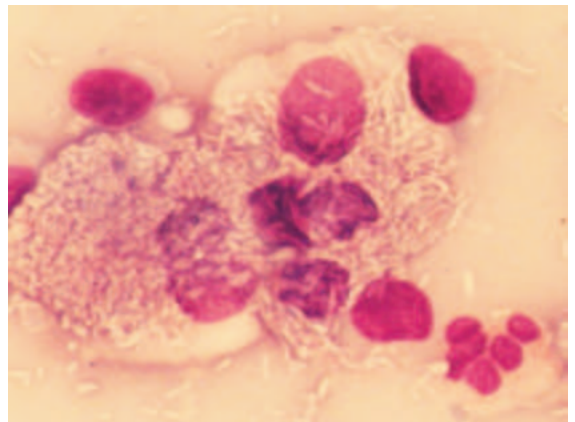


FIGURE 16-9 *Mycobacterium* does not take up any stain, and so appears as a clear bacillus that is negatively stained by color to material around it. This lymph node aspirate from a dog has two large macrophages filled with clear rods. Clear, unstained rods are also in the protein-rich fluid in the background. There are also about five lymphocytes and a neutrophil.

advantages; however, this author usually was able to make a diagnosis with NMB and Wright-stained smears and write out the report before the technologists could finish staining smears with Pap's stain. Therefore we ceased using Pap's staining. Specific malignant criteria have been established for cells stained with these stains. Pap's stain is a transparent stain that permits evaluation of thick tissue fragments and fine evaluation of nuclear characteristics. Smears need to be immediately fixed in alcohol before Pap's staining.

Microscopes

A good-quality, well-maintained microscope is needed for cytology. An ergonomic binocular microscope is more comfortable for long viewing periods. Four objectives are recommended: a 4× and a 10× objective are used for scanning a smear and quickly finding likely diagnostic areas, which are then examined with 50× oil and 100× oil objectives for fine details. A properly equipped, good-quality microscope is expensive but will have multiple uses in a clinic and should last a lifetime. The microscope cost per each slide is examined below.

A 50× oil plan achromat objective (i.e., 40× to 60× oil) pays for itself (\$350 to \$1000) by the time it saves to examine a slide. Magnification is sufficient for most detail, and more cells can be seen in a shorter time. "Plan" means the whole field of view is in focus. Having more cells in the larger field of view allows better comparison of variations among cells and easier identification. Achromat corrects spherical aberration for 1 color. Apochromat corrects for 3 to 4 colors but is more expensive and not needed for diagnosis. A 50× oil objective avoids the need to coverslip smears.

Wright-stained cells observed with most 40× high-dry objectives appear fuzzy, because the cells are surrounded by air. Using mounting media with a coverslip or oil eliminates the air-cell interface and allows good cellular detail with a high-dry lens. Adding a drop of oil is much faster than permanently coverslipping smears. Oily smears are messy to store and less permanent, however. High-dry 45× objectives are easily contaminated with oil on smears and the convex lens is difficult to clear of oil. A microscope with only oil objectives avoids this problem and loss of time.

Oil should be removed from lenses at the end of a work period, because oil can penetrate behind some lenses to render them useless or dry to become a hard coating on the lens. Immersion oil is not removed by alcohol but is removed by gasoline or xylene, which should be used instead of alcohol for routine cleaning. Kimwipes are lint-free tissues that are satisfactory for cleaning microscope objectives not used for photomicroscopy. Kimwipes absorb oil better than does lens paper and thus clean more effectively. Concave lenses require a cotton-tipped swab moistened with gasoline to clean the recessed area. Final polishing of the objective lens should be with lens paper. One should clean filters, light sources, stage, and condenser as needed. Complete covering of the microscope prevents accumulation of dust in places hard to clean. Sharp vibrations (e.g., dragging the microscope along the surface of a

desk, setting it down hard) should be avoided. This can knock the prism out of alignment and cause a double image.

The condenser must be in the proper position for optimal detail (e.g., for finding small bacteria). The condenser is near the optimal setting (i.e., Köhler illumination), if it is close beneath the glass slide on the stage. More specifically, several simple steps should be performed daily. While at a high magnification (e.g., 40× objective), one should focus on the cells on a smear. The field diaphragm at the bottom of the microscope is completely closed, and the condenser is moved slightly up or down until the circle of light in the field has sharp edges. The condenser should be then left at this optimal height. One should then center the circle of light to the center of the field and reopen the field diaphragm fully so that no shadow appears in the field. The diaphragm in the condenser can be adjusted to optimize contrast but, more simply, it should be fully open for most users. High-quality oil objectives available today, function well with the condenser diaphragm fully open. A common error is closing the aperture diaphragm in the condenser too much, causing too much contrast. This is usually done (incorrectly) to reduce light intensity. The proper way to reduce light intensity is to use a neutral-density filter, a dimmer bulb, or a more controllable light source rather than to cause poor cell detail. Improved contrast may be obtained by slightly closing the aperture diaphragm in the condenser, but this should be left to experienced microscopists using photomicroscopy.

When morphologic detail is not needed and the goal is only to easily find objects such as parasite ova in a fecal exam, urinary casts in urine sediment, or platelets in a hemocytometer, the condenser is moved to the lowest position that still provides adequate illumination. This position gives clear structures more contrast and they are easier to find.

Some common problems with microscopy include placing the slide upside down on the stage. In this case one can focus on cells at medium magnification (i.e., 10×, 40×) but not with the 100× oil objective, because cells are on the underside of the slide. If the coverslip or mounting medium is too thick, one also cannot focus at high magnifications. If the fine focus will not turn any farther in the direction needed, one should adjust the coarse adjustment past the plane of focus needed, and then turn the fine focus knob in the opposite direction to regain focusing ability. If the cells look refractile and have poor detail, the lighting is probably wrong. In this case one should adjust the microscope to Köhler illumination. If cells are in focus with the 100× objective but not the high-dry 45× objective, the 45× objective is likely contaminated with oil and must be cleaned.

CYTOLOGIC CONCLUSIONS

The usual composition of a mass is a proliferation of tissue cells, an accumulation of inflammatory cells, or both. Miscellaneous masses include hematomas, cysts, or focal areas of necrosis. A general approach to cytologic

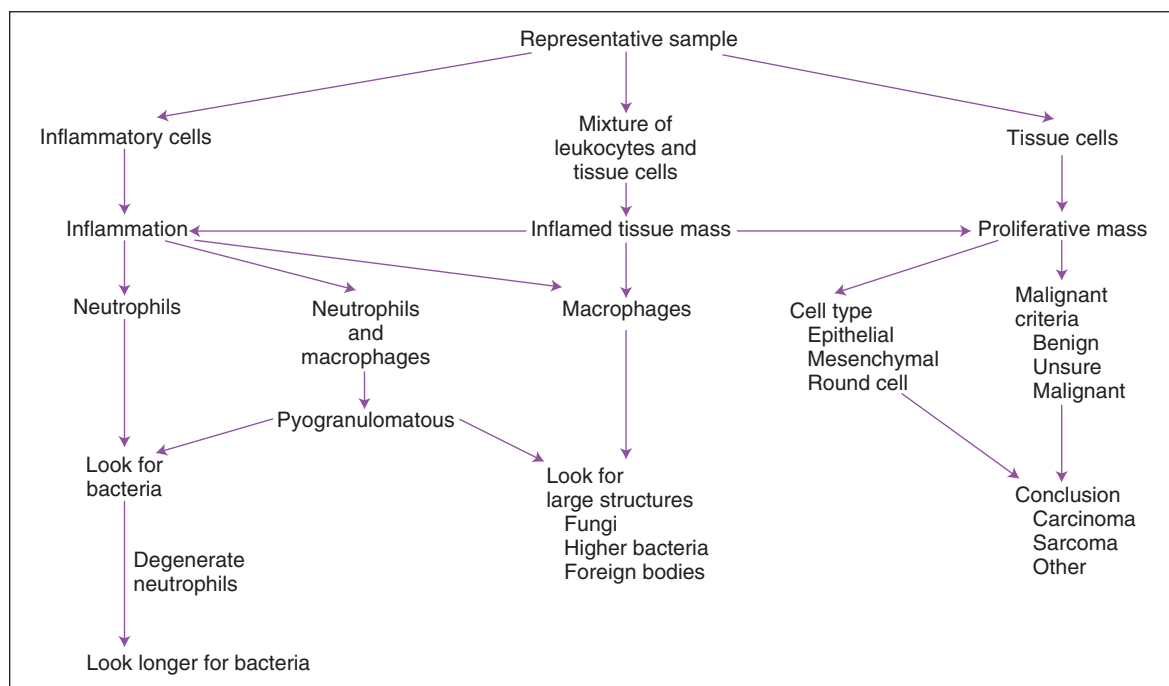


FIGURE 16-10 General cytologic approach. Most diagnostic samples are initially divided into an inflammatory or a proliferative pattern or both. Inflammatory samples are examined for likely causative organisms. Degenerative changes in neutrophils stimulate a longer search for bacteria. Proliferative patterns are evaluated for cell type and evidence of malignancy. Summary statements are then made. If there are both inflammatory cells and proliferating tissue cells, then interpret the balance of evidence for a primary inflammatory lesion or if evidence on the smears indicates a primary proliferative mass (neoplasia) with secondary inflammation. The cytologist should summarize in the report how confident he or she was in the final conclusion. No diagnosis is 100%.

interpretation of a case is simplified in Figure 16-10. More complete description of many cytologic diagnoses is available.¹¹

Slide Reading Approach

Most American veterinarians have ample microscopy, histology, and pathology training, so most can learn to make many cytologic diagnoses as long as they recognize their limitations and continue to learn from their cases. Cytologic evaluation may be performed on excised masses and then the descriptions and conclusions may be compared with histopathology reports. Cytologic evaluation is a visual task, so one should obtain one or more cytologic atlases for frequent reference in the lab.^{2,5,11} An organized approach to an aspirate or impression smear of an abnormal mass is necessary for consistent conclusions. A summary of steps follows, and details are provided in later discussions.

One should first determine if the cytologic specimen likely represents the lesion. Adjacent structures may be sampled inadvertently. For example, a common error is to aspirate the salivary gland instead of the submandibular lymph node. The salivary gland has normal, mature acinar and ductal structures with foamy epithelial cells. The conclusion should be that the sample was not representative, not that an adenoma

or metastatic carcinoma was present. Other examples include inadvertently sampling the liver while obtaining “thoracic” aspirates and having the needle pass all the way through a small mass and to only aspirate subcutaneous (SC) fat. One may contaminate a cystocentesis urine sample with gut bacteria by inadvertently puncturing the intestine. Correct conclusions often require intuition and experience.

1. Establish that a sufficient number of intact, properly stained cells are present and properly represent the mass.
2. Scan smears at low power to determine variation in distribution and content. Look for large structures (e.g., fungus, bacterial colony, parasite eggs, or larvae).
3. Begin fine evaluation of cells in a thin area with intact cells of good staining quality.
4. Determine whether the cell population is primarily inflammatory. If so, attempt to identify the etiologic agent.
5. Determine whether enough tissue cells of one type are present to indicate a noninflammatory, proliferative tissue mass.
6. With proliferative tissue masses (e.g., neoplasia or hyperplasia), determine cell types (connective tissue, epithelial, round cell) present and amount of evidence indicating malignancy.

The initial effort should be to screen smears grossly for those areas most likely to be diagnostic. Smears that likely have small tissue fragments appear granular and should be stained with NMB or other semi-transparent stain. Smears that stain dark blue are the most cell-rich smears and are most likely diagnostic. The intense blue color is because they have the most nuclei. Hemodiluted smears with few nucleated cells appear orange (like a blood smear) with Wright's stain, suggesting reduced chance of diagnosis.

Too often one goes too quickly to the 100× oil objective and stays at that power until fatigued. The scanning objectives (4×, 10×) must be used first, and often, to locate productive areas of the smear, which are then evaluated with an oil objective power (50×, 100×). Promising areas are thin and have intact, well-stained individualized cells. Cells poorly stained with Wright stain have an altered, pale, diffuse, blue color. The color and streaked-out appearance of necrotic, lysed cells indicates an area to avoid. Diagnostic structures (e.g., bacterial and fungal colonies) may be rare and isolated, so one should invest one's time to scan smears and not waste time at high magnification in a few areas. Similarly, tissue fragments that have the valuable architectural patterns are irregularly distributed and found by scanning. Tissue particles appear as dark granules often at the feathered edge.

An accurate and complete description makes conclusions easier. Performing a differential count of cells with a hematology differential counter forces the cytologist to classify each and every cell and not be biased by prominent cells such as eosinophils, plasma cells, and large cells that seem more numerous than small cells such as lymphocytes. One should not expect to identify all cells. It is common to have a few unidentified cells (i.e., fibroblasts, monoblasts, lymphoblasts) in inflammatory masses, and these few cells may look immature and have cytologic characteristics of malignancy. If they are few, they may be accepted as reactive cells secondary to inflammation. Recall that a confident cytologic report of a malignant neoplasm often leads the veterinarian to kill the patient without further testing that may give a better diagnosis. No cytologist likes killing dogs and cats.

Inflammatory Masses

Inflammation is diagnosed much more frequently and easier with cytology than is neoplasia. Cytologic diagnosis simply requires an adequate number of inflammatory cells. The number of cells sufficient for diagnosis varies with the sample. A rare plasma cell and phagocytic macrophage aspirated from inside the eye indicates inflammation, whereas thousands of neutrophils are found in pus. In hemodiluted samples, one considers the number and type of WBC usually found in blood. Blood has about a 500:1 ratio of red blood cells (RBCs) to WBCs, with mainly neutrophils and lymphocytes. More WBCs (e.g., 20:1 or 1:20 ratio) or the presence of a WBC not found in blood (e.g., plasma cells, phagocytic macrophages) is used with hemodiluted samples to diagnose inflammation (e.g., hepatic cytology). Based on predominant WBC type, different terms are used and different causes are suspected.

NOTE: Inflammation is much more commonly diagnosed by cytology than neoplasia.

Neutrophilic Inflammation

Neutrophilic infiltrates (e.g., exudation, suppuration, abscess formation, purulent inflammation) are so frequently seen that they are almost synonymous with inflammation. Neutrophils are the most motile WBCs and the first to infiltrate an area or exude out on a surface. Some call neutrophilic inflammation "acute inflammation," even though neutrophils may be prominent in chronic but active inflammation. Therefore the term *acute* may refer to a cell type (e.g., predominance of neutrophils) and not always to a time interval. Pus is proteinaceous fluid with many neutrophils and cell debris. Aspiration readily collects this fluid material; therefore many neutrophils are often present on smears. Other cells in an inflammatory mass may not exfoliate as easily (especially fibroblasts) if scarring and fibrosis are present. Neutrophils are associated with bacterial infections and some yeast infections (e.g., *Candida*), but nonseptic causes include immune-mediated processes (e.g., lupus polyarthrititis) and chemical irritation (e.g., pancreatitis, bile peritonitis). A neutrophil migrating between stratified squamous epithelial cells may indent into the surface of a squamous cell and appear as if it is within the squamous cell when it exfoliates (see Figure 16-2).

Bacterial Sepsis • Neutrophilic inflammation indicates a search for bacteria. The best place to search is in cytoplasm of neutrophils (see Figure 16-4). The neutrophil's cytoplasm is usually clear and free of granular debris. Macrophages often contain phagocytized cell debris that can mimic bacteria. Bacteria are more prominent in the clear neutrophilic cytoplasm, and the phagocytic vacuole may help outline the organism. Bacteria have uniform shapes and sizes, in contrast with granular debris. Formation of uniform pairs, tetrads, and chains identifies structures as bacteria. Rods are more confidently identified as bacteria than cocci. Wright stain precipitate is coccoid in appearance and may mimic coccoid bacteria. However, the irregular size of the precipitate, a more purple color, and a refractile appearance will differentiate stain precipitate from bacteria. Bacteria have a more dull blue color (see Figure 16-4). Stain precipitate may be on neutrophils, suggesting phagocytosis, but will also be elsewhere on the glass slide including where no sample was applied.

The description of bacteria should include number, location (e.g., free in the smear, phagocytized by neutrophils, or on epithelial cells), appearance, and whether a pure or mixed population is present. These observations permit certain conclusions. For example, a pure population of small cocci in chains within neutrophils from an abscessed lymph node suggests an infection (e.g., *Streptococcus*), whereas a mixed population of variably sized, large rods and even cocci in neutrophils in abdominal fluid suggests a ruptured gut. Beaded filamentous organisms indicate higher bacteria (e.g., *Actinomyces*) (Figure 16-11; see also Figure 16-8).

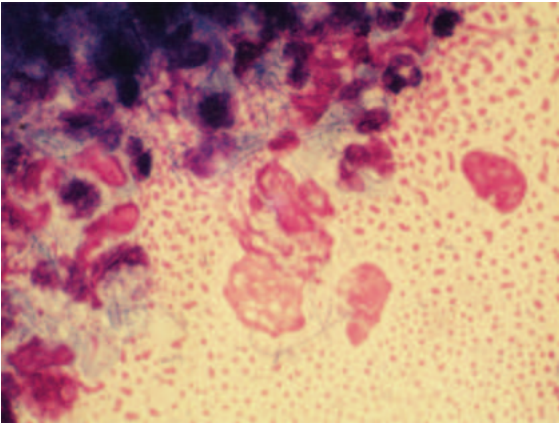


FIGURE 16-11 Septic exudate in thoracic fluid illustrates degenerative neutrophils and *Actinomyces*. Degenerative neutrophils have karyolysis, indicated by their chromatin “dissolving” into the background, and often suggest a bacterial effect. *Actinomyces* is a long, thin, filamentous, “beaded” bacteria that branches and also divides into the small rod-shaped “diphtheroidal” forms in the background.

NOTE: Bacterial infection is best shown by finding intracellular bacteria in neutrophils combined with neutrophilic inflammation. However, extracellular bacteria in fresh samples with neutrophilic inflammation also likely indicate infection. Infection is not ruled out by a lack of bacteria in cytologic samples with neutrophilic inflammation, because bacterial numbers may be below detectable levels of a microscopic examination, especially during antibiotic treatment.

Phagocytosis of bacteria by neutrophils is a better indication that there was bacterial infection than are free bacteria. But even free bacteria combined with neutrophilic inflammation support probable infection, especially if in a fresh sample. Bacteria or fungi free in the background may have been bacterial or fungal contamination in the stain or with older samples. Contamination is especially likely in samples that were moist (e.g., tracheal wash) and stored for hours or days before a smear was made. Bacteria or fungi in a sample without an inflammatory response are usually contamination, with some exceptions (e.g., ear swabs, diabetes, Cushing disease).

Bacteria on stratified squamous epithelial cells are usually normal flora from a body surface. A normal flora of the oropharyngeal area of dogs is *Simonsiella*. Finding this characteristic, huge, flat multicellular form (called trichome) of 12 to 20 bacterial cells indicates at least part of the sample came from the mouth or pharynx. Finding *Simonsiella* and a mixture of other bacteria on squames indicates that one cannot trust finding bacteria in another area of a transtracheal wash or BAL as indication of infection of the lower respiratory tract. *Bordatella bronchiseptica* has a predilection for cilia of respiratory epithelial cells, so finding small rods on cilia suggests *Bordatella* infection (Figure 16-12).

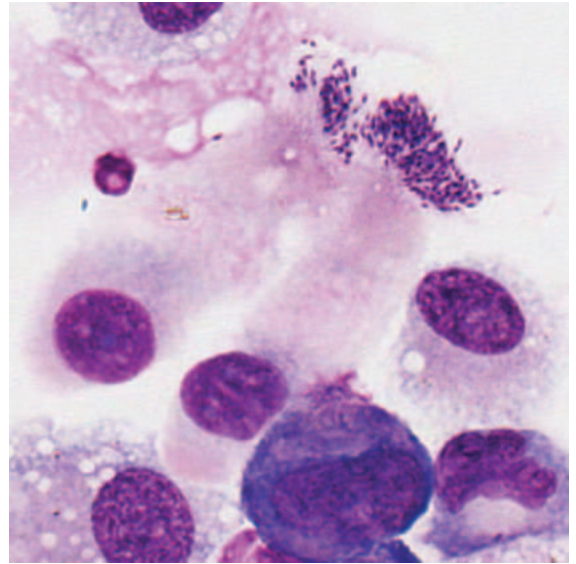


FIGURE 16-12 *Bordatella bronchiseptica* are attached to cilia of two respiratory epithelial cells in a BAL from a dog. There are very many small rods in this photo. Bacteria need not only be inside neutrophils to indicate pathogenicity. Note also the mature, cylindrical shape of the epithelial cells with nuclei located at the base of the cells. (Courtesy of a digital case from Dr. Mike Scott.)

Degenerative Neutrophils • How long should one search for bacteria in a sample with neutrophilic inflammation? Bacteria may be in low concentrations in chronic infections, with antibiotic therapy, and in some samples such as joint fluid and cerebrospinal fluid (CSF). Five to 10 minutes is a reasonable time limit unless something suggests searching longer. For example, one should search for bacteria longer than usual if neutrophils appear degenerate. Bacterial toxins often cause rapid neutrophil death (karyolysis). Degenerative changes in neutrophils suggest but do not prove sepsis. Some bacteria seem less toxic to neutrophils, however, and bacteria may be found in nondegenerate neutrophils (see Figure 16-4).

Morphologically degenerate neutrophils are characterized by swelling of the nucleus (karyolysis) and cytoplasm. Karyolysis appears as a wider, more irregularly shaped, lighter-staining nucleus lacking the dark, distinctly granular chromatin pattern and thin lobulated shape of viable nuclei (see Figure 16-11). Severely degenerate neutrophils may barely resemble neutrophils as they swell and lyse into “globs” of nuclear debris. Degenerative changes caused by bacteria must be differentiated from swelling due to sample storage, trauma to fragile cells during streaking of the smear, or nonbacterial toxic effects (e.g., urine). Inexperienced cytologists tend to over-identify degenerative neutrophil changes by examining damaged cells (see Figure 16-4). One should evaluate only intact, undamaged cells. If the neutrophils with intact cell boundaries appear nondegenerate, lysed neutrophils on the slide are probably artifactually broken rather than degenerate from bacterial toxins.

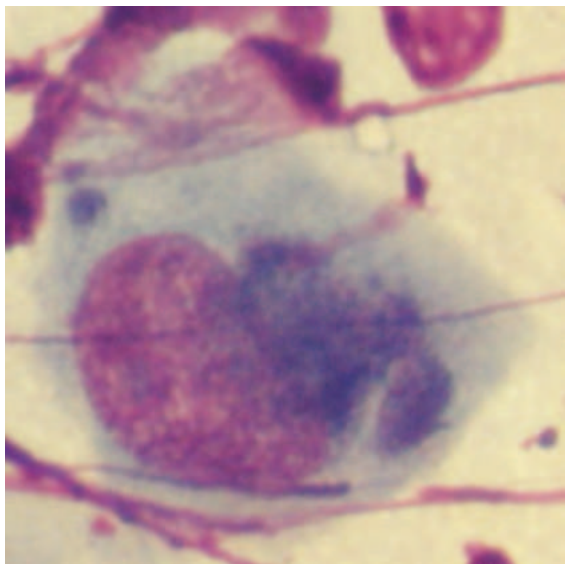


FIGURE 16-13 *Chlamydia psittaci* may be detected on conjunctival swabs of cats with acute infections. This epithelial cell has a somewhat triangular reticulate body to the right of the nucleus containing many elementary bodies. There is a second smaller reticulate body further to the right and probably a basophilic initial body in the upper left of the cell. Chlamydia are often located near the nucleus while *Mycoplasma* are found more often at the edges of the cells. There are also strands of DNA from damaged nuclei crossing the photo. (Courtesy of Dr. Anna Hillström.)

Nondegenerate neutrophils resemble normal neutrophils in fresh blood smears (i.e., clear cytoplasm; a dark, thin, lobulated nucleus). A lack of bacterial toxins permits cells to live longer. Old neutrophils become hypersegmented and are evidence of a nontoxic environment. Nondegenerate neutrophils die slowly of old age and develop pyknotic or karyorrhectic nuclei. Pyknosis (one very dark, homogeneous nucleus) and karyorrhexis (variably sized dark, homogeneous, round fragments of nuclear material) are evidence for nondegenerate neutrophils.

Necrosis • Necrosis is a common pathologic change. Necrosis occurs with inflammation and neoplasia and may be the only change seen on a cytologic sample. Necrosis is suggested by numerous lysed cells but is best proven by phagocytosis of cellular debris inside macrophages. Necrotic nuclear material is viscous. Long streaks of viscous, purple, nuclear debris are often misidentified as mucus or fungal hyphae (Figure 16-13). The streaks often extend back to a damaged nucleus of the same color and texture. Hyphae, in contrast, have parallel cell walls and a different dull blue color. Granular necrotic debris should not be misidentified as bacteria or yeast. Cholesterol crystals reflect breakdown of certain lipids and appear like panes of glass (i.e., clear rectangular crystals). The crystals are clear and “negatively stained” by other materials beside them that are stained.

Pathologic Bleeding • Blood pigments (e.g., hemosiderin and hematoidin) or erythrophagocytosis (Figure 16-14) found in macrophages proves local breakdown of

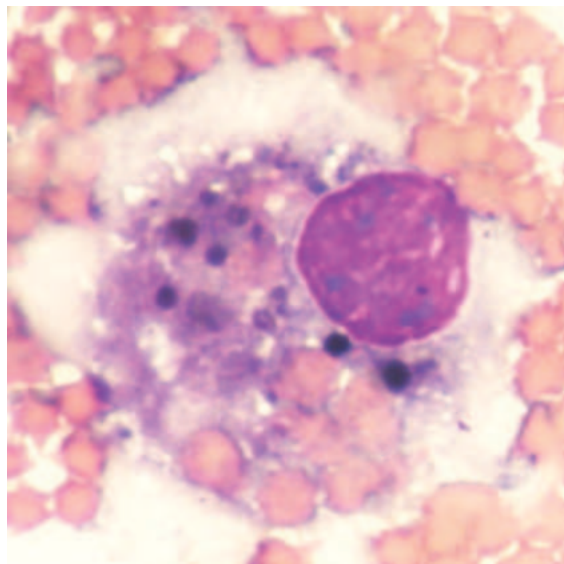


FIGURE 16-14 Pathologic bleeding (due to preexisting disease) is differentiated from artifactual bleeding due to sample collection by finding erythrocytes (erythrophagocytosis) or blood pigments (hemosiderin or hematoidin) in macrophages. There are several erythrocytes in the macrophage (especially at the bottom of the cell) and moderate dark pigment (hemosiderin) indicating pathologic hemorrhage.

RBCs and is evidence of pathologic bleeding. Pathologic bleeding can be due to primary or pure bleeding (e.g., hematoma), but bleeding often accompanies inflammation and neoplasia as a secondary problem. Blood is present in most cytologic samples, and one should determine if it is artifact of collection or evidence of preexisting disease.

Granulomatous and Pyogranulomatous Inflammation

When the inflammatory population is mainly macrophages (granulomatous) or a mixture of neutrophils and macrophages (pyogranulomatous), one should consider a causative agent larger than bacteria (e.g., fungus, foreign body). Fungi, higher bacteria, foreign bodies, and cell debris are predominantly phagocytized by macrophages. If an agent is not found, only a morphologic conclusion of pyogranulomatous or granulomatous inflammation is commonly reached. Neutrophils exfoliate more easily than do macrophages and therefore are more numerous in exudate or impression smears than in histologic tissue sections. Similarly, joint fluid cytology in immune-mediated polyarthritis may indicate an exudate (mainly neutrophils), while a synovial biopsy may report a lymphocytic plasmacytic synovitis. Both are correct, but reflect cell types found in different areas of the body.

NOTE: Granulomatous inflammation (i.e., >50% macrophages) suggests the cause was large structures such as foreign bodies, necrotic debris, fungi, and certain types of bacteria that stimulate macrophage proliferation rather than pus formation.

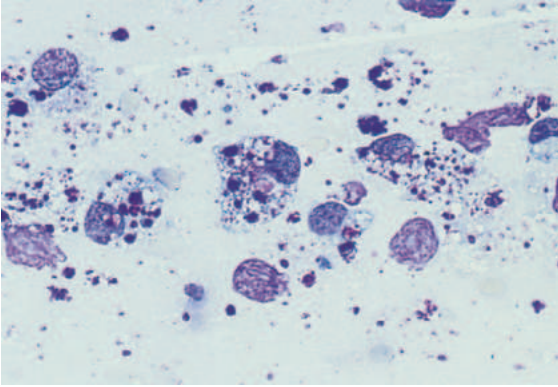


FIGURE 16-15 Granulomatous inflammation at the site of an injection. There is abundant debris in the background and, more importantly, phagocytized by macrophages. (Courtesy of Dr. Rick Cowell.)

Phagocytized material in macrophages often indicates the diagnosis. Injection-site reactions usually have high numbers of macrophages containing amorphous debris (Figure 16-15). Macrophages from necrotic lesions contain nuclear fragments and cellular debris. In organized hematomas, macrophages contain phagocytized RBCs and RBC breakdown products (e.g., hemosiderin, hematin) (see Figure 16-14). In steatitis, smears contain oil droplets or, rarely, yellow fat crystals. Yeast and hyphae usually stain blue with NMB and Wright stain, but when they stain poorly, their shape may be outlined by darker background material. To confirm the presence of an unstained organism, one should try another stain. Mycobacteria do not stain and appear as clear slits (i.e., rod-shaped ghosts) in macrophages (see Figure 16-9).

Large structures stimulating a macrophage response may be numerous, such as fat droplets in aspiration pneumonia, and found in most macrophages (see Figure 16-7), but more often the diagnostic structures are few in number and easy to miss. Scanning of the smears is critical to find large, abnormal structures such as burdock awns (golden-brown, linear, barbed plant material) in samples from a lingual foreign-body granuloma. Similarly, a fungal colony may appear as a spot in one area of one smear. One can improve the chances of having fungal or bacterial colonies in smears if they were initially detected by gross inspection of aspirated fluid or a flush. Off-colored white, yellow, or green flecks found in a fresh sample should be collected and included in smears.

Chronic Inflammation

Chronic inflammatory lesions that lack the abundance of macrophages described in the preceding granulomatous category have a mixture of inflammatory cells, including plasma cells, lymphocytes, macrophages, neutrophils, and occasional fibroblasts. This pattern is expected, for example, in canine lick “granulomas,” in later stages of healing of an inflammatory lesion (e.g., an old abscess), or in a chronic low-grade inflammatory disease (e.g., proliferative synovitis). Smears from very fibrous lesions are often poorly cellular and might be mistaken for a poor aspirate. Repeat cytologic sampling yields often similarly

poorly cellular samples, so histopathology should be used instead. Because fibroblasts exfoliate poorly, one should use plasma cells as a cytologic indicator of chronicity. It takes days to weeks for plasma cells to proliferate in an inflammatory lesion.

Eosinophilic Inflammation

Inflammation is usually classified by the most numerous type of WBC. Because eosinophils are normally rare, they need only exceed 20% to 30% of an inflammatory population to indicate eosinophilic inflammation as at least part of the classification. Eosinophilic granuloma complex in cats and Siberian husky dogs is diagnosed when smears from a typical lesion in the expected area indicate eosinophilic inflammation with a variable component of macrophages, plasma cells, and mast cells. In cats, eosinophilic plaque and linear granuloma usually have eosinophilic infiltrates and fibroblasts, whereas eosinophilic ulcers may not. Parasites and allergic reactions are usually the first rule outs, but causes include a wide variety of various infectious, inflammatory, and neoplastic diseases and syndromes (see Eosinophilia, Chapter 4).

Lymphocytic Inflammation or Hyperplasia

Focal lymphoid hyperplasia may appear as a small mass or elevation of a mucosal surface consisting of mainly small lymphocytes and a variable number of plasma cells. These occur in the nasopharyngeal area, vagina, and intestine, where one might not expect a lymph node or focal lymphoid tissue. Lymphoid hyperplasia may be focal (e.g., focal lymphoid hyperplasia in vagina, spleen, or intestine) or diffuse (e.g., lymphocytic rhinitis). Diffuse submucosal or subsynovial lymphocyte proliferation is more often called inflammation, especially if mixed with proliferation of other types of leukocytes.

Selected Causative Agents

Descriptions of several organisms follow. One or more cytology atlases and microbiology texts should be available near the microscope.¹¹ Granulomatous or pyogranulomatous inflammation suggests larger organisms, but the type of inflammation is not a major differentiating feature of various agents. Diagnosis by microscopic identification is emphasized here. Culture of the organisms and immunologic testing are more specific methods discussed in Chapter 15. Many organisms have distinct geographic distributions (e.g., salmon disease in Washington, Oregon, and northern California). Anatomic location of the infection (e.g., *Cryptococcosis* in feline nasal cavity) also aids in diagnosis.

Dimensions of organisms are often given in micrometers (μm). Some photomicroscopes allow precise measurement of structures. A micrometer may be inserted in the microscope's eyepiece. The micrometer's units may be calibrated for each objective with the grid of a hemocytometer. RBCs and WBCs on the smear are more likely available and may also be used to estimate size. Canine erythrocytes are about 7 μm , and feline erythrocytes are about 6 μm . The approximate diameters of leukocytes on blood smears are as follows: neutrophils, 14 μm ; eosinophils, 16 μm ; small lymphocytes, 6 to 10 μm ; large lymphocytes, 12 to 15 μm ; blast-transformed lymphocytes,

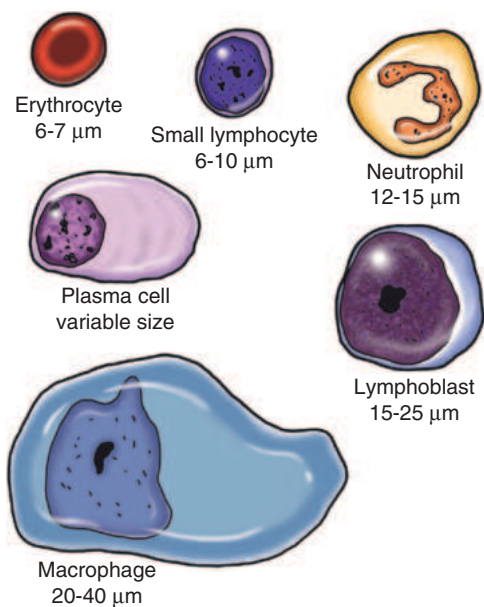


FIGURE 16-16 Cell sizes. Certain cells on smears (especially erythrocytes) may be used as micrometers to judge the size of infectious agents, and nuclei of cells in question. Cells in the figure are drawn to scale. Erythrocytes are less subject to size changes due to thickness of the smear than are neutrophils. Thus for example, the size of nuclei in lymphoma cases are often described as how many times larger in diameter the lymphoma cell nucleus was than an erythrocyte.

15 to 25 µm; monocytes, 14 to 20 µm; and macrophages, 20 to 40 µm (Figure 16-16). Depending on the thickness of the smear, cells vary in diameter (see Figure 16-2), so these values are only approximate.

NOTE: One should judge the size of organisms by comparing them to the size of erythrocytes or neutrophils.

Fungal Characteristics

Yeast are characterized by the formation of buds on uniformly sized, round to oval structures (see Figure 16-5). Spherules such as *Coccidioides*, *Rhinosporidium*, and *Prototheca* form endospores. The organisms are differentiated by size, appearance of buds or endospores, shape, capsule, and location in the body (Figure 16-17). Other structures may mimic yeast, such as fat droplets in urine, especially when two adjacent droplets resemble budding. Unlike yeast, oil droplets are of various sizes, are refractile and are not stained with Wright stain. Similar droplets can be ultrasound gel (red droplets) or medications.

Fungal hyphae have two parallel cell walls and form branches. Hyphae are 3 to 20 µm thick, may have distinct septa, and may form spores or fruiting bodies.

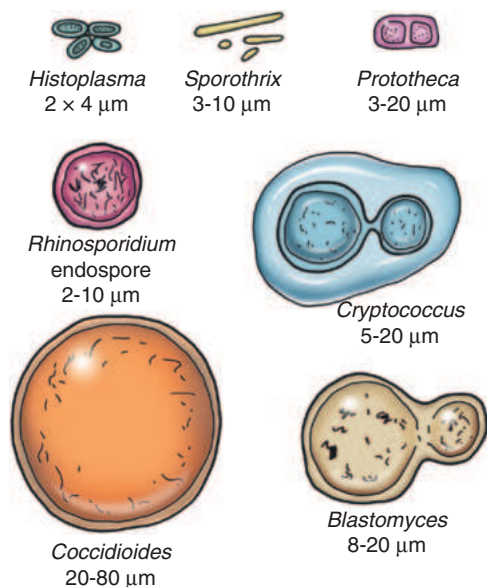


FIGURE 16-17 Comparative morphology of selected yeast and spherical organisms. These are drawn to scale except for *Coccidioides*. Size is useful for differentiation. Shape of organisms and budding of yeast aid in identification.

Aspergillus is characterized by uniformly thick septate hyphae (Figure 16-18). Streaks of nuclear debris, protein that has been rolled up into cylindrical forms, fibers, and lint may mimic hyphae. *Alternaria* is a common contaminant in the air, dust, and smears (Figure 16-19). It has golden septate hyphae (which may stain green with Wright-Giemsa) and diagnostic club-shaped macroconidia.

Small pathogenic fungi (e.g., *Histoplasma*, *Sporothrix*) are in macrophages, whereas larger fungi are

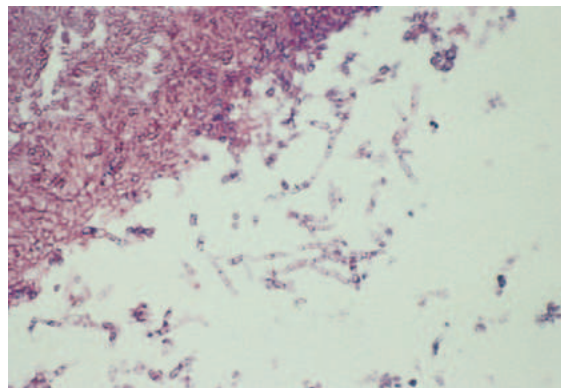


FIGURE 16-18 Canine nasal mycosis illustrates a colony of *Aspergillus* that has uniformly thick septate hyphae. These colonies may be few in number in the sample, and one needs to screen the smear for an odd-colored structure that may easily be considered debris.

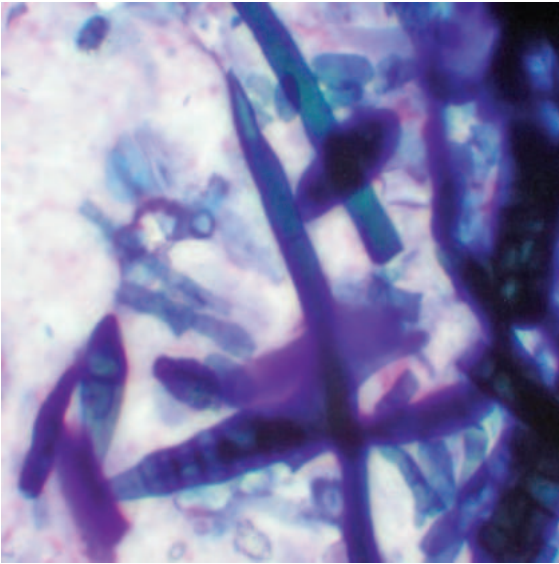


FIGURE 16-19 *Alternaria* contamination in a BAL sample is shown by finding large green septate hyphae and the typical club-shaped macroconidia of *Alternaria* that are subdivided into internal chambers. *Alternaria* is a common contaminant of cytologic samples.

usually not phagocytized. Contaminant fungi are found anywhere on a slide, including areas away from tissue imprints or fluid smears. If a colony of fungi is found, one should check the stain for fungal contamination by applying the stain to a blank slide.

Histoplasmosis

Histoplasma capsulatum in cats is most consistently found in bone marrow aspirates.⁴ Blood smear analysis is an insensitive diagnostic test, but yeast may be found in phagocytes in any body fluid. Buffy coat smears concentrate WBCs for examination. *Histoplasma* may be diagnosed by cytology or histopathology of enlarged lymph nodes, liver, or other organs (e.g., colonic scrapings). The small (i.e., 2- to 5- μ m) yeast are in macrophages and occasionally in neutrophils. A phagocyte may contain a few too many yeast (Figure 16-20). Budding may be observed.

Sporotrichosis

Sporothrix schenckii is abundant in samples of draining, ulcerated feline lesions, but the yeast is difficult to find in canine lesions. In cats, the yeast is very pleomorphic with round, oval, and fusiform (i.e., classically described as cigar-shaped) 3- to 10- μ m forms (Figure 16-21) in macrophages and neutrophils, and free in the background.

Warning: People acquire sporotrichosis from animals more easily than they do other mycoses.

Cryptococcosis

Cryptococcus neoformans may be found in various tissues. A nasal mass in cats is a classic presentation. This yeast is best identified by the variably thick gelatinous capsule that often doubles the size of the cell (Figure 16-22). Rare

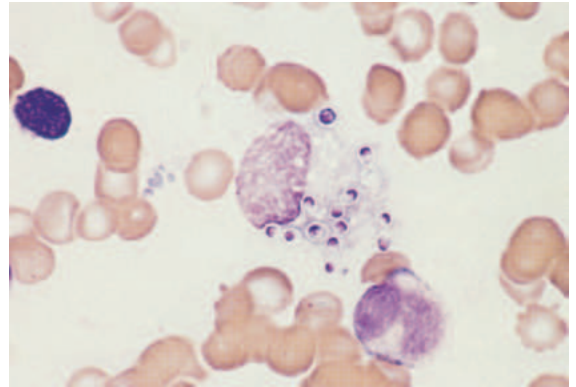


FIGURE 16-20 Canine histoplasmosis is identified by small (2- to 4- μ m) oval, budding yeast with distinct cell walls in macrophages. This macrophage was a bone marrow aspirate.

strains of *Cryptococcus* lack a capsule. The yeast itself usually stains clear to eosinophilic. Budding is from a narrow base, in contrast with *Blastomyces*, which has broad-based buds. Cells vary from 8 to 20 μ m in diameter. *Rhinosporidium seeberi* occurs rarely in the nose of dogs and produces endospores (2 to 5 μ m) that may be confused with cryptococcosis. *Cryptococcus* yeast is well demonstrated on smears stained with Wright or NMB. India ink preparations are messy and unnecessary. Inflammation may be absent, and the lesion just a glistening mass of yeast.

Rhinosporidiosis

R. seeberi infects the nasal cavity of dogs with the formation of recurrent non-neoplastic polyps. The huge trophic stages (i.e., 60 to 120 μ m) and sporangia (i.e., 100 to 300 μ m) are diagnostic on histologic sections and occasionally visible on cytologic specimens. The smaller 2- to 10- μ m endospores from ruptured sporangia are

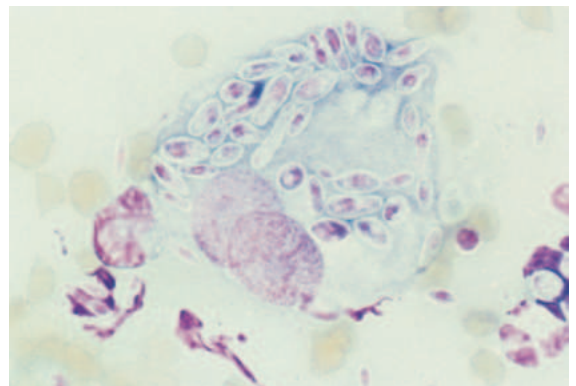


FIGURE 16-21 Feline sporotrichosis is identified by pleomorphic "cigar"-shaped yeast with distinct cell walls in macrophages. This sample was from an infected wound in a cat after surgically removing the claws with inadequately sterilized equipment.

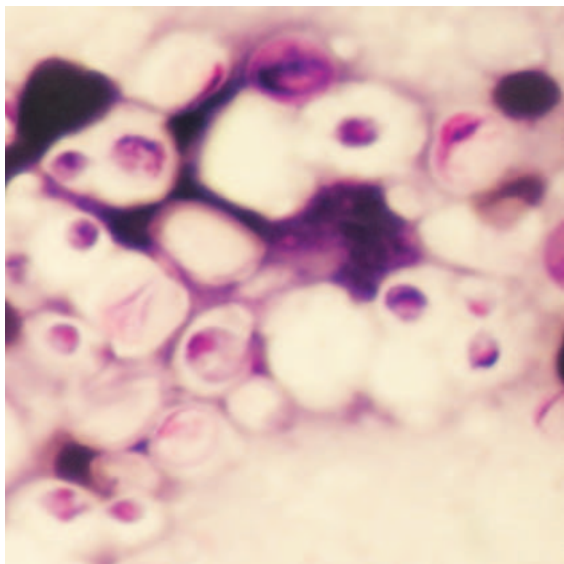


FIGURE 16-22 Feline throat mass with a large colony of *Cryptococcus*. The yeast (8 to 20 μm) characteristically have a large clear capsule or halo and form narrow-based buds.

commonly visible on cytologic preparations (Figure 16-23). The spherical endospores lack both budding and the characteristic clear capsule associated with *Cryptococcus*.

Coccidioidomycosis

Coccidioides immitis is usually recovered from pulmonary or disseminated lesions in dogs and rarely from cats. It is characterized by large size and internal endospores. Spherical sporangia range from 10 to greater than 100 μm in diameter (see Figure 16-17). Endospores (2 to 5 μm in diameter) are usually in bigger spherules. *Coccidioides* organisms tend to be surrounded by inflammatory phagocytes on smears.¹⁰ Arthrospores formed in fungal

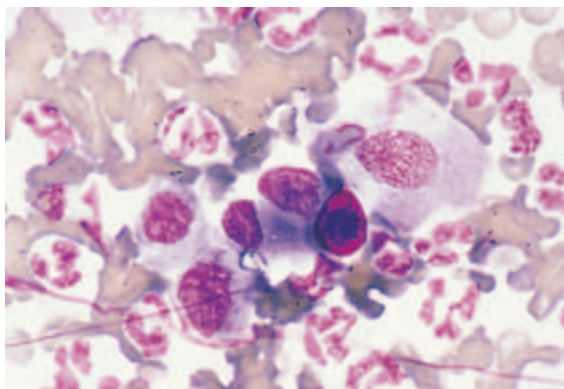


FIGURE 16-23 Besides the neutrophils, blood, and epithelial cells in this nasal mass cytology from a dog, there is a reddish endospore of *Rhinosporidium* just to the right of center. (Courtesy of Dr. Rick Cowell.)

cultures are highly infectious; therefore, the microbiologist should be warned if *C. immitis* is possible.

Blastomycosis

Blastomyces dermatitidis is a thick-walled budding yeast (approximately 20 μm in diameter) infecting lungs or other tissues in dogs and occasionally cats (see Figure 16-17). With Wright stain, the yeast cells are dark blue and are often collapsed and wrinkled from the alcohol dehydration during staining, but they appear more typical with NMB stain. Because the yeasts are large, they are often pushed to the end of the smears and are best found by scanning the slides.

Candidiasis

Candida albicans is usually a normal flora. It may be an overgrowth on a surface (i.e., thrush, moniliasis) but rarely is a systemic infection or is disseminated. It is a typical thin-walled budding yeast about 2 to 6 μm in diameter. It is unique from other yeasts in the formation of pseudohyphae (3 to 4 μm thick), which look like short septate hyphae in cultures and occasionally in tissue samples.

Malassezia

Malassezia (Pityrosporum) (see Figure 16-5) is a small budding yeast similar to *Candida*. It is commonly found in ear swabs of dogs with chronic otitis that have been nonresponsive to antibiotic therapy. Diagnosis of chronic yeast otitis should occur routinely in private practice. Karen Moriello (Dermatologist, University of Wisconsin) suggests using a stainless steel “flat, round-ended micro spatula” to harvest yeast from between the toes of dogs that persistently lick their paws. The spatula is designed for transferring small amounts of chemical to weighing scales (and may be purchased from chemical distributors). It is effective, easily cleaned and not traumatic in skin scrapings. Tape preparations are also effective in collecting yeast. *Malassezia* in cats may be smaller and resemble a large bacterial coccus. A separate staining system should be used for ear swabs to avoid finding yeast contaminating other cytologic samples.

Protothecosis

Prototheca is a rarely disseminated canine infection. Signs are related to lesions in the skin, eyes, and intestine. The round to oval algae are 3 to 20 μm in diameter (usually just smaller than a neutrophil), have a clear cell wall, and may have two or more endospores (Figure 16-17 and Figure 16-24). Cytologic and histologic diagnosis can be further confirmed by culture and immunofluorescent tests on tissues.

Aspergillosis

Aspergillus spp. is the most common fungus associated with canine nasal infections (see Chapter 11). It is critical to scan many smears to find a fungal colony, because most of the sample is exudate with secondary bacterial sepsis. Finding a septic exudate may suggest a bacterial cause and terminate the search before the primary problem is found. Fluid from a nasal flush should be grossly examined for a green (*Aspergillus fumigatus*) or brown (*Aspergillus niger*) mass (i.e., the fungal colony).

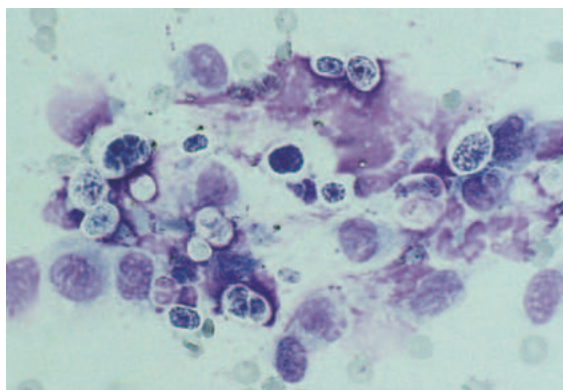


FIGURE 16-24 Canine rectal scraping has inflammation, but also *Prototheca*, which are the spherical organisms with a clear halo.

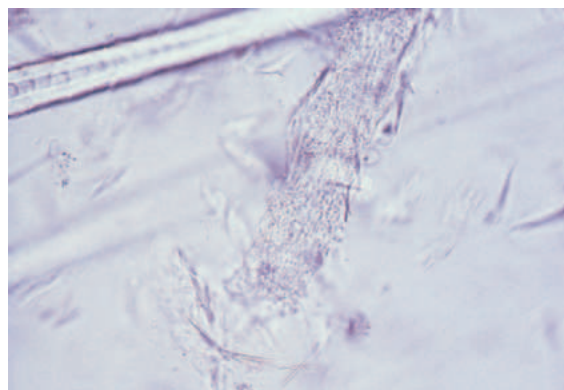


FIGURE 16-25 Canine skin scraping with one normal hair shaft and another swollen hair shaft (center) destroyed by a proliferation of arthrospores of *Microsporum canis*.

Any odd-colored clump is placed on a smear to improve the probability of diagnosis. Fungal colonies are found by scanning the smear. A colony resembles a clump of debris, but should be examined with high magnification. Colonies are a mass of septate, branching hyphae of comparably uniform thickness (i.e., 3 to 4 μm) (see Figure 16-18). Uniformly thick hyphae are good evidence of aspergillosis. The center of an *Aspergillus* colony may not stain, thus requiring examination of its perimeter. The large (i.e., 300- μm) branching conidiophore with 2.5- to 3- μm spherical conidia is most diagnostic but most often seen in fungal colonies grown on agar. Occasional cases may only have the small, clear, bluish spherical spores that may have a thin, clear halo. See the earlier discussion on hyphae under Fungal Characteristics to differentiate nonpathogenic forms such as *Alternaria*.

Mycetoma

Cutaneous fungal granulomas have a confusing array of names (e.g., eumycotic mycetomas, maduromycosis, chromoblastomycosis) and are caused by a wide variety of fungi (e.g., *Drechslera*, *Allescheria*, *Madurella*, *Cladosporium*, *Fonsecaea*). Expectation for cytologic diagnosis is limited to identifying granulomatous or pyogranulomatous inflammation with one or more fungal forms (i.e., hyphae, spherules). Shape, size, and color of fungi on an unstained slide should be recorded, but it is unlikely the type of fungus will be recognized by morphology. Fungal culture is used for specific causative diagnosis.

Mucormycosis

Mucor and similar relatively nonpathogenic fungi may be found in gastric ulcers or other tissues. The hyphae are nonseptate, branching, and wider (i.e., 15 to 20 μm) than those of *Aspergillus* spp. Type of fungus is identified by culture.

Alternaria

Alternaria is a ubiquitous contaminant found in various microscopic preparations that must not be confused with truly pathogenic fungi. *Alternaria* organisms have large, golden-brown septate hyphae and characteristic club-shaped conidia with longitudinal and transverse septae

(see Figure 16-19). *Alternaria* is rarely pathogenic. Documenting that it has invaded deeper tissues indicates infection and not just surface contamination.

Dermatophytes

Dermatomycosis may be identified by finding swollen abnormal hair shafts filled with arthrospores (Figures 16-25 and 16-26), or septate hyphae on squames or hair shafts, or the rectangular arthrospores as hyphae breaks into arthrospores (see Chapter 15).

Higher Bacteria

Actinomyces, *Nocardia*, and *Dermatophilus* are higher bacteria. They branch but are much thinner (width of 0.5 to 1.0 μm) than fungal hyphae. Fungal hyphae, in contrast, have two distinct cell walls separated by obvious space

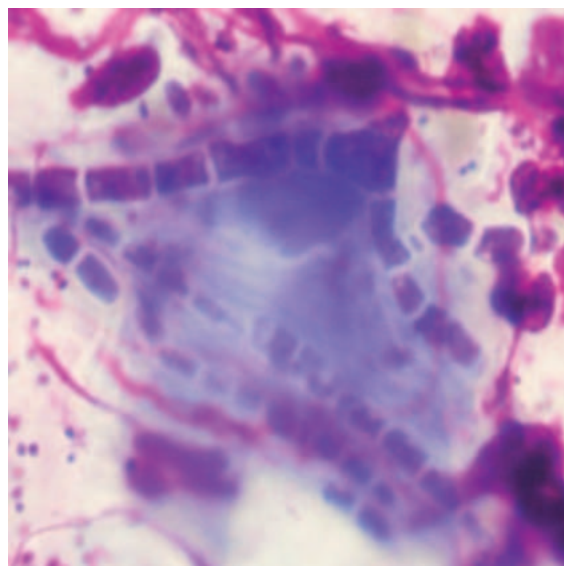


FIGURE 16-26 This skin scraping had a dermatophyte infection on a squame in which the septate hyphae of *Trichophyton mentagrophytes* have broken into rectangular arthrospores. There was also a *Staphylococcus* infection.

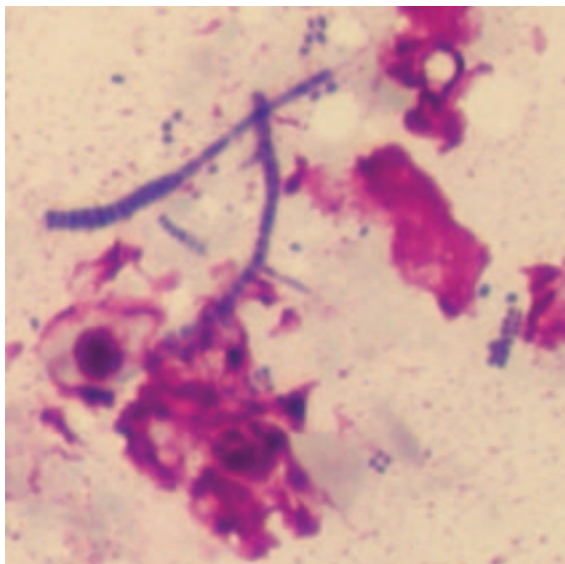


FIGURE 16-27 *Dermatophilus congolensis* is a higher bacteria with zoospores that divide into vertical and horizontal rows (railroad track pattern). The structures also branch. The bacteria may be seen on impression smears from undersides of skin scabs that had been moistened with saline and stained with a Wright or Giemsa stain.

(see Figure 16-18). *Actinomyces* has long filaments that occasionally branch and have a beaded appearance on Wright stain (i.e., variable staining intensity along the filaments) (see Figure 16-8 and Figure 16-11). *Actinomyces* and *Nocardia* are pleomorphic organisms that may seem to be a mixed bacterial infection with branching filamentous forms and smaller rod-shaped diphtheroidal forms. *Actinomyces viscosus* was cultured from 11 consecutive Michigan cases with clinical diagnoses of canine “nocardiosis” without identifying one case with *Nocardia*. Colonies in exudate appear grossly as white to yellow (called “sulfur granules”) and should be collected for making the smear.

Dermatophilus congolensis causes cutaneous infections in dogs less frequently than in other species. One can identify it by soaking skin crusts in saline and then rubbing the underside of the crusts on glass slides. On Wright stain, characteristic railroad track-like patterns of parallel and longitudinal rows of zoospores (0.5 to 1.0 μm) (Figure 16-27) forming variably thick branching structures are found on squamous cells.

Mycobacteriosis

Mycobacterium lepraemurium, *Mycobacterium fortuitum*, *Mycobacterium bovis*, and some other species cause cutaneous or SC masses in cats and less frequently in dogs.⁸ Feline leprosy (*M. lepraemurium*) lesions occur anywhere on a cat; atypical mycobacteriosis tends to cause fistulous tracts on the ventral abdomen. The organisms are demonstrated by impression smears of the granulomas or smears of the draining tracts stained with acid-fast stains (Figure 16-28). Mycobacteria do not stain with Wright

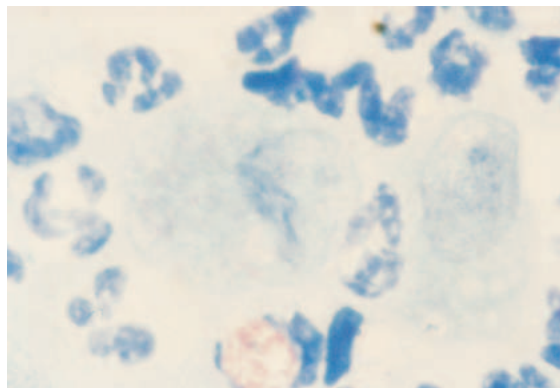


FIGURE 16-28 Pyogranulomatous inflammation with red bacilli (acid fast stain positive rods) in a neutrophil at the bottom. *Mycobacterium* was cultured from this subcutaneous lesion in a cat.

stain, and negatively stained rods appear as clear slits or “ghosts” in macrophages (see Figure 16-9).

Notify the histopathologist, because samples for identifying mycobacteria require special processing. Organisms tend to localize in clear lipid droplets in the center of granulomas, and they are lost during alcohol and xylene steps if routine slide preparation is performed. Frozen sections retain the organisms so they can be displayed in the section. Culture is required to differentiate feline leprosy from atypical mycobacteriosis. One should advise the microbiologist to use media for atypical mycobacteria. Cytology, histopathology, and culture may need to be repeated.

NOTE: The pathologist should be advised of the possibility of *Mycobacterium* infection, because special processing of histopathologic samples improves the chance of finding the organisms.

Salmon Disease

Neorickettsia helminthoeca may be identified in macrophages from lymph nodes of infected dogs. A moderate to large number of coccoid to rod-shaped bodies (0.3 μm in diameter) are spread through the cytoplasm of macrophages and may form morulae. Macchiavello’s stain is excellent for rickettsiae, but Wright-type stains are suitable. Trematode eggs are in a dog’s feces 1 week after infested fish are eaten.

Ehrlichiosis

Finding an *Ehrlichia canis* morula (i.e., raspberry-like cluster of tiny bodies) in circulating WBCs, cytologic smears of lung, or synovial fluid is diagnostic but is rare except in highly endemic areas such as Brazil. (See *E. canis* diagnosis discussion in Chapters 5 and 15.) Canine granulocytic “ehrlichiosis” is now called *Anaplasma phagocytophilum*.

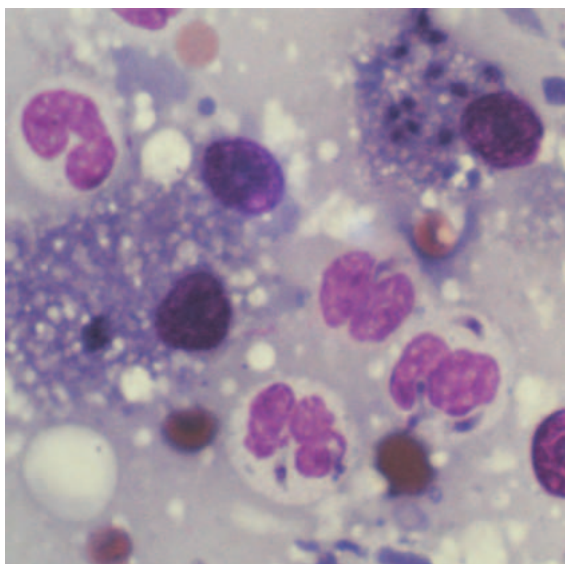


FIGURE 16-29 *Toxoplasma gondii* tachyzoites are crescent shaped (i.e., 2 to 4 μm $\times$ 4 to 7 μm), with a nucleus at one end. Three neutrophils (lower center) contain one to three tachyzoites each in this photo of an impression smear of lung of a cat with toxoplasmosis.

Toxoplasmosis

Toxoplasma gondii is rarely found in macrophages and neural, ocular, or muscle tissue. The actively dividing forms (i.e., tachyzoites) are crescent shaped (i.e., 2 to 4 μm $\times$ 4 to 7 μm), with a nucleus at one end (Figure 16-29). The shape may not be discerned when several are packed in a cell, but it becomes apparent when freed from a ruptured cell. Smaller bradyzoites in stable tissue cysts remain infective for a long time. *Neospora caninum* tachyzoites look the same. Similar protozoa to consider are *Sarcocystis* and *Leishmania*. Active *Toxoplasma* infection is diagnosed serologically (see Chapter 15). Small (i.e., 10- to 12- μm) coccidial oocysts of *Toxoplasma* are briefly (i.e., 2 weeks) shed in feces by cats after ingestion of infected meat. This suggests the enteric infection but is too transient for consistent diagnosis. Other coccidial oocysts are usually larger (i.e., 20 to 40 μm).

Leishmania

Leishmania may be found in macrophages in bone marrow, lymph nodes, or splenic aspirates of dogs with visceral *Leishmania* infection (Figure 16-30). The oval protozoa have a round nucleus and characteristically a rod-shaped kinetoplast. Infected dogs often come from countries around the Mediterranean (e.g., Greece, Spain). PCR testing is specific and sensitive (see Chapter 15). *Trypanosoma cruzi* is a protozoa which also has a nucleus and rod-shaped kinetoplast. It looks similar to *Leishmania* but rounder in cell shape and a larger, rounder kinetoplast.

Cytauxzoonosis

Cytauxzoon felis is a tick-transmitted, highly fatal protozoal disease of domestic cats. Cytauxzoonosis is diagnosed antemortem by finding the small signet-ring

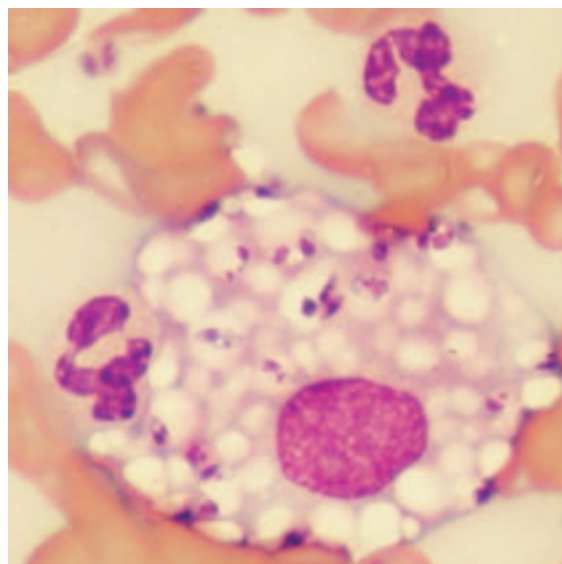


FIGURE 16-30 *Leishmania* is a common infection in Southern Europe. This macrophage from an enlarged canine lymph node contains about 15 protozoa that are oval and contain a nucleus and a rod-shaped kinetoplast.

intraerythrocytic stage (50% of cases) or finding large macrophages containing schizonts of developing *Cytauxzoon* organisms in lung, liver, spleen, lymph node, or bone marrow aspirates or impression smears.

Parasitic Infestations

Adult parasites or various life stages found in cytologic samples should be identified as well as possible but may need to be sent to a parasitologist for final classification. Ectoparasites and endoparasites include too large a group to address in this chapter. Some parasites found in the lower respiratory tract and respiratory cytology of dogs and/or cats include *Crenosoma vulpis*, *Oslerus* (*Filaroides*) *osleri*, *Filaroides hirthi*, *Paragonimus kellicotti*, and *Capillaria aerophila*. If an eosinophilic inflammatory response suggests a parasitic cause, all cytologic samples should be screened at low magnification for parasites. Figure 16-31 illustrates the characteristic copulatory organ of a male parasite. Baermann's fecal analysis may be used to identify larvae. *Oslerus osleri* forms submucosal nodules, which may be seen by bronchoscopy. When these are sampled for cytology, the coiled first-stage larvae may be found (Figure 16-32). Screening magnification can be used to find the larvae or ova.

Ocular Cytology

Conjunctival swabs often identify the primary inflammatory reaction in the conjunctiva and may identify an organism. Besides common bacterial infections, fungal infections such as *Aspergillus* may be found in necrotic lesions treated with antibiotics. Primary infections include *Chlamydia psittaci*. *C. psittaci* may initially have basophilic



FIGURE 16-31 *Crenosoma vulpus* is a common lungworm of foxes and causes eosinophilic bronchitis in dogs. The golden rod-shaped structure is the copulatory organ of a parasite found in a canine lung washing. *Crenosoma vulpus* should be considered in dogs with eosinophilic inflammation of their lungs.

initial bodies, which appear as small to medium, round bluish bodies in the cytoplasm; these can seem somewhat nonspecific, like debris. A reticulate body filled with coccoid *Chlamydia* elementary bodies is less likely confused for debris (see Figure 16-13). *Mycoplasma* bacteria lack a cell wall and appear as round to pleomorphic, dull blue organisms on conjunctival epithelial cells (Figure 16-33). *Mycoplasma* is seen more at the edge of epithelial

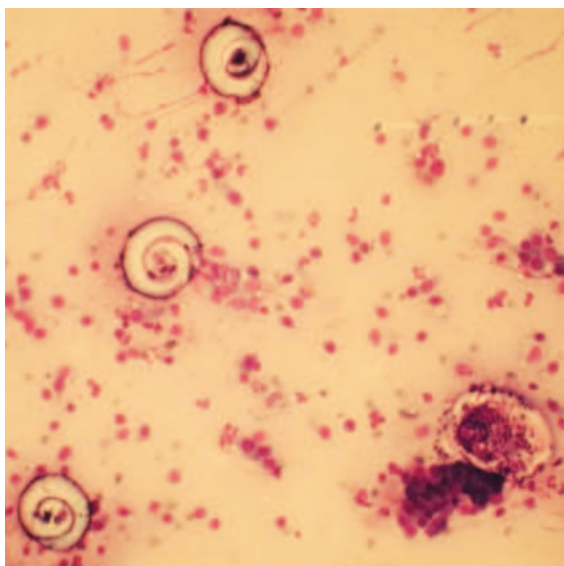


FIGURE 16-32 *Oslerus (Filaroides) osleri* appeared as "bubbles" under the tracheal mucosa of a dog. Several of these large coiled first-stage larvae (four in this photo) were easily found at screening magnification. The neutrophils in the background can be used for size comparison.

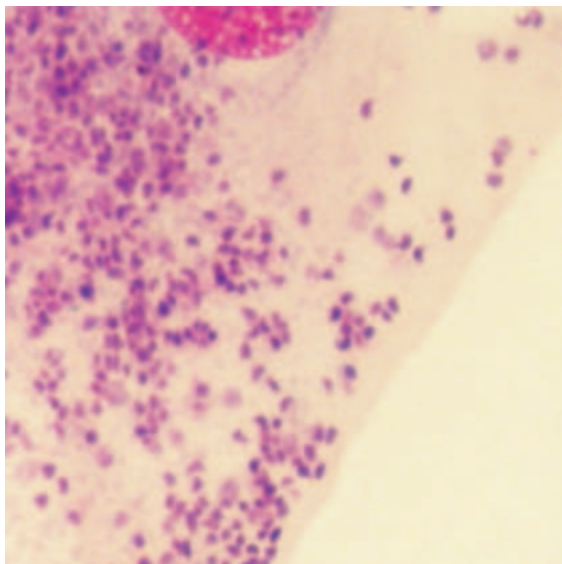


FIGURE 16-33 *Mycoplasma* appear as rounded, pleomorphic bacteria on conjunctival epithelial cells because they lack a cell wall and thus firm structure.

cells in contrast to *Chlamydia*, which is more often perinuclear in location.

Proliferative Masses (Neoplasia)

Cytology of neoplasms is more difficult than cytology of inflammatory and infectious lesions. The decisions based on the cytology result can be devastating for the patient. Therefore the veterinarian making the treatment decision needs to know how much to trust the sample and how much to trust the opinion of the cytologist interpreting the sample. A cytology report should describe the sample well enough to indicate if it was adequate for a diagnosis. The report needs to describe the evidence for the conclusion well enough to indicate how much weight should be placed on the cytologic conclusion.

Initial Decisions

The two major initial conclusions are: (1) the smears represent the structure, and (2) the structure is composed of a proliferation of noninflammatory tissue cells of one type. The minimum number of cells needed to permit a firm conclusion is subjective, but for a mammary neoplasm cytology study, more than 100 cells per slide were considered necessary for evaluation.¹ When a small number of tissue cells are present, the risk is increased that they do not represent the mass. This is especially so when the sample is diluted with blood, exudate, or other fluid. Diagnosis is often made difficult by concurrent inflammation, fibrosis, and necrosis. The higher the percentage and total number of inflammatory cells in the population, the lower is one's confidence that the mass is not primarily inflammatory. Diagnosis of neoplasia also requires identifying the cell type and determining the strength of cytologic evidence of malignancy.

Cell Typing

Shape of the cells, their association with other cells (especially in tissue fragments), and cytoplasmic features are used to indicate the cell type of origin. Classification of cell type by cytology into three general categories is the usual and reasonable goal. Cells are classified as epithelial, mesenchymal (i.e., connective tissue, spindle cell), or round cells. Enough information (e.g., site of collection) plus cell morphology may be sufficient to be certain the cells are of one specific type, such as perianal gland cells from a mass by the anus. However, if cytology can identify, for example, that a mass is epithelial in type and has strong criteria of malignancy allowing a reasonably firm conclusion of a carcinoma, this should be an acceptable cytologic result. Histopathology should be used for more specific classification of type and malignancy of most neoplasms, while cytology should be considered a tentative diagnosis. Description of cytologic features of neoplasia in this chapter will be an overview. For detailed descriptions of tumor types, the reader should refer to a reference text.¹¹

Epithelial cells are best indicated by distinct, tight, cell-to-cell junctions (Figures 16-34 and 16-35; see also Figure 16-12). Epithelial cells form surfaces with tight cell junctions that persist to a variable degree on smears. Epithelial cells line a basement membrane, and so often form cell clusters with a uniform edge (along the basement membrane). One should be cautious of interpreting adjacent, crowded cells on a thick part of the smear as having epithelial junctions where the cells simply flatten along edges of contact. Instead, one should look for distinct linear junctions, complete with formation of angles and

corners. Epithelial cells may form layers, so an epithelial cell may be identified in a pocket formed by an adjacent epithelial cell (the way one hand fits in the palm of another). Tissue fragments in smears may retain structures such as bands or acini with lumina (glandular origin) or papillary structures (i.e., fingerlike epithelial projections with a central core of connective tissue stroma). Glandular tumors are called adenomas or adenocarcinomas. Tissue architecture is best shown with semi-transparent stains such as NMB. Well-differentiated epithelial cells may retain squamous, polyhedral, cuboidal, or columnar shapes and cilia. Secretory material such as mucus, granules, or vacuoles suggests glandular epithelium.

Macrophages have many appearances that routinely confuse cytologists. Epithelioid macrophages can bind together and lack phagocytic vacuoles, and therefore appear like epithelial cells. A common error is to misdiagnose a granuloma as a carcinoma on cytology or histopathology. Synovial cells, endothelial cells, and melanocytes may also mimic epithelial cells. Mesothelium (and synovium) forms a surface like epithelial tissue. Mesothelial cells often form epithelial-type papillae, which are mesothelial cell-lined, fingerlike projections formed during irritation of a surface (e.g., villous proliferation in *Actinomyces* pleuritis or hemopericardium). The frequently anaplastic appearance of reactive mesothelial cells, especially after swelling in the fluid, has often caused a misdiagnosis of carcinoma or mesothelioma in pleural, pericardial, and peritoneal fluids. No cytologist can consistently differentiate between very reactive and often swollen mesothelial cells in fluids and neoplastic epithelial cells or neoplastic mesothelial cells (Figure 16-36). One should not waste time and money sending these types of fluids to an “expert,” expecting to get an accurate answer. No veterinarian should kill a dog or cat based only on a fluid cytology diagnosis of

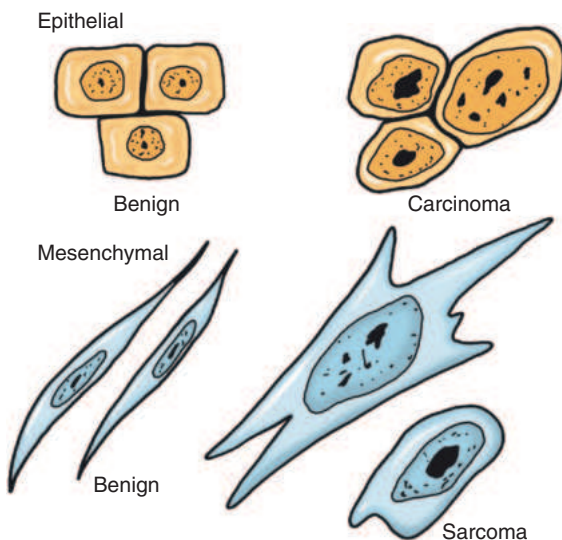


FIGURE 16-34 Benign and malignant examples of epithelial and mesenchymal cells. The reader should note the tight intercellular junctions of the two clusters of three epithelial cells (top). The two pairs of individualized cells (bottom) have the elongated spindle or stellate shapes of mesenchymal cells. Nuclei of malignant cells shown on the right are larger, more variable, and have more irregular nucleoli and chromatin.

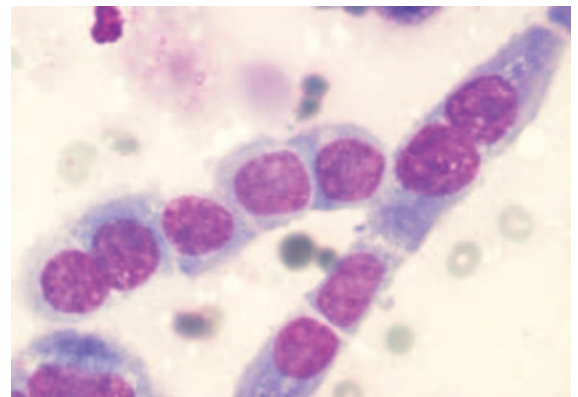


FIGURE 16-35 Canine mammary tumor aspirate smear shows a typical epithelial cell pattern. There are distinct and angular cell-cell junctions that appear as white lines between cells. Additionally, the cells look cuboidal and are in rows, giving distinct evidence that the cells are epithelial. The tumor was malignant, but the nuclei in this field are fairly uniform, about two times an RBC in diameter, and have one or two medium-sized nucleoli.

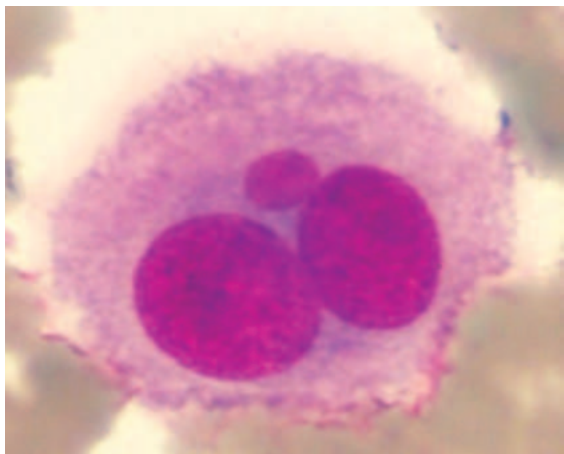


FIGURE 16-36 Mesothelial cells in fluids (abdominal, thoracic, pericardial) are often activated (reactive) and have large nuclei with anisokaryosis and variable numbers of nuclei, large nucleoli, and mitoses. They are often swollen, which makes the cells, nuclei, and nucleoli look larger. No cytologist can consistently differentiate reactive from malignant cells in many fluid preparations. These changes are often misinterpreted to indicate carcinoma or mesothelioma. This mesothelial cell has a pink fringe to indicate it is a mesothelial cell. It contains a micronucleus indicating an abnormal mitotic division of chromosomes. There was no malignant tumor in the pericardial sac of this dog.

carcinoma or mesothelioma, without other confirming evidence such as histopathology. Certain neoplasms (e.g., lymphoma) can be diagnosed consistently in fluid cytology, but not carcinoma, in which the cells are similar to reactive mesothelial cells.

NOTE: No cytologist can consistently differentiate very reactive and swollen mesothelial cells in peritoneal, pleural, or pericardial fluid from neoplastic cells (e.g., carcinoma, mesothelioma). It is a waste of time and money to send fluid cytology smears with this type of large anaplastic cell to a cytologist for a diagnosis that cannot be accurately made. No dog or cat should be killed based on only a fluid cytology report.

Cells are classified as mesenchymal (e.g., fibrous, osseous, muscle, or neural connective tissue) mainly by an elongated shape forming tail-like or conic extensions (see Figure 16-34). Two tails are found on spindle-shaped cells (see Figure 16-3). Stellate-shaped cells have three or more tails. Nuclei tend to be more oval than with epithelial cells. Mesenchymal cells tend to have more indistinct “wispy” cell margins that fade into the background (Figure 16-37). Matrix produced by the mesenchymal cells (e.g., osteoid, cartilage, collagen strands) may be found among cells (Figure 16-38).

Cytoplasmic structures suggest the specific cell type. Melanocytes usually have a fine golden-brown to black pigment. Melanin granules may be rod shaped in melanocytes or round and larger in melanophages.

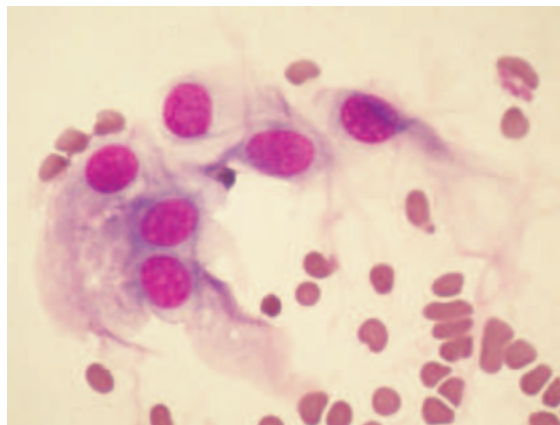


FIGURE 16-37 Canine perivascular wall tumor (hemangiopericytoma) aspirate. These mesenchymal cells have indistinct, wispy cell margins and round to oval nuclei.

Hemosiderin may be mistaken for melanin but is usually larger and may be mixed with green or yellow pigment that is more easily recognized as hemosiderin or accompanies erythrophagocytosis. Osseous cells sometimes have prominent pink cytoplasmic granules. Columnar epithelial cells may mimic spindle cells, because the point where the base of the cell pulled away from its attachment to the basement membrane often pulls out to a thin, pointed tail. The other end of the columnar cell has a flat surface with cilia to indicate its true type.

Four tumors classically placed in the round cell category are (1) lymphoma and other hematopoietic cell neoplasms, (2) mast cell tumor, (3) transmissible venereal tumor, and (4) canine histiocytoma (Figure 16-39). Most veterinarians should be able to recognize mast cell

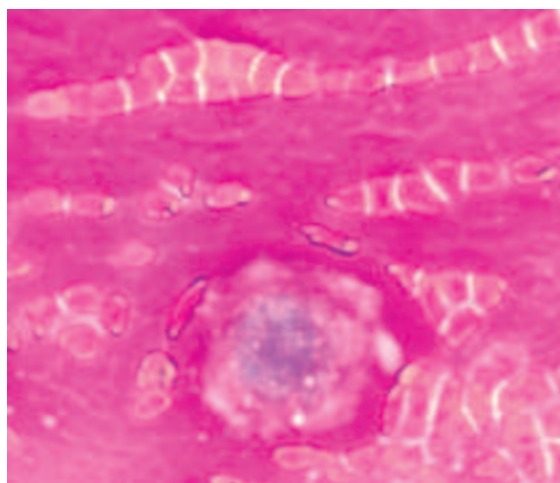


FIGURE 16-38 Canine chondrosarcoma has an abundant red matrix of cartilage in the background. Various shades of blue to purple are possible with cartilage. Note also the chondrocyte at the bottom is a round cell and not spindle shaped. The rule of thumb differentiating mesenchymal and “round” cell tumors is a good basic rule, but exceptions are common.

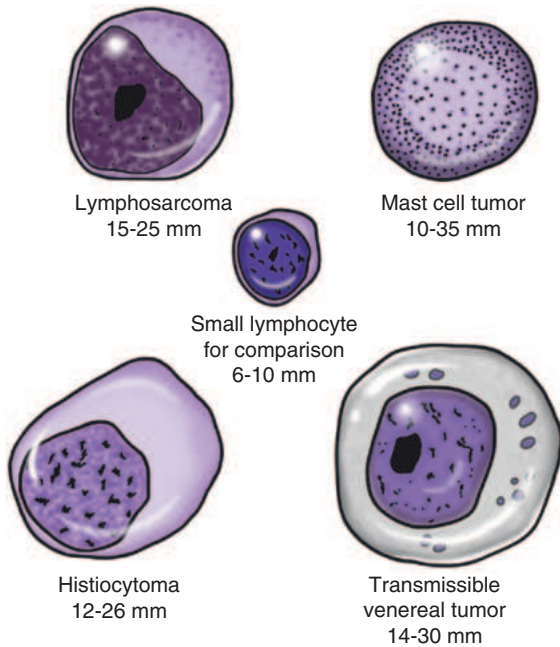


FIGURE 16-39 Classic round cell tumors. Cell characteristics of the four classic round cell tumors are illustrated with a small lymphocyte for size comparison. See text for descriptions.

tumors (at least the well-differentiated type) by cytology (Figures 16-40 and 16-41). Other neoplasms, including poorly differentiated tumors (e.g., malignant melanoma), may have mainly round cells (see Figure 16-37). “Malignant large round cell neoplasm” is a descriptive and adequate cytologic diagnosis.

NOTE: Round cell neoplasms include not only the classic four (lymphoma, mast cell tumor, transmissible venereal tumor, and cutaneous histiocytoma) but also anaplastic melanoma, plasmacytoma, anaplastic carcinoma, and others. Many neoplasms may appear as only discrete, round cells on cytologic smears.

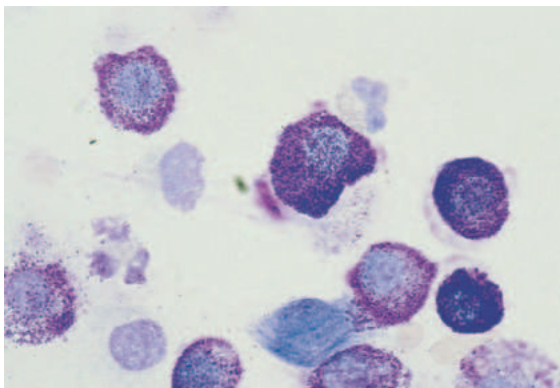


FIGURE 16-40 Canine mast cell tumor diagnosis is usually straightforward, because an aspirate usually has a large number of mast cells. Mast cells in this example are well differentiated, round cells filled with purple granules. Eosinophils are expected and support the diagnosis.

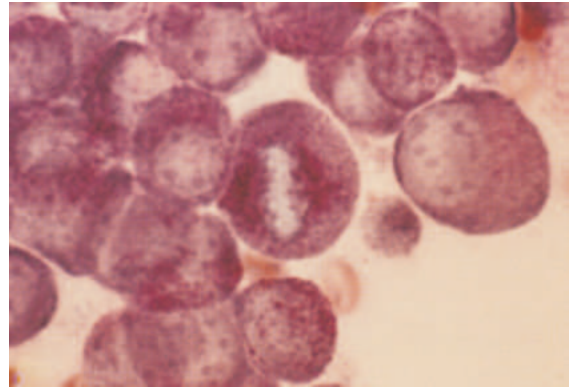


FIGURE 16-41 This canine malignant mast cell tumor should be compared to Figure 16-40. Malignant mast cells may have few to no granules and not clearly resemble mast cells. Mast cells in this photo have obvious criteria of malignancy, such as large and variably sized nuclei, variable numbers of nucleoli, and a mitotic figure.

Malignant Criteria

Cytologic diagnosis of neoplasia requires an estimate of malignancy. The type of malignant criteria present in the sample and strength of those observations (i.e., small, moderate, great) are listed in the description of the sample and summarized in the conclusion—for example, “epithelial proliferation with strong cytologic evidence of malignancy.” Calling the conclusion an “impression” or “interpretation” indicates to the veterinarian submitting the sample that cytology provides a less specific tumor diagnosis than does histopathology. Speculations on specific type of neoplasm and likely malignancy may be made under “Comments.”

Grading systems attempt to convert subjective observations into quantitative measures. A scoring system for malignancy of canine mammary neoplasm was reported.¹ One point was given for the presence of each of 10 cytologic criteria that had been previously been shown to correlate significantly with histopathologic conclusions of malignancy. A score of 0 to 3 was benign, 4 to 7 was inconclusive, and 8 to 10 was malignant. The authors required several malignant criteria, and many samples had inconclusive evidence for malignancy. This system had few false-malignant diagnoses (i.e., high positive predictive value), but the sensitivity in identifying malignancy was only 17% to 25% (i.e., low negative predictive value). One should not expect to count malignant criteria until a simple threshold number is reached. Even with rigid definitions of each malignant criterion, the mammary neoplasm scoring system had a wide inconclusive range. The number of malignant criteria noted in each case varies, because cytologists vary in what they see. A nucleus that appears large to one observer may seem normal to another. People also vary in thoroughness. Use of a numbering system is weakened if equal weight is given when a few cells barely have the change, compared with when most cells have great alterations. No single feature or group of features always proves malignancy, and the conclusion is a subjective impression. Computer

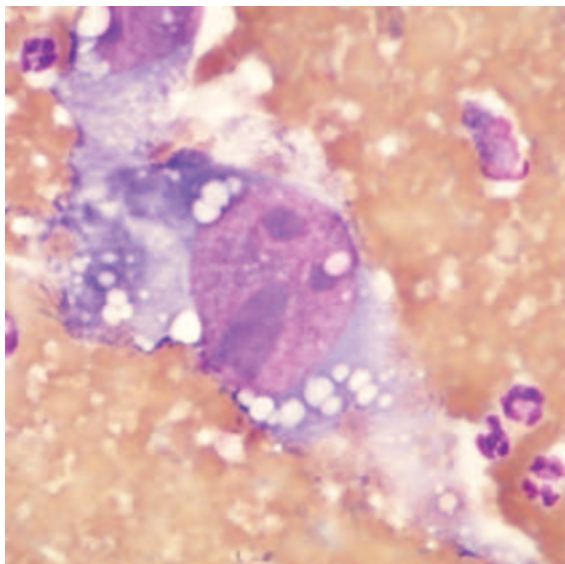


FIGURE 16-42 Large size of nuclei and nucleoli are strong evidence of malignancy. The three nucleoli in the nucleus of this hemangiosarcoma cell are as large as or larger than a single erythrocyte and vary in size. The nucleus is about four neutrophils in diameter. These changes may be exaggerated by swelling of the cell.

diagnostic programs similarly never succeeded in replacing the human mind.

Different types of neoplasms have different criteria for diagnosis of malignancy. Cytologic criteria of malignancy usually indicate whether the tumor is likely to be malignant, but there are exceptions. With canine mammary tumors, it is histological evidence of invasiveness of tumor cells that is the criterion that most closely correlates to biological tendency of the neoplasm to metastasize. With canine thyroid tumors, most are biologically malignant, but there may be little cytologic evidence of malignancy on the smears. Thus interpretation varies with tumor type.

Malignancy is best indicated by nuclear variability (Figure 16-42; see also Figures 16-2 and 16-41). Variation in nuclear size (i.e., anisokaryosis) and very large nuclear size are obvious even on poor-quality smears. Greater deviations from normal (e.g., greater increases in nuclear size and anisokaryosis) indicate greater risk for malignancy. *Anaplasia* means becoming more undifferentiated and immature. Anaplastic cells tend to have large nuclei and minimal cytoplasm, resulting in a high nuclear:cytoplasmic (N:C) ratio. Bizarre nuclear shape variation, such as with pseudopods and marked convolutions, is strong evidence of malignancy. Multinucleation in cells not normally multinucleated occurs in malignant and reactive cells. Molding of a nucleus around the nucleus of an adjacent cell is evidence that the cells grew next to each other rather than a strong criterion of malignancy.

Malignant chromatin patterns are characterized by variability in size, shape, and distribution of chromatin granules. The active proliferation of DNA occurs in the clear area of the nucleus (euchromatin), while inactive

DNA is in the clumped chromatin (heterochromatin). Thus immature nuclei appear lighter staining due to a greater area of euchromatin. Active nuclei have fine chromatin (i.e., small granules of heterochromatin) and increased clear space between chromatin (i.e., euchromatin area). A resting nucleus appears dark and has condensed chromatin (i.e., more heterochromatin). A resting nucleus is smaller and has more even chromatin distribution. Chromatin distribution, size, and shape may be strong evidence of malignancy. It includes irregular parachromatin clearing, which is an uneven area of clear space separating the chromatin granules. This plus variation in size of heterochromatin clumps gives a coarse, irregular chromatin pattern. Variation in chromatin and nucleoli is better evaluated with a stain such as NMB, Sano's trichrome, or Pap's stain than the Wright-type stains that give indistinct nuclear detail (see Figure 16-3).

Malignant cells often have prominent nucleolar variation, such as excessive variation in the number of nucleoli (i.e., varying from one to five or more), large nucleolar size (may be larger than an RBC), variation in nucleolar shape (i.e., jagged, sharply pointed, irregular nucleoli), and variation in size of nucleoli even within the same nucleus (see Figure 16-42). Nucleolar variability is strong evidence of malignancy and easily recognized on NMB-stained smears. Benign cells have more consistent numbers (e.g., one to three) of small- to moderate-sized round or indistinct nucleoli without irregular angular shapes.

Mitoses are often more plentiful in malignant neoplasms, and truly atypical mitotic figures are strong evidence of malignancy. However, mitoses are often given excessive weight as malignant criteria and normal mitoses are often incorrectly classified as atypical. Mitoses are routinely found in non-neoplastic macrophages.

Abnormal mitotic shapes can be classified into a few types (Figure 16-43). Some of these are well identified by routine cytology or histology, while some require techniques of cell biologists.¹² One should note that normal mitoses (e.g., metaphase) can be seen parallel or perpendicular to the spindle apparatus; thus the plate of chromosomes may appear as a circle (parallel) in a row (perpendicular). Normal mitoses are often incorrectly classified as atypical. Spindle multipolarity indicates that there were more than two poles (centrioles). Tripolar asymmetry is common (Figure 16-44). Chromosome lagging (i.e., one or chromosomes separated from main cluster of chromosomes in the mitotic figure) is also easy to recognize on routine cytologic samples (see Figure 16-44). Polar asymmetry indicates a division of chromosomes as seen in anaphase. This is indicated by an unequal number of chromosomes in each side of a cell, but caution is suggested, because as a cell collapses on a glass slide, uneven separation of chromosomes may be only an artifact. Errors in mitosis with uneven division of chromosomes may be seen at interphase as micronuclei (see Figure 16-36). Anaphase bridging is not commonly detected by cytology.

The appearance of the cytoplasm can indicate that the cell is well differentiated if granules, cilia, or other cell shapes (i.e., columnar) show maturity (see Figure 16-12). But cytoplasmic changes are weak indicators of malignancy. A basophilic cytoplasm indicates active protein

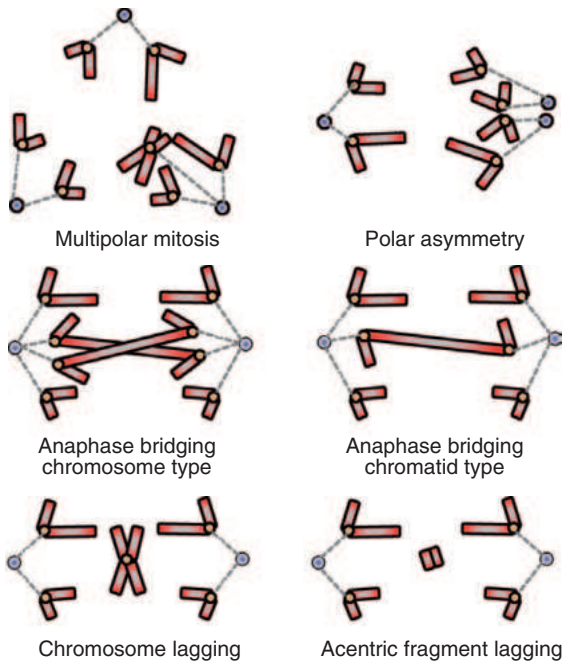


FIGURE 16-43 Correct, consistent classification of atypical mitoses is aided by a classification suggested by Gisselsson.¹²

synthesis and abundant RNA. A large nucleolus also indicates active RNA synthesis and thus protein synthesis. Protein synthesis is often high in malignant cells but also in active non-neoplastic cells (e.g., hyperplasia, reactive change). Normal hepatocytes, for example, have large nucleoli reflecting active production of messenger RNA (mRNA) and protein synthesis. Malignancy of a mast cell tumor may be suggested by variability in the number, size, and distribution of cytoplasmic granules (see Figures 16-40 and 16-41). Degenerative changes mimic

malignant changes. Nuclei and nucleoli swell with cell damage and degeneration (see Figures 16-2 and 16-42), and the larger nuclear size, light nuclear color, and nuclear prominence make the damaged cell appear malignant. One should not evaluate cells with broken cytoplasmic boundaries or with partial leakage of nuclear material out of the nucleus! Even apparently intact cells may be swollen and degenerating. The best indication of cell death is chromatin that has lost its granular, stippled appearance on a Wright stain and appears streaked or smudged. The degeneration is less obvious on NMB-stained cells, so it is easier to mistake the degeneration for malignancy. Cells degenerate quickly in certain fluids such as unbuffered saline and urine. Cells collected directly from bronchial brushings, for example, have much better cell detail than do cells collected with a saline flush. Cells degenerate within minutes in regular saline, which is acidic (i.e., pH 6) and lacks inorganic ions and glucose for cellular metabolism. Hanks' balanced salt solution (HBSS) preserves cell morphology and is a better fluid to retrieve cells when cellular detail is critical (e.g., neoplastic diagnosis). The cost of HBSS prevents its routine use in veterinary medicine. Mediocre cellular detail is adequate for evaluation of exudates and sepsis but not for diagnosis of neoplasms. To prevent excessive cell degeneration, one should make direct smears as quickly as possible and avoid prolonged cell storage in fluids. Adding protein, such as 5% to 10% bovine serum albumin solution, or several drops of serum or plasma to a fluid (e.g., BAL) before centrifugation (e.g., cytocentrifuge) minimizes cell degeneration.

NOTE: Degenerative changes in cells from necrotic areas, in old samples, or during cell processing mimic malignant changes. Debris may also resemble infectious agents.

Selected Cytologic Diagnoses

Mammary Neoplasms

The most specific evidence of biologic malignancy of canine mammary tumors is histologic evidence of invasion of tumor cells into normal tissues. About half of canine mammary tumors may look malignant based on cellular changes and are called carcinomas by many pathologists. However, only half of those "carcinomas" (about one quarter of all mammary tumors) will have metastasis and are biologically malignant. Histologic tumor invasion is the criterion that identifies those truly likely to metastasize. Cytologic evaluation of mammary masses still is requested to plan surgical removal despite inability to demonstrate invasiveness. Draining lymph nodes should be checked cytologically for metastasis. Infiltration of epithelial cells into lymph nodes smears gives convincing proof of malignancy and metastasis, whereas canine mammary tumor cytology gives more equivocal proof of malignancy.

Ten cytologic criteria were significantly correlated to histopathologic conclusion of malignancy.¹ It is well worth describing this study because the criteria of malignancy hold true for cytology of neoplasia in general. Cytologic evaluation was performed on modified Sano's

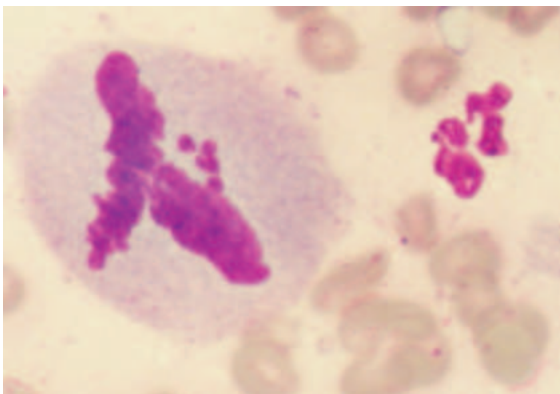


FIGURE 16-44 The atypical mitosis on the left shows two types of atypia. It is a multipolar (tripolar in this case) mitosis with three plates of chromosomes. There are also four lagging chromosomes (center right of cell) separate from the main plates of chromosomes.

trichrome stain of smears fixed in 95% ethanol while still wet. The malignant criteria were (1) variable nuclear size, (2) giant nuclei (more than twice normal), (3) distortion of nuclear or cytoplasmic membranes, (4) high N:C ratio (i.e., >1:2), (5) irregular chromatin shapes, (6) variable chromatin size, (7) parachromatin clearing (i.e., a discrete pale area in a more dense chromatin pattern), (8) variable nucleolar number (i.e., >3), (9) abnormal nucleolar shape, and (10) macronucleoli (more than two times normal). Abnormal nucleolar shape was defined as "not round or oval." Nuclear membrane distortion was indentation of nuclear shape by a cytoplasmic organelle, such as a vacuole, in contrast with nuclear molding, which was the molding of a nucleus about another cell's nucleus without cell crowding.

Abnormal mitotic figures (e.g., spindle multipolarity, tripolar) always denoted malignancy but were rare. Neither nuclear molding, irregularly thick nuclear margins, cellularity of the smear, poor intercellular cohesion, nor multinucleation allowed differentiation between benign and malignant neoplasms. Fine-needle aspirates of mammary tumors were more cellular and had better tissue architecture than impression smears, scrapings, or nipple secretions. Spindle cells on smears did not differentiate simple from complex or mixed tumors. In another survey, only 8 of 19 histologically confirmed mammary carcinomas were identified as such by fine-needle cytology.⁷

NOTE: Mammary tumor cytology is insensitive and may be misleading; therefore histopathology should be required for determination of malignancy.

Perianal Gland Tumor

Cells of perianal gland tumors (i.e., hepatoid tumor) closely resemble hepatocytes, with a polyhedral shape and abundant cytoplasm. Round nuclei have one or two prominent nucleoli. The cytoplasm has characteristic granularity with Wright stain. Cell morphology and obtaining them from a mass near the anus allows a consistently correct tumor type diagnosis. Cytologic appearance may not reflect biological malignancy, because benign-appearing cells may be found at metastatic sites (e.g., lung). Metastasis is fortunately rare, but cytologic diagnosis should not include a conclusion of adenoma versus carcinoma.

NOTE: Malignancy of perianal gland neoplasms (like canine mammary tumors) is best determined by histologic evidence of vascular invasion. Cytology consistently identifies the tumor as of the perianal gland (i.e., hepatoid) but may not identify the degree of malignancy.

Lipoma

Lipoma diagnosis by cytology is rapid and simple, and so should be routinely done by veterinarians. Diagnosis does not require an experienced cytologist. Smears grossly have clear droplets of lipid that do not dry prior to staining. Staining with Sudan's or another fat stain is

unnecessary. The smears may lack cells or have a variable number of adipocytes singly or in tissue fragments. Adipocytes are large cells with a small dark nucleus on one edge. The thin cell membrane may appear wrinkled as cells shrink when alcohol in Wright stain removes intracellular lipid. Mature fat has the same appearance as a lipoma. Lipomas may be traumatized, hemorrhagic, fibrotic, or inflamed. Infiltration by macrophages, other inflammatory cells, and fibrosis may confuse diagnosis. Infiltrative lipoma has mature-appearing adipocytes, but these appear aggressive in infiltration of muscle or other tissues. This infiltration of adipocytes is better appreciated with histopathology, but fat cells tightly bound to muscle fibers may be found in cytologic samples.

Mast Cell Neoplasms

Well-differentiated mast cell neoplasms are consistently well identified by veterinarians. Aspirates usually have a moderate to large population of typical mast cells with a variable number of eosinophils from a canine tumor. The round, distinctly granular cells of more differentiated tumors are easily identified on Wright stain (see [Figures 16-40 and 16-41](#)). Some water-based, modified Wright stains may fail to stain mast cell granules. Alcohol-based stains color mast cell and basophil granules better. Focal dermatitis with mast cell hyperplasia occurs; therefore small numbers of mast cells are not diagnostic for mast cell tumors.

Histologic grading of canine cutaneous mast cell tumors has prognostic value. Classification is from grade 1 (i.e., well-differentiated, round monomorphic cells with no mitotic activity and round nuclei with condensed chromatin) to grade 2 (i.e., intermediate) to grade 3 (high grade malignancy). Grade 3 has pleomorphic cells with irregularly shaped cells, more open [more euchromatin] nuclei with one or more prominent nucleoli, binucleation, frequent mitotic figures, and cytoplasmic granules that were indistinct, fine, or not obvious). Differentiation between grades 1 and 2 is indistinct, and histopathologists tend toward classification as grade 2. Cytologically, cutaneous mast cell tumors are subclassified by degree of differentiation. Some nuclear criteria of malignancy may be hard to identify on Wright-stained cytologic smears, because the nuclei may be obscured by granules and often stain poorly owing to the mast cell's heparin content. NMB is better for nuclear detail. Cytoplasmic characteristics of malignancy in dogs include variation in the size, density, and prominence of the granules. Malignant cells have fewer and finer granules (see [Figure 16-41](#)). Granules may polarize to one end of malignant cells. Anaplastic cells are larger (i.e., 12 to 35 μm instead of the normal 10 to 20 μm), have larger nuclei, prominent nucleoli, more euchromatin (more open nucleus), mitotic figures, and binucleation.⁶ Anaplastic, large, poorly granular mast cells with nuclear criteria of malignancy clearly indicate malignancy, but some well-differentiated mast cell tumors have metastatic potential. All mast cell tumors should be regarded as potentially malignant. Free collagen bundles and large immature mesenchymal cells are often seen in mast cell tumor cytology samples and are evidence of necrobiosis and part of mast cell tumors and not a concurrent sarcoma.

Solitary feline cutaneous mast cell tumors without splenic involvement are usually benign. Survival in a small survey of 14 cats did not correlate with a grading system similar to that used in dogs.³ In histiocytic-type cutaneous mast cell tumors in young Siamese cats, granules may be difficult to find (see discussion of mastocytosis in Chapter 4). Anaplastic mast cell tumors do occur in cats and can have metastasis.

Histiocytoma

Canine histiocytoma cytologic diagnosis may be difficult and uncertain. Cells may exfoliate poorly, and secondary inflammation may occur. Cells are not-so-unique round cells with round nuclei with a moderate amount of fairly clear cytoplasm. Many other cell types will look the same. Cells are 12 to 26 μm and have distinct cell boundaries. They occasionally have indented nuclei without apparent nucleoli.⁶ Chromatin is fine, and mitotic activity is variable. Cytoplasm on Wright stain is pale blue. The N:C ratio varies and is usually 1:1. Human pathologists unfamiliar with this benign tumor often consider it malignant. Having a history of a dome-shaped (button-shaped tumor), hairless skin tumor in a young dog helps the diagnosis. Lymphoma or other histiocytic tumors and even focal mononuclear cell dermatitis may be confused with a canine cutaneous histiocytoma, so caution is advised if the patient is older than 5 years, though older dogs may have histiocytoma. Cutaneous canine plasmacytoma is a round cell tumor of older dogs. Seeing cells with double nuclei and eccentrically placed nuclei suggests plasmacytoma.

Transmissible Venereal Tumor

Transmissible venereal tumor is a round cell tumor with discrete cells that may show moderate to marked variation in cell size. The round cells are 14 to 30 μm in diameter. Round to oval nuclei have a prominent nucleolus and linear, cordlike chromatin. The cytoplasm is a pale hyaline blue with distinct vacuoles.⁶ Mitotic activity is common. Location of the mass on the body (i.e., genital area, mouth), plus the patient being from an endemic area, supports the diagnosis.

Follicle or Epidermal Inclusion Cyst

Epidermal inclusion (follicle) cysts are common skin lumps in dogs and should be diagnosed frequently by cytologists. Contents of the cyst mainly consist of an accumulation of squames in a cystic hair follicle. Diagnosis is made by finding large numbers of squames (often in large accumulations) and keratinized debris on aspiration smears. Squames are very mature, thin, non-nucleated cells from the surface of stratified squamous epithelium. (Note: Occasional squames are common contaminants on smears, mainly from fingerprints.) Other debris, including cholesterol crystals, may be present in the cysts. The cysts often rupture, and keratin is highly irritating, so a strong pyogranulomatous inflammatory response is also typical. Rupture and inflammation often cause a rapid increase in size of a skin lump, which may alarm the owners, and inflammation irritates the dog. The primary lesion is shown to be a follicular cyst by finding moderate numbers of squames often surrounded by macrophages. Epithelial cells can contain abundant melanin,

but this does not indicate the lump is a melanoma. Furunculosis looks very similar due to rupture of hair follicles and release of keratin into the dermis.

NOTE: An epidermal inclusion cyst is a common skin mass easily diagnosed by finding large numbers of anuclear squames and often cholesterol crystals in aspirates. These often have strong pyogranulomatous inflammation when they rupture.

Hematoma and Seroma

Hematomas are fluctuant subcutaneous masses containing fluid with a variable amount of blood in variable states of degeneration. Seroma is similar to a hematoma but has more fluid than blood. Evidence for both is aspiration of a variably bloody fluid with evidence of erythrophagocytosis or hemosiderin-laden macrophages (see Figure 16-14). Blood pigments are granular or crystalline pigments that have various colors (i.e., blue, green, black, gold, yellow). Artifactual bleeding due to sample collection is common, but fresh blood often includes platelets.

Hepatic Cytology

Hepatocytes normally have abundant granular cytoplasm and a round nucleus with a prominent nucleolus. Binucleation (i.e., diploid) is normal in a minority of hepatocytes. Increased numbers of binucleated hepatocytes may suggest hyperplasia. Common alterations include vacuolar degeneration (e.g., fatty liver, glucocorticoid hepatopathy), inflammation (e.g., lymphocytic plasmacytic hepatitis), cholestasis, and neoplasia. Cytology may identify these processes, though histopathology better identifies the presence, location, and amount of inflammation (i.e., cholangiohepatitis). When well-outlined, variably sized hepatocytic vacuoles are visible, fatty change is expected. Neutral fat in the vacuoles can be proven using Sudan's stain. Canine glucocorticoid hepatopathy has accumulation of glycogen and not fat. Glycogen accumulation appears as light pink, cytoplasmic clearing instead of distinct vacuoles. Cholestasis is indicated by swollen, green bile canaliculi between hepatocytes. The dark blue-green granular pigment commonly seen within hepatocytes is lipofuscin (old age) pigment and of no diagnostic significance. Light blue-green, refractile granules suggest copper accumulation, which occurs in the breed-associated copper hepatopathies and secondary to cholestasis (Figure 16-45).

Inflammation may be difficult to prove cytologically, because the number of leukocytes in the liver in hepatitis or cholangiohepatitis is often few. There is usually blood with a hepatic aspirate, and neutrophils may tend to pool in some capillary beds. If only a few neutrophils or lymphocytes are on a hemodiluted smear, it is hard to determine whether leukocytes are from peripheral blood contamination or a mild inflammatory infiltrate (e.g., cholangiohepatitis). The capillary collection (not aspiration) technique or packing technique described earlier are recommended to harvest hepatocytes with minimal hemodilution, so the presence of leukocytes better reflects

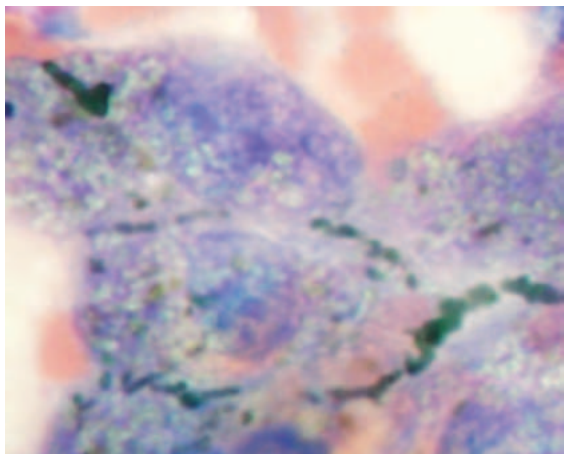


FIGURE 16-45 Canine copper hepatopathy is indicated by the refractile, light blue-green granules in hepatocytes. The focus of the photo is on the granules and not hepatocyte nuclei. There are also several swollen biliary canaliculi that look like green tubes with small cylinders or droplets of inspissated, green bile between hepatocytes.

a hepatitis. Leukocytes and collagen found within hepatic tissue fragments also support inflammation. Because plasma cells and vacuolated macrophages are not found in peripheral blood (and do not contaminate a sample by hemodilution), they are more consistent indicators of inflammation than are neutrophils and lymphocytes. Extramedullary hematopoiesis is indicated by nucleated RBCs and megakaryocytes. Neoplasia is diagnosed by previously described criteria though hepatoma samples may have mainly hepatocytes without cytologic criteria of malignancy.

Lymph Node Cytology and Lymphoma

Aspiration cytology is the preferred test to evaluate enlarged lymph nodes. Cytology may provide evidence of metastasis and other disorders even in normal-sized nodes. Because lymphoid cells are fragile and large lymph nodes are often necrotic, many smears are not diagnostic because inadequate numbers of intact cells are present. Very gentle smearing techniques such as using a coverslip or drawing out fluid in the “starfish” method must be learned to make diagnostic smears. A differential lymphoid cell count should be performed in a thin area with good cell morphologic detail. This forces the cytologist to identify each cell and therefore not risk reporting a conclusion if at least 100 intact cells can be not identified. Other areas and smears should be examined to ensure that the differential count is representative. It is typical that lymphoid cells in some areas or smears may be swollen and thus appear more immature, while in other smears or areas lymphocytes stain more typically and may appear normal. A representative differential lymphoid cell count (Diff) allows various conclusions and description of different patterns.

Lymphoid cells in a normal lymph node are about 2% to 15% lymphoblasts and 60% to 90% small- to medium-sized lymphocytes. Plasma cells are infrequent

(i.e., 0% to 3%) except in some commonly reactive nodes (e.g., mesenteric, submandibular). There are obviously more than three types of lymphoid cells in a lymph node, but this three-cell lymphoid cell (lymphoblast, plasma cell, lymphocyte) Diff quickly and consistently describes the maturity and diversity of the lymphoid cells. “Lymphoblast” in this hematology-type definition is a cell that looks larger and clearly more immature than the lymphocytes. A prominent nucleolus (or nucleoli) clearly identifies a hematopoietic cell as a blast. Lymphoblasts are as large as or larger than neutrophils. Nuclei are larger (>1.5 times an erythrocyte in diameter) and have finer, smaller chromatin granules, more euchromatin, and thus a lighter, more open-appearing nucleus. In contrast, small lymphocytes are just larger than an erythrocyte but smaller than a neutrophil. The lymphocyte nucleus has coarser and darker chromatin (more heterochromatin) and no visible nucleolus (see Figure 16-16). Medium-sized lymphocytes are grouped with small lymphocytes as well-differentiated (resting) cells if they have a mature, coarse chromatin pattern and no visible nucleolus. With reactive lymph nodes and some types of lymphoma, there is a predominance of more medium-sized lymphoid cells or no clear division among small, medium, and large cells. Neutrophils are fewer than 5%, with only occasional macrophages, mast cells, and eosinophils.

Lymph node cytology is routinely used for diagnosis of lymphoma (i.e., lymphosarcoma [LSA]) (see Chapter 4). Complete lymphoma diagnosis should include methods to identify 15 to 30 types of lymphoma in the dog.¹³ For the following discussion, a greatly simplified system where lymphoid cells are divided into lymphocytes, plasma cells, and lymphoblasts is used. A predominance of lymphoblasts or monomorphic population of one type of lymphoid cell is the major diagnostic criterion of lymphoma. Lymphoblasts greater than 50% of cells is a common criterion for diagnosis of lymphoma. Confidence in a diagnosis of lymphoma increases with the percentage of large immature lymphoid cells and how distinctly the cells appear clearly immature on the smear. The most common lymphoma¹³ is the diffuse large B-cell lymphoma, which is easy to diagnose on cytology or histology. It has a predominance of large pleomorphic lymphoblasts with one or more large and prominent nucleoli. There are frequent mitoses and many tingible body macrophages, indicating rapid cell proliferation and death.

Fewer than 50% lymphoblasts do not exclude other forms of lymphoma. Several forms of lymphoma have small to medium lymphoid cells that lack prominent nucleoli and that are more difficult to diagnose cytologically. Some examples are pleomorphic small T-cell lymphoma, mycosis fungoides, pleomorphic medium to large T-cell lymphoma, and chronic lymphocytic leukemia.¹¹ Immunophenotyping aids in classification and prognosis of lymphomas. A prominent population of lymphoid cells of one type may suggest neoplasia. Atypical morphology, other than immaturity, is not a consistent or sensitive indicator of lymphoma. When present, features such as bizarre nuclear and cytoplasmic shapes (e.g., “hand mirror cells” with cytoplasmic pseudopods) or unusual chromatin patterns add to one’s confidence in a diagnosis of lymphoma.

The reader should note that the term “lymphoblast” in the previous description refers to a large, immature lymphoid cell more along hematologic classification of hematologic blast cells. In the updated Kiel classification of lymphoblastic lymphoma, cells classified as lymphoblasts do not have a prominent nucleolus.¹¹ Thus *lymphoblast* refers to another cell type (and type of lymphoma) to those specializing in lymphoma diagnosis.

Frequent necrosis in lymph nodes with lymphoma can cause aspiration of mainly loose necrotic debris. A thick background of excessive cell debris on aspirate and impression smears interferes with staining. One may find only a small percentage of intact and well-stained cells around the thin perimeter of the smear. Lysed cells (i.e., “naked” nuclei) and partially lysed normal cells have swelling of the nucleus and nucleolus. This creates a larger, lighter-stained nucleus with a prominent nucleolus resembling a lymphoblast. Failure to ignore these damaged cells leads to an erroneously high percentage of lymphoblasts and perhaps an incorrect conclusion of lymphoma. One should not look at damaged cells. Repeated aspiration and very careful streaking methods may be required to obtain a diagnostic sample.

Chronic lymphocytic leukemia (CLL) is discussed in Chapter 4 as a hematologic diagnosis. Cytologic diagnosis of CLL is difficult because there is a predominance of lymphocytes with mature chromatin patterns. A prominent lymphocytosis in the blood aids diagnosis. CLL should be considered as a cause of lymphadenopathy if a monotonous lymphoid population of small to medium-sized lymphocytes is present. The lymphocytes in some types of CLL have moderate to abundant cytoplasm and are very consistent in appearance. CLL and small cell lymphoma may be diagnosed by additional methods.

Classification of lymph nodes as reactive and hyperplastic means essentially the same thing. Both are a benign, expected response to some irritant in their drainage field (e.g., Demodex). A hyperplastic lymph node has an increased number of lymphoid cells in normal percentages, but the node is larger in size, owing to an immunologic stimulus. If there is some hemorrhage with hemosiderin-laden macrophages or a slight increase in neutrophils and eosinophils, or lymphoblasts, the diagnosis tends to be reactive lymph node and not just hyperplasia.

Highly reactive lymph nodes may have an increased percentage (e.g., 15% to 30%) of lymphoblasts (and large to medium lymphoid cells), or some lymphoblasts may be so large that it might suggest lymphoma. Heterogeneity of the lymphoid population with variation in size and maturity of lymphoid cells and often abundant plasma cells, however, still indicates more likely a reactive node instead of lymphoma.

Greater than 10% neutrophils, 5% macrophages or greater than 3% to 5% eosinophils indicates lymphadenitis. Macrophages may appear in small aggregates and contain material such as hemosiderin. These are minimal numbers and much greater numbers of neutrophils and macrophages will be present in an abscessed lymph node or systemic granulomatous disease. The predominant type of WBC determines the classification of inflammation (i.e., neutrophilic, granulomatous,

pyogranulomatous, eosinophilic). Myeloid leukemia infiltration occurs rarely, and the diagnosis is based on maturity of the WBCs. Chronic granulocytic leukemia is strongly supported when a prominent granulocytosis in the blood is accompanied by a lymph node aspirate that looks like bone marrow with mixed granulopoiesis with mature neutrophils but extension back to progranulocytes and myeloblasts.

Metastatic neoplasia is diagnosed if adequate numbers of malignant-appearing nonlymphoid cells are present. A few large anaplastic cells can occur in non-neoplastic reactive nodes; unless cells are very distinctive (e.g., forming definite epithelial patterns), a confident diagnosis requires many cells (e.g., 50 to 100). Occasional mast cells are normal in lymph nodes, and lymph nodes may have mast cell hyperplasia. Hence many mast cells or very atypical mast cells must be found to diagnose mast cell neoplasia. Macrophages with hemosiderin mimic melanocytes, so one should be careful diagnosing metastatic melanoma. Inflamed lymph nodes may be fibrotic; therefore one must accept a few fibroblasts without diagnosing sarcoma. In contrast, only a few cells may indicate metastasis if they are foreign to the lymph node, such as stratified squamous epithelial cells, perianal gland cells, and mucin-secreting epithelial cells.

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Laboratory Diagnostic Toxicology

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PRINCIPLES OF TOXICOLOGIC DIAGNOSIS

Toxicologic diagnosis is based on four principal types of evidence: an accurate and thorough history, evaluation of clinical signs and physical examination, prudent use of clinical pathology laboratory tests and/or gross/histologic evaluation of lesions, and appropriate use of specific toxicologic tests. Each of these is important, and a diagnosis is almost never obtained from only one of these four pillars of diagnosis. This chapter will focus on appropriate laboratory testing.

Choosing appropriate specimens and laboratory tests almost always requires attention to a thorough history of actual or potential exposures. This requires effective questioning of owners or caretakers, some knowledge of the animal environment and care, and a reasonable account of the time course of a potential toxicosis. Important historic findings include changes in location or in food source, recent chemical applications (e.g., spraying for insects, fertilizing a lawn, changing radiator coolant), administration of medications, whether the animal is free to roam, and distance to public or commercial areas.

In most cases of acute poisoning, a combination of history and clinical findings must be used to initiate life support and effective detoxification of the animal. One can rarely wait for results of a laboratory chemical test. Prompt clinical laboratory testing (complete blood count [CBC], serum clinical chemistry, blood gas analysis, electrolytes, and urinalysis) can quickly detect disturbances in major metabolic pathways or specific organ damage. Such information can also be correlated with known laboratory effects from specific toxicants.

To assist with clinical observations and help determine organ systems affected, [Tables 17-1 through 17-6](#) show probable toxicoses associated with different organ systems and clinical signs (e.g., seizures, hepatic failure, vomiting); however, very few toxicoses have pathognomonic signs. While many signs of poisoning (e.g., vomiting, seizures) are also caused by infectious, metabolic, and endocrine diseases, knowledge of high-risk toxicology differentials is the first step in working through the diagnostic process. A thorough history and physical

examination should be completed to determine potential exposure to toxicants. Telephone instructions should include having the owners collect any potential baits, vomitus, or other suspect materials for possible chemical analysis.

CLINICAL LABORATORY TESTS

After clinical data are obtained from history and physical examination, laboratory testing is used to identify and characterize pathophysiologic effects typical of specific toxicants (e.g., basophilic stippling and nucleated red blood cells [RBCs] without reticulocytosis suggest lead toxicosis; high serum osmolality, hypocalcemia, and an increased anion gap are consistent with ethylene glycol [EG] toxicosis). [Table 17-7](#) summarizes clinical laboratory tests that may be altered by specific toxicoses. Several clinical laboratory parameters may be altered by some toxicants, although others may cause no significant changes.

A major use of the toxicology laboratory is to determine the presence and possibly the amount of a specific toxicant. Two levels of toxicant testing are suggested by various authorities. The first is clinical evaluation (as in the previous paragraph). These include blood gases, electrolytes, glucose, blood urea nitrogen (BUN), creatinine, hepatic function tests, anion gap, osmolality, and renal function. The second level is specific drug or toxicant assays.⁶ After clinical laboratory evaluation, clinicians may utilize some form of “screening” test for rapid turnaround to suggest potential toxicant groups. Depending on results of screening procedures, a specific confirmatory instrumental or immunologic procedure may be chosen to support the initial diagnosis. These are often most important when there is potential product liability or a need for forensic evidence (as in suspected malicious poisoning). Quantification of a positive test may be important for interpretation of some toxicoses, but not for others. For example, any amount of strychnine in stomach contents is considered diagnostic of a toxicosis while other toxicant diagnoses depend on quantitation (e.g., lead) above a threshold to establish toxicosis.

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TABLE 17-1. COMMON TOXICANTS OF THE NERVOUS SYSTEM BY MAJOR EFFECT(S)

TOXICANT	COMMON SOURCES	ADDITIONAL SIGNS
EXCITATION		
Aminopyridine	Bird control baits.	Tremors, ataxia, may be hyperexcitable.
Caffeine, other methylxanthine alkaloids	Chocolate, coffee, tea; stimulant or anti-drowsiness pills sold OTC.	Excitement, exaggerated reflexes, hyperreflexia; cardiac arrhythmia, hyperpnea, vomiting. Alkaloids detectable in GI contents, blood, or urine.
Cyanide	Cyanogenic plants (apple, peach, cherry seeds); fumigants; rodenticides (rarely); industrial chemicals.	Excitement, agitation, seizures progressing to ataxia, weakness, and collapse; also salivation, hyperpnea, pink mucous membranes. Very rapid course. Test stomach contents, blood for cyanide, or liver and muscle from dead animals.
Lead	Paint (pre-1970) or specialty paints, lead objects (drapery weights, fishing sinkers).	Intermittent vomiting and behavioral changes; tremors, ataxia, seizures, altered vision, anorexia.
Metaldehyde	Snail and slug baits; solid fuels sold OTC.	Continuous tremors, occasional seizures; incoordination and cerebellar ataxia; nystagmus, salivation also prominent.
Pyrethrins, pyrethroids	Active ingredients in insecticides for pets and homes.	Tremors, hyperexcitability, excitement, occasional seizures; sometimes alternate with depression.
Strychnine	Mole and gopher baits, usually less than 0.05% for below-ground use.	Acute onset of hyperesthesia, hyperreflexia progressing to tetanic seizures exacerbated by external stimuli; rapid shallow respirations, tachycardia.
Tremorgenic mycotoxins	Penitrem A on moldy walnuts, other nut products; less commonly, spoiled dairy products.	Tremors, ataxia, and hypermetria are characteristic and may be continuous; exacerbated by exercise or stress. Test stomach contents or suspect materials for penitrem A or other tremorgens. Available laboratory testing is limited.
Zinc phosphide	Used as alternative to anticoagulants; sold OTC in some states. Bait has mild garlic or acetylene odor.	Excitement, stimulation, and seizures; may include tremors and "running fits." Alternative sign may be depression. Vomiting, colic often accompany the neurologic signs.
DEPRESSION OR COMA		
Alcohols	Beverages, disinfectants, brewery residues.	Depression, disorientation early, followed by coma, respiratory depression, acidosis, cardiac arrest. Alcohol residues appear in blood and urine.
Barbiturates	Access to tablets or capsules; occasionally in meat from animals euthanatized with barbiturates.	Depression, loss of reflexes, hypothermia, hypotension, coma, respiratory failure. Barbiturates in blood or urine of living animals; liver or kidney of dead animals.
Bromethalin	Relatively new rodenticide, often used as an alternative to anticoagulant rodenticides.	Signs may include posterior ataxia and weakness, sometimes vomiting resulting from cerebral edema. Available laboratory testing is limited.
Citrus oils	Citrus extracts used in insecticides and repellents (e.g., D-limonene). Most hazardous to cats.	Depression, ataxia, coma, and pronounced hypothermia. Citrus extracts are conjugated in the liver and excreted in urine. Few laboratories offer routine testing for these products.
Carbon monoxide	See Table 17-5 .	See Table 17-5 .
Ethylene glycol	Automobile radiator antifreeze solutions. Also used to prevent freezing in water pipes of vacant houses or mobile homes.	Profound depression early is due to alcohol-like inebriation; later signs of depression are due to severe metabolic acidosis.
Hydrocarbons (aliphatic and aromatic)	Petroleum distillates and pine oil compounds; most available in paint thinners, mineral spirits, cleaning solutions; exposed by spilling on skin, footpads, or by inhalation of fumes.	Generally there is initial depression and ataxia similar to ethanol intoxication. High dosage leads to vomiting, possible liver and/or kidney damage, severe depression, coma, respiratory depression, and death.

Continued

TABLE 17-1. COMMON TOXICANTS OF THE NERVOUS SYSTEM BY MAJOR EFFECT(S)—CONT'D

TOXICANT	COMMON SOURCES	ADDITIONAL SIGNS
SIGNS OF PARASYMPATHETIC STIMULATION		
Blue-green algae	Blue-green algae (<i>Anabaena</i> spp. and <i>Microcystis</i> spp.) are most prevalent in lakes and farm ponds during late summer.	In addition to sudden death (neuromuscular paralysis) and hepatotoxicity, blue-green algae may inhibit cholinesterase, causing signs of salivation, vomiting, tremors, and dyspnea. Cholinesterase inhibitor testing requires whole blood sample. Water samples for blue-green algae should be submitted fresh and fixed (addition of 1 part formalin/9 parts water).
Ivermectin, milbemycin, moxidectin	Anthelmintics, ectoparasite control, both small animal and large animal (higher concentration products).	Ataxia, hyperesthesia, salivation, vomiting, bradycardia, recumbence. See <i>MDR1</i> gene general description in text.
Opioids	Prescription or recreational drugs.	Miosis, salivation, arrhythmia, vomiting, defecation, urination. Urine samples may test positive.
Organophosphate and carbamate insecticides	Home and garden pest control; limited use for small animal ectoparasite control.	Salivation, lacrimation, miosis, vomiting, diarrhea, bradycardia, dyspnea, urination, ataxia and muscle weakness. Whole blood sample for confirmation of cholinesterase inhibition.
Nicotine	Tobacco products, specialty insecticides (e.g., nicotine sulfate). Also used occasionally in capture guns.	Initial signs are due to early depolarization (tremors, excitement, lacrimation, vomiting), followed by paresis, ataxia, and complete collapse with death from respiratory paralysis. Nicotine alkaloid is readily detected in stomach contents, blood, or urine.
SIGNS OF PARASYMPATHOLYTIC AGENTS		
Amphetamines	Prescription or recreational drugs, including methamphetamine.	Mydriasis, tachycardia, but lack other predominant effects of anticholinergics.
Atropine	Plant, <i>Atropa belladonna</i> , may be grown as ornamental in gardens.	Causes dry mucous membranes, mydriasis, tachypnea, tachycardia, hyperthermia, disorientation, visual dysfunction, GI stasis. Readily detected in blood and/or urine.
Ephedra/ma huang	Herbal product for weight loss, decongestant, recreational.	Mydriasis, tremors, seizures, hyperthermia, salivation, death.
Scopolamine	<i>Hyoscyamus niger</i> and <i>Datura</i> spp. are common plant sources. Also available as pharmaceutical.	Signs and effects are similar to those with atropine (above).
SSRIs (selective serotonin reuptake inhibitors)	Prescription drugs. Veterinary use for urine spraying, lick granuloma, separation anxiety.	Mydriasis, autonomic instability, tremors, seizures, bradycardia, vomiting, diarrhea, colic.
ATAXIA, INCOORDINATION, WEAKNESS, OR PARALYSIS		
Aminoglycoside antibiotics	Kanamycin, neomycin, streptomycin, gentamicin.	Effects are due to post-synaptic receptor blockade of neuromuscular junctions, results in paresis, paralysis, and death from respiratory failure. Testing of blood and/or urine may establish exposure.
Botulism	<i>Clostridium botulinum</i> growth in decaying organic matter, especially with high protein content.	Lower motor neuron paralysis results in muscle weakness, difficult deglutition, progressive paresis and paralysis, mydriasis, dysphagia. Suspect material from stomach contents may be cultured or injected in mice as a bioassay, followed by protection tests with antitoxin.
Cholinesterase inhibitors	See <i>Signs of parasympathetic stimulation</i> .	Test for confirmation of cholinesterase inhibitors as described above for organophosphate and carbamate insecticides in <i>Signs of parasympathetic stimulation</i> .
Ivermectin, milbemycin	See <i>Signs of parasympathetic stimulation</i> .	
Mycotoxins, tremorgenic	Moldy refrigerator foods (e.g., cream cheese, walnuts, peanuts, high-protein products).	Vomiting, diarrhea, agitation, tremors, hyperthermia, tachycardia, pigmenturia from rhabdomyolysis.
MODIFICATION OF BEHAVIOR		
Atropine/scopolamine	See <i>Signs of parasympatholytic agents</i> .	Behavioral effects of disorientation and possible loss of vision appear secondary to overdose of atropine. Diagnosis of atropine overdose is described in <i>Signs of parasympatholytic agents</i> .
Ethanol	See <i>Depression or coma</i> .	See <i>Signs of depression or coma</i> .

TABLE 17-1. COMMON TOXICANTS OF THE NERVOUS SYSTEM BY MAJOR EFFECT(S)—CONT'D

TOXICANT	COMMON SOURCES	ADDITIONAL SIGNS
Lead	See <i>Excitation</i> .	Lead exposure may cause changes in mental abilities, lost recognition of familiar persons, belligerence, and hysteria.
Lysergic acid diethylamide (LSD)	Sources are some plant forms (morning glory seeds, ergotized grains) or illicit street drugs.	May be profound behavioral changes ranging from excitement and hallucinations to deep depression. Analysis of vomitus or stools may help to establish exposure.
Marijuana	Illicit street drugs, prepared from leaves or seeds of <i>Cannabis sativa</i> . Sometimes prepared in foods such as brownies.	Behavioral changes may include depression, excitement, hallucinations with barking at unknown stimuli; nystagmus, vomiting, and diarrhea. Diagnosis by recognition of suspect material and/or testing of blood or urine for cannabis alkaloids.

GI, Gastrointestinal; OTC, over-the-counter.

TABLE 17-2. TOXICANTS OF THE DIGESTIVE SYSTEM

TOXICANT	COMMON SOURCES	COMMENTS
DIRECT IRRITANTS		Very nonspecific. Expect acute onset, potential oropharyngeal lesions, gagging, colic, emesis and/or diarrhea (possibly hemorrhagic); bloat or flatulence.
Acids, alkalis, aldehydes	Batteries, cleaners and bleaches, disinfectants.	Salivation, dysphagia, acute glossopharyngeal swelling, cough, nasal discharge. Laboratory testing of suspect materials may establish source, but animal testing for these substances is usually not practical or helpful.
Petroleum distillates	Solvents, paint thinners, furniture cleaners, gasoline, kerosene.	Coughing, vomiting, choking. Possible sequel is aspiration pneumonia and central nervous system depression. Agents are difficult to detect in blood or tissues by laboratory tests.
Volatile oils	Turpentine, gum spirits, pine oil, eucalyptus oil, pennyroyal oil, lemon oil.	Acute gastroenteritis with vomiting and possibly diarrhea. Systemic effects can include seizures, delirium, depression, and coma.
Gastroenteritis, hemorrhagic	Aflatoxin, aspirin, NSAIDs.	Aflatoxin is also hepatotoxic; Aspirin and NSAIDs may include gastric ulcers, platelet dysfunction, and/or renal damage.
GASTROENTERITIS, HYPEREMIC/NECROTIC		
<i>Amanita phalloides</i> (death cap, death angel)	Wild, toxic mushroom. Most prominent in eastern or western seacoast regions.	Acute clinical signs are hemorrhagic gastroenteritis with vomiting and bloody diarrhea, hepatosis after a latent period of 12–24 hours. Available laboratory testing is limited.
Arsenic, antimony, bismuth	Older insecticides or herbicides, including ant baits; old paint pigment. Most uses of arsenic are restricted or canceled. Antimony may be present in caustic pastes.	Acute vomiting, followed by moderate to severe diarrhea changing from watery to necrotizing and hemorrhagic within 24–48 hours. Hypotension, shock and renal tubular damage are additional effects. Analysis of urine or gastrointestinal contents can help to establish exposure. Liver and kidney concentrations from postmortem specimens can be helpful.
Iron	Accidental access to dietary iron supplements.	Acute gastroenteritis, shock, vascular collapse and death, liver damage 1–2 days later. Test for liver function, assay total serum iron and total iron-binding capacity.
<i>Staphylococcus</i> toxins	Spoiled foods, especially egg and high-protein products left at room temperature.	Severe vomiting and diarrhea may develop after short latent period (<3 hr). Cultures may identify the potential bacterium but alone are not confirmatory.
GASTROENTERITIS, NONHEMORRHAGIC		
Digitalis glycosides	Accidental access to prescription medicines; plant sources including foxglove, oleander.	Cardiac signs may be preceded or accompanied by colic and vomiting.
Lead	See Table 17-1 .	Vomiting is intermittent.

TABLE 17-3. TOXICANTS OF THE HEPATOBILIARY SYSTEM

TOXICANT	COMMON SOURCES	COMMENTS
Acetaminophen	Over-the-counter pain killer and anti-inflammatory drug.	Initial signs of vomiting, cyanosis, and facial edema are followed by icterus, depression, and mild methemoglobinemia.
<i>Amanita phalloides</i>	Wild mushroom, most common in East or West coast areas.	Acute vomiting is followed in 1–2 days by liver failure.
Blue-green algae	Algal forms, commonly concentrated by low water and prevailing winds.	Acute gastroenteritis, vomiting, and hemorrhagic diarrhea. Water fixed in formalin 1:10 can be examined for presence of blue-green algae. Frozen samples are used for toxic activity by mouse bioassay.
Iron	Dietary supplement pills.	See Table 17-2.
Petroleum distillates	Solvent in paints, paint thinners, paint strippers; fuels including gasoline, kerosene.	Liver damage may accompany initial signs of neurologic dysfunction.
Sago palm	Toxic plant outdoors in tropical climates, indoors as potted plant.	Icterus, severe liver damage, secondary signs from liver failure. Often fatal if left untreated.

TABLE 17-4. TOXICANTS OF THE CARDIOVASCULAR SYSTEM

TOXICANT	COMMON SOURCES	COMMENTS
Albuterol	Inhalers, decongestant.	Arrhythmias, tachycardia.
Alpha ₂ -adrenergic agents	Xylazine (often iatrogenic), detomidine, romifidine for veterinary use.	Arrhythmias, bradycardia, hypotension, AV block, vomiting, occasional apnea. Hyperglycemia is common.
Amphetamines, methamphetamine	Prescription of recreational drugs.	Tachycardia. Accompanied by seizures, tachypnea, mydriasis.
Azalea	Ornamental shrub.	Arrhythmias, often preceded by vomiting and gastroenteritis.
Baclofen	Human prescription drug. Skeletal muscle relaxant.	Arrhythmias, bradycardia in severe overdose. Other signs are vomiting, ataxia, salivation, hypothermia.
Calcium channel blockers	Human and veterinary cardiovascular prescription.	Bradycardia, arrhythmia, hypotension, vomiting, weakness.
Cardioactive glycosides	Digoxin and related prescription drugs; foxglove, oleander, <i>Bufo</i> spp. toads.	Vomiting, colic, diarrhea, followed by weakness, bradycardia, and arrhythmia.
Cholecalciferol	Vitamin supplements, rodenticides. High dosages cause signs in 12–36 hr.	Hypercalcemia, azotemia, bradycardia, arrhythmias, dystrophic calcification.
Cocaine	Illegal street drug.	Tachycardia and arrhythmias. Test plasma or urine.
Ionophores	Poultry coccidiostats; ruminant feed supplements. Dangerous to dogs.	Trembling, weakness, stiffness, recumbency, cardiovascular insufficiency. Analysis of stomach contents; cardiac muscle biopsy for diagnosis.
Pimobendan	Veterinary prescription for congestive heart failure—usually accidental overdose.	Large overdose causes arrhythmias, hypotension, tachycardia, vomiting, diarrhea.
Yew bushes	Ornamental evergreen shrubs with strap-like, two-ranked, dark-green leaves.	Sudden death several hours after ingestion; nervousness, trembling, bradycardia and arrhythmia. Test for yew alkaloids in stomach.

AV, Atrioventricular.

TABLE 17-5. TOXICANTS OF THE BLOOD AND BONE MARROW

TOXICANT	COMMON SOURCES	COMMENTS
ANEMIA, HEMOLYTIC		
Methionine	Urinary acidifier (given to dogs to decrease brown spots in lawn).	Heinz body anemia, also methemoglobinemia in cats; abdominal pain, agitation, hyperactivity. CBC, chemistry panel for low BUN, hypoglycemia.
Mothballs, naphtha	Home care product; exposure possible by oral or inhalation routes.	Heinz body anemia. Confirm with hematology examination.
Onions, garlic	Accidental access to large amounts in prepared foods or by direct ingestion.	Acute signs are typical of hemolytic crisis.
Phenothiazine anthelmintics	Older, little-used anthelmintics.	Acute hemolytic crisis may occur. Phenothiazine in urine becomes oxidized to a red color. Phenothiazine can be detected in blood or urine, but routine testing is not offered by many laboratories.
Propylene glycol	Component of many consumer products. Alternative antifreeze.	Heinz body anemia (cats). Also depression, weakness, hypotension, acidosis.
Zinc	Zinc metal objects, galvanized food or water containers, zinc ointments, pennies minted after 1983.	Vomiting and diarrhea in combination with pale mucous membranes and icterus.
METHEMOGLOBIN		
Acetaminophen	OTC analgesic and anti-inflammatory. See Table 17-3.	Hepatotoxicosis is prominent. Blood may display dark-brown color due to methemoglobinemia.
Aniline dyes	Shoe polish, inks, paints. More prominent in older products.	See Nitrites, below.
Chlorates	Soil/contact herbicide or soil sterilant. Used where prolonged suppression of plant growth is desired. Accidental ingestion of granules may occur.	See Nitrites, below.
Nitrites	Lawn fertilizers, explosives, meat-curing agents, contaminated well water.	Methemoglobinemia, cyanosis, weakness, depression, hyperpnea. See Acetaminophen in text for analysis of methemoglobin.
CARBOXYHEMOGLOBIN		
Carbon monoxide	Poorly vented heaters, automobile exhaust.	Depression, weakness, somnolence, coma, and death. Mucous membranes are bright pink and blood is cherry-red to pink.
APLASTIC ANEMIA AND THROMBOCYTOPENIA		
Benzene	Gasoline and industrial or commercial products as a solvent. Small animals could be at risk by inhalation exposure.	Causes initial central nervous system signs of tremors, ataxia. Cardiac fibrillation may occur at high acute exposures. Prolonged exposures usually cause pancytopenia. Serum iron is increased and fetal hemoglobin may be increased. Anemia is macrocytic with relatively few reticulocytes that are generally immature.
Estrogens	Used to correct mismating, treat prostatic hyperplasia, induce abortion, or reduce urinary incontinence.	Lethargy, weakness, pale mucous membranes, petechial hemorrhages, hematuria, melena. Pancytopenia, reduced reticulocyte count.
COAGULOPATHY		
Anticoagulant rodenticides	OTC baits for rodent control in homes and business.	Dermal and mucosal petechiae and ecchymoses, hemoptysis, epistaxis, melena, pale mucous membranes, weakness, dyspnea, subcutaneous hematoma, muffled heart and lung sounds, depression, and death.

BUN, Blood urea nitrogen; CBC, complete blood count; OTC, over-the-counter.

TABLE 17-6. TOXICANTS OF THE KIDNEYS

TOXICANTS	COMMON SOURCES	COMMENTS
Aminoglycoside antibiotics	Primarily kanamycin, neomycin, and gentamicin.	Usually occurs with extended treatment. Initial findings are polydipsia/polyuria followed by vomiting and azotemia.
Ethylene glycol	Automobile radiator antifreeze fluids.	Initial signs are in central nervous system with ataxia and depression, followed by metabolic acidosis, oxalate nephrosis, and azotemia.
Fungal toxins	Ochratoxin or citrinin in refrigerated table foods (cream cheese, nuts).	Signs are polydipsia, polyuria from renal tubular nephrosis.
Grapes/raisins	Accidental access to large amounts by dogs.	Anorexia, lethargy, vomiting and diarrhea. Confirm with renal function tests and hypercalcemia/hyperphosphatemia.
Halogenated hydrocarbons	Solvents in commercial products, paints, cleaning agents.	Initial signs may include inebriation, incoordination, and depression followed in 1–3 days by toxic tubular nephrosis. Clinical laboratory findings reflect acute tubular injury.
Metals	Many metals and metalloids are potent tubular toxicants.	Effects and signs are typical of tubular nephrosis. Samples for analysis of metals should include blood, urine, kidney, and liver.
Nonsteroidal anti-inflammatory agents	Ibuprofen, naproxen, usually accidental or owner administered.	Renal damage and clinical laboratory findings are similar to those for the aminoglycoside antibiotics. See Table 17-1.
Plants (non-oxalate)	Easter lily (<i>Lilium</i> spp.), daylily (<i>Heemerocallis</i> spp.).	Cats appear most at risk to these plants. The lilies have a potent tubular toxicant. Suspect animals should be tested for renal function. No toxicant analyses currently available.
Vitamin D ₃	Vitamin supplements and vitamin D ₃ (cholecalciferol)-based rodenticides.	Signs after latent period of 18–24 hr; progress over 1–3 days to polyuria, polydipsia, renal pain on palpation.

TABLE 17-7. CLINICAL LABORATORY TESTS THAT MAY BE ALTERED BY TOXICOSES

LABORATORY PARAMETER	TOXICANT(S)	EFFECT ON VALUES
Alanine aminotransferase	Acetaminophen, aflatoxin, benzimidazole anthelmintics, halogenated hydrocarbons (e.g., halothane, chloroform) hydrocarbon solvents, iron, melaleuca oil, mushrooms (<i>Amanita</i> spp.), phosphides, sago palm, xylitol, zinc	Moderate to massive increase depending on dosage and toxicant.
Ammonia	Ammonium-based fertilizers Toxic liver injury	Increased. Increase associated with signs of hepatic encephalopathy.
Anemia, nonregenerative	Cadmium, lead, zinc Cancer chemotherapy (e.g., 5-fluorouracil ointments for skin cancer) Phenylbutazone Chloramphenicol Estrogen therapy	Varies in severity; lead usually mild anemia, zinc often is clinically important. Dosage dependent: 5-fluorouracil patients may die acutely before anemia develops. More likely in cats than dogs. Usually only at high dosage over extended time period. Ferrets may be unusually sensitive.
Anemia, regenerative	Acetaminophen, aspirin, copper, methionine, mothballs (naphtha), onions/garlic, phenols, propylene glycol	Heinz bodies indicate oxidant injury from toxicants listed.

TABLE 17-7. CLINICAL LABORATORY TESTS THAT MAY BE ALTERED BY TOXICOSES—CONT'D

LABORATORY PARAMETER	TOXICANT(S)	EFFECT ON VALUES
Azotemia	Calcium supplements Methionine Mothballs NSAIDs, veterinary Paintballs Paraquat Sago palm Salt Zinc	Also decreased GFR, nephrolithiasis. Also elevated serum ammonia and lipase. Also hyperbilirubinemia and acidosis. Also anemia, oliguria, hypoalbuminemia. Also lactic acidosis and hypovolemia. Also oliguria. Also hypoalbuminemia and hypoglycemia. Also acidosis and hypernatremia. Increased urinary hemoglobin and bilirubin.
Basophilic stippling	Lead	Accompanied by nucleated red cells. Mild anemia of low clinical significance.
Bile acids	Acetaminophen, aflatoxins, corticosteroids, diethylcarbamazine, 5-fluorouracil, sago palm, thiacetarsamide (Caparsolate)	Early onset of mild to moderate increase, often preceding other parameters of liver injury.
Bilirubin	Acetaminophen, aflatoxins, thiacetarsamide (Caparsolate)	Increased.
Blood pH		
Acidosis	Alcohols, aspirin, bread dough, cholecalciferol, essential oils, ethylene glycol, grapes/raisins, hops, iron, metaldehyde, methionine, oxalates, paintballs, phenol/pine oils, phosphides, propylene glycol	Acidosis, usually resulting from metabolic disturbance or less commonly from acidic nature of the toxicant.
Alkalosis	Calcium supplements, diuretics, foreign bodies, phenols/pine oil,	
Calcium, serum	Calcipotriene, cholecalciferol rodenticide, diuretics, grapes/raisins, hops, xylitol	Increased.
	Ethylene glycol, fluorides, hydrofluoric acid, oxalates, phosphate enemas, rhubarb, soaps	Decreased.
Casts, renal	Aminoglycoside antibiotics, arsenic (cats), cadmium, lilies (most species), nonsteroidal anti-inflammatory drugs	Renal epithelial damage as sequel to renal tubule toxicosis.
Chloride, serum	Ammonium chloride	Mechanisms of elevation include loss of concentrating ability, laboratory interference.
	Amphotericin	
	Bromides	Watch for potential laboratory error.
	Lithium	
Cholinesterase in RBCs	Blue-green algae, hydrofluoric acid, ivermectin, organophosphate/carbamate insecticides, phosphides	
Coagulation factors	Anticoagulants for human cardiovascular disease ("blood thinners"); coumarin-based rodenticides (e.g., warfarin, brodifacoum, bromadiolone)	Prolonged PT and aPTT associated with loss of factors II, VII, IX, X.
Creatine kinase	Ionophores (e.g., monensin, lasalocid, salinomycin)	Increased.
Crystalluria	Oxalate-containing plants (e.g., rhubarb, oxalis)	Oxalate crystals in urine sediment.
	Ethylene glycol metabolites	Oxalate crystals appear in urine, also from impression smears of kidney at necropsy.
	Melamine/cyanuric acid in contaminated commercial foods.	Outbreak reported in 2007. Usually accompanied by advancing signs of renal failure.
Gamma-glutamyl transpeptidase	Barbiturates, glucocorticoids	Moderate increase.

Continued

TABLE 17-7. CLINICAL LABORATORY TESTS THAT MAY BE ALTERED BY TOXICOSES—CONT'D

LABORATORY PARAMETER	TOXICANT(S)	EFFECT ON VALUES
Glucose	Albuterol, alcohols/aldehydes, α_2 -adrenergic agonists (detomidine, dexmedetomidine, xylazine), amitraz insecticide, calcium channel blockers, ephedra/ma huang, iron, pyrethrins/pyrethroids, scorpion envenomation, sodium monofluoroacetate (compound 1080)	Increased. Causes of hyperglycemia are varied, primarily related to blocking of TCA cycle or sympathetic stimulation of glucose release.
Hemoglobinuria	Acetaminophen, brown recluse spider venom, chlorate herbicides, copper, crotalid snake (pit viper) venom, mothballs, onions/garlic, propylene glycol (cats), Zinc (metallic)	Elevated, mild to severe. Often a result of strong RBC oxidant and hemolytic properties of the toxicant.
Icterus, serum	Acetaminophen, aflatoxin, benzodiazepines, blue-green algae, club drugs, mothballs, mushrooms, phosphides, sago palm, veterinary NSAIDs, xylitol, zinc	Also reflected at lower concentrations as hyperbilirubinemia in clinical laboratory profile.
Leukocyte count	Benzene Chloramphenicol Estrogens (dog) Phenylbutazone	Neutropenia with likely left shift. Chemical neutropenia and/or pancytopenia is generally due to inhibition or destruction of stem cells after prolonged use.
Magnesium, serum	Gentamicin	Decreased, secondary to nephrosis or hypercalcemia.
Methemoglobin	Vitamin D-induced hypercalcemia Acetaminophen, benzocaine, chlorate herbicides, copper, meat curing salts (nitrites), methionine, nitrites, onions/garlic, phenols, smoke inhalation	MetHb increased more than 40% causes clinical signs. MetHb may be metabolized rapidly or change after collection; contact laboratory for instructions on preservation and shipping.
Osmolarity	Aspirin, ethanol, ethylene glycol, phosphate enema	Hyperosmolarity and high osmolar gap.
Porphyrinuria	Hexachlorobenzene, lead	Moderate increase from inhibition of porphyrin synthesis pathway.
Pancytopenia	Benzene derivatives, cancer chemotherapy, chloramphenicol, 5-fluorouracil, thallium	
Phosphate, serum	<i>Cestrum diurnum</i> (night blooming jessamine), cholecalciferol rodenticides Xylitol	Increased due to vitamin D effect. Xylitol is reported to cause either hyperphosphatemia or hypophosphatemia.
Phosphate, serum	Albuterol	Decreased.
Potassium, serum	Angiotensin-converting enzyme inhibitors, calcium channel blockers, digitalis glycosides, diuretics, grapes/raisins, matches/fireworks, NSAIDs	Increased.
Potassium, serum	Albuterol	Decreased. Albuterol also may cause decreased serum phosphate and serum magnesium, elevated lactate, and secondary hyperglycemia resulting from sympathetic stimulation.
Sodium, serum	Aspirin, chocolate/cafeine, dehydration, excess salt intake, grapes/raisins, paintballs	Increased.
Thrombocyte count	Aflatoxin, cephalosporins, crotalid (pit viper) snakes, estrogens, 5-fluorouracil, IFE therapy, NSAIDs, phenols, sago palm, xylitol, zinc	Decreased.

aPTT, activated partial thromboplastin time; GFR, glomerular filtration rate; IFE, intravenous fat emulsion; MetHb, methemoglobin; NSAIDs, nonsteroidal anti-inflammatory drugs; PT, prothrombin time; RBC, red blood cell; TCA, tricarboxylic acid.

Quantification of toxicants is greatly influenced by passage of time and the half-life of that specific toxicant. Thus if specific confirmatory tests are anticipated, early collection of vomitus, feces, blood, and urine may be the key to a good chemical laboratory confirmation.

OBTAINING AND SHIPPING SPECIMENS

Blood or plasma is the major vehicle for body transport of toxicants. Whole blood should be collected in anticoagulant (ethylenediaminetetraacetic acid [EDTA] or heparin unless otherwise specified). Vomitus or feces may provide evidence of recent oral exposure, biliary excretion, or both. Many organic toxicants are excreted in urine by direct secretion from renal tubule transport systems. Hair may document chronic accumulation of a toxicant (especially for metals or sulfur-containing compounds), showing prior exposure even when gastrointestinal and organ concentrations are no longer elevated or detectable. However, hair values must be carefully interpreted because assay may be influenced by external contamination rather than systemic accumulation. Baits and other environmental samples may establish a source of poison or probable route of exposure. Environmental samples include food, water, suspected toxic plants, baits, pesticides, household products, medications, and solvents. Empty containers may also be useful.

When animals die, clinicians should recommend a thorough necropsy and collect appropriate specimens to examine for lesions and analyze for toxicants. Stomach contents may contain plants, foreign objects, abnormal colors (e.g., marker dyes associated with pesticides), tablets, or capsules. Specimens of appropriate organs and tissues should be (1) saved fresh by freezing and (2) placed in 10% neutral buffered formalin for microscopic examination. Specimens should include brain, liver, kidney, cardiac muscle, stomach and intestines, lung, urine, feces, and any other tissues appearing abnormal. Many toxicoses can be presumptively diagnosed by a characteristic lesion or lesions (e.g., coumarin anticoagulants cause coagulopathy, usually with hemothorax; EG toxicosis is routinely diagnosed at postmortem by microscopic examination of renal impression smears or routine hematoxylin and eosin [H&E] histopathology). If there is contention of liability or malicious poisoning, it may be well to request submission of the entire animal to a laboratory with qualified veterinary pathologists. Gross and especially microscopic lesions are often valuable procedures to eliminate certain toxicoses or to suggest others, and this information is usually relatively cost-effective.

Appropriate samples free from contamination (e.g., environmental agents, medications, preservatives) and sealed individually in glass or plastic containers can be submitted for chemical assay. Although standard evacuated blood or serum tubes are adequate for most body fluids, some trace elements (e.g., zinc) require special trace element tubes (i.e., Vacutainer, royal-blue cap). Clinicians should never use preservatives unless laboratory instructions specifically recommend them. Serum should be separated from the clots before shipping.

Unless otherwise indicated, freezing of samples for toxicologic analyses is the best means of preservation. For unusual analyses, or if in doubt about specimens needed, clinicians should consult a toxicologist or call the laboratory for information.

Applicable U.S. Postal Service (USPS) rules regarding biologic, infectious, and toxic materials should be followed. This generally means protective bagging around the entire submission and use of United Nations–approved packaging materials (usually indicated in catalogs for packaging materials; see also U.S. Department of Energy, Office of Environmental Management, at http://www.em.doe.gov/Transportation/PMC_PackagingSpecs.aspx). If sample integrity and chain of custody are important (e.g., insurance claims, litigation), the clinician should seal the shipping box, place a copy of the transmittal letter in an envelope marked “Invoice,” stick the envelope to the outside of the sealed box, and, if possible, ship it directly to a specific receiving person who has been notified in advance. USPS regulations and recommendations for biological specimens with potential toxic or infectious contaminants are extensive and beyond the scope of this chapter. They can be found currently at http://pe.usps.com/text/pub52/pub52c3_021.htm. Veterinary hospitals submitting specimens routinely through the USPS or via commercial carriers should be aware of current practices and regulations; it may be helpful to assign this responsibility to one or two employees in a practice who can stay current on these regulations.

SELECTING AN ANALYTICAL LABORATORY

In selecting and using an analytical laboratory or referral laboratory, certain criteria should be considered. First, the laboratory should be accredited or certified by an external group that audits and approves procedures and methodologies. Specimen and sample tracking within a laboratory information management system (LIMS) is essential to maintaining clear records of receipt, storage, and analysis. Procedures for sample tracking must maintain an unmistakable connection between the analytical data and the sample obtained. This is especially true for legal samples, which may involve “chain of custody” where the data from the laboratory must be able to stand up in court. Analysis of specimens should be done using standard operating procedures (SOPs) that have been validated in-house or from the literature or both. For good quality control, performing the method should involve an internal standard, a control sample, or a spiked sample. An *internal standard* is a compound added to the sample that is similar in nature to the compound of interest. A *control sample* is a sample that contains the compound of interest in the same or similar matrix as that which is being analyzed. A *spiked sample* is when two samples are weighed and to one is added the compound of interest so a recovery from the matrix can be determined. Analytical instruments used in the procedure must be validated to be operating within the control limits to obtain verifiable data for reporting. Good documentation and maintenance of records must be kept to assure the validity of the data.

Finally, it is important that a toxicologist or specialist be able to consult the client, review the case and make appropriate test suggestions after analyzing all the data.

Below is a brief review of techniques used in an analytical toxicology laboratory for investigation and identification of compounds of interest. They are intended to help the clinician understand some information about techniques used in a modern analytical laboratory.

Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a technique to separate compounds most typically using normal phase (silica) or reverse phase (silica modified to contain a C18 arm). In using TLC, the sample is spotted on the plate (stationary phase) along with standards and placed in a tank with the developing solvent (mobile phase). The solvent moves up the plate by capillary action and the compounds are separated. Depending on the compound, the plate can be observed by ultraviolet (UV) light or fluorescence, or sprayed to form chromophores visibly. This technique is mainly used for screening, although quantification can be done using densitometry when the compound of interest is well separated from the matrix. TLC is relatively versatile and inexpensive, but less specific than many instrumental methods.

Gas Chromatography

Gas chromatography (GC) is a technique used for the analysis and quantification of volatile compounds. Compounds are injected onto the gas chromatograph and flash evaporated onto the column. This can be done using a packed or capillary column. The column is the stationary phase and the gas is the mobile phase. The compounds are separated on the column by how they interact with these phases; heat also moves the compounds along the column. After leaving the column, the compounds can be detected by various detectors. Some of these detectors commonly used include the flame ionization detector (FID), the thermoselective detector (TSD) or flame photometric detector (FPD), and the electron capture detector (ECD).

High-Pressure Liquid Chromatography

High-pressure liquid chromatography (HPLC) is a technique used for separating and quantifying mainly non-volatile compounds. Compounds are injected onto a column and the mobile phase is pumped into the column under high pressure to separate the compounds. The compounds are detected typically using UV or fluorescence. In some cases compounds can be derivatized to absorb in the UV or fluoresce.

Mass Spectrometry

Mass spectrometry (MS) is an analytical technique that measures the mass-to-charge ratio of charged particles. This is used in measuring the masses of particles from a compound. There are two general types: gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS); the names

denote how the sample is introduced. GC-MS uses a gas chromatograph and fragments the compound, giving a characteristic pattern specific to the compound. LC-MS uses HPLC to introduce the sample and detects the whole mass of the compound. For further confirmation of the compound, one can do liquid chromatography-tandem mass spectrometry (LC-MS/MS) in which the compound mass is detected and then hit with a specific collision energy and fragmented into daughter ions, which form from the compound. These techniques are used for confirmation of the compound and may be justified in a legal case.

Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (AAS) is a technique used to quantify a specific metal element in a sample. There are two types: flame AAS and graphite furnace AAS (GF-AAS). These techniques can typically only analyze one element at a time. In flame AAS, the sample is aspirated into the flame, where the element of interest is absorbed and detected to be quantified. In GF-AAS, the sample is placed in the graphite furnace and heated to extremely high temperatures to vaporize the element, which can be absorbed and detected to be quantified.

Inductively Coupled Plasma Emission Spectroscopy

Inductively coupled plasma emission spectroscopy (ICP-ES) is a technique used for analyzing metals and minerals. The sample is aspirated into the plasma, which excites the atoms, which then emit characteristic electromagnetic radiation the wavelength of which can be measured. Using this technique, one can measure many elements at once.

Inductively Coupled Plasma Mass Spectrometry

Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry that measures metals and nonmetals. Similar to ICP-ES, the sample is aspirated into the plasma, but the technique is more sensitive (parts per billion [ppb] to parts per trillion [ppt]) since one is measuring the actual mass of the elements. This technique can be used for confirmation and speciation of elements and may be justified in a legal case.

DIAGNOSIS OF SPECIFIC TOXICANTS BY LABORATORY ANALYSIS

Chemical analysis is the final and most important confirmatory procedure for toxicoses when a chemically confirmed diagnosis is needed. Specific selected common toxicants are addressed in the next section. In addition, a differential list of toxins is provided for selected organ systems, along with common sources for each toxicant and comments about distinguishing clinical or laboratory features of the toxicant (see [Tables 17-1 to 17-6](#)). Refer

also to Table 17-7, which compares specific clinical laboratory changes associated with common veterinary toxicants.

Acetaminophen

Indications • Intoxication with acetaminophen (ACM) is likely in any cat with a history of ACM administration (especially if dyspnea or cyanosis is present). One 325-mg tablet may be toxic to cats.

Sample Collection • Plasma (from EDTA or heparin-preserved blood) or serum and urine are the preferred antemortem samples. Blood concentrations usually peak 4 to 6 hours after ingestion. Samples should be refrigerated if analyzed within 24 hours or frozen if longer delays are expected. A blood smear should be examined for Heinz bodies. Antemortem whole blood can be tested for methemoglobin. Methemoglobin is unstable and must be tested within 4 hours or stabilized by hemolysis of the whole blood with an equal amount of sterile water. The clinician should contact the laboratory in advance for recommendations for handling and submitting samples for methemoglobin analysis. Toxic concentrations in tissues have not been established.

Analysis • ACM is measured in serum or plasma typically using HPLC. If the test is available, blood glutathione values are depressed. Moderate (i.e., 10% to 50%) to marked (i.e., >50%) numbers of large Heinz bodies are expected in intoxicated cats. In normal cats, up to 10% of RBCs may contain small Heinz bodies (see Chapter 3). Hemoglobinuria or hematuria may be present; methemoglobin concentration is increased.

Normal Values • Data for animals are scarce, but ACM values less than 100 µg/ml likely do not suggest toxicosis.

Danger Values • Not established in cats; human ACM values greater than 300 µg/ml are indicative of hepatopathy.

Artifacts • Other agents producing methemoglobinemia or hemoglobinuria (e.g., aniline dyes, copper salts, nitrites, onions; see Table 17-5) can mimic ACM's effects.

Causes • ACM toxicosis is most common in cats; dogs are less commonly poisoned and more likely than cats to have prominent liver toxicosis. Exposure is usually due to persons not familiar with ACM toxicosis to cats. Other causes of Heinz bodies and methemoglobinemia should be eliminated (see Table 17-5 and Chapter 3). Evidence of methemoglobinemia, moderate to marked numbers of large Heinz bodies, or both accompanied by positive test results for ACM or its metabolites support a diagnosis of ACM toxicosis.

Aflatoxins

Indications • Dogs have been most commonly affected. Acute signs of vomiting, hemorrhage, and hepatic failure or a subacute response that includes progressing hepatic

failure, icterus, hypoproteinemia, coagulation failure, and weight loss are indications to suspect aflatoxin poisoning. Aflatoxins are potent hepatotoxins formed by *Aspergillus flavus* and other *Aspergillus* spp. They infrequently have caused large-scale contamination of pet foods when moldy grain has been used in pet foods. Clinical laboratory changes suggestive of aflatoxicosis include hypoproteinemia, coagulation dysfunction and hepatic insufficiency or failure. Hepatic enzymes (alanine aminotransferase [ALT], sorbitol dehydrogenase [SDH], and gamma-glutamyl transpeptidase [GGT]) are increased, and elevated serum bile acids are a sensitive indicator of hepatic aflatoxicosis. Abdominal ultrasound and hepatic biopsy have been used to detect patterns of hepatic aflatoxicosis, typically centrilobular necrosis and hemorrhage.

Sample Collection • Suspect pet foods should be sampled from several bags and the lot number of each bag recorded. Store the food in a cool dry place, or for longer holding, freeze it to preserve the status at time of sampling. Urinary metabolites of aflatoxin are indicative of recent exposure, and residues of aflatoxin metabolites such as aflatoxin M1 can be found in tissues (liver and kidney) of animals that diet because of aflatoxin poisoning.

Analysis • A range of analytical procedures on feed are available, from enzyme-linked immunosorbent assay (ELISA) kits and/or lateral flow rapid tests that could be run in a clinical laboratory to more sophisticated and quantitative assays by TLC, HPLC, or LC-MS/MS when quantification and confirmation of contaminated food is warranted.

Normal Values • Aflatoxins are regulated by the U.S. Food and Drug Administration, and grain values above 20 ppb are not permitted in interstate commerce.

Danger Values • Aflatoxin concentrations greater than 60 ng/g (ppb) have been associated with aflatoxicosis in dogs. Serious subacute toxicosis is more likely when food concentrations are above 200 ppb.

Artifacts • Not all aflatoxins are of equal toxicity, and detection of total aflatoxins by some tests may nonspecifically overestimate the true level of the most toxic metabolite, aflatoxin B.

Causes • *Aspergillus* fungi (*A. flavus*, *A. parasiticus*, and *A. nomius*) may contaminate grains during their formation or grow in improperly stored grains at moisture levels above 14%. Most at risk are corn, cottonseed, and peanuts; corn is a common constituent of many pet foods.

Albuterol

Indications • Acute onset of apprehension, agitation, and cardiac arrhythmia combined with tachypnea and collapse are indications of albuterol poisoning. Effects are due to excessive beta₂ agonist activity, and there is profound hypokalemia as a result of Na⁺/K⁺-ATPase activation, and hyperglycemia in response to sympathetic stimulation.

Sample Collection • Blood collection for glucose and serum electrolytes is indicated when albuterol exposure or typical clinical signs occur.

Analysis • A routine electrolyte panel including serum potassium, phosphorus, magnesium, lactate, AST and glucose is recommended. In addition, monitoring of blood pressure and electrocardiogram is warranted based on presenting signs and potential for serious hypokalemia. Albuterol can be measured in plasma, serum, or liver by HPLC or LC-MS.

Danger Values • Marked hypokalemia and probably hypophosphatemia and hypomagnesemia and increased aspartate aminotransferase (AST), blood lactate, and blood glucose are indicators of albuterol toxicosis.

Artifacts • Many toxic agents can affect blood glucose and serum potassium (see Table 17-7). The acute beta₂ agonist signs coupled with hyperglycemia and hypokalemia would suggest albuterol as a valid differential diagnosis.

Causes • Albuterol is most commonly encountered when dogs chew and puncture pressurized canister inhalers that are normally used by humans or used off-label in veterinary patients.

Alkaloids

Indications • Intoxications with alkaloids should be considered in patients with central nervous system (CNS) excitation, including seizures (see Table 17-1), with or without vomiting, and possible exposure to alkaloids. High-risk alkaloid poisons include 4-aminopyridine (Avitrol), amphetamines, caffeine, cocaine, theobromine, nicotine, and strychnine. Some of these have been used as malicious poisons, and theobromine is the major toxic alkaloid in chocolate products.

Sample Collection • After absorption, alkaloids may be concentrated in the liver and eventually excreted in urine. Plasma values may be low compared with urine values, unless death occurred very rapidly before excretion in urine (i.e., <1 hour). Preferred samples are vomitus or urine from live animals and any suspect baits (e.g., bird toxicants [4-aminopyridine]). Blood or plasma may contain significant levels of some alkaloids (e.g., the methylxanthines [caffeine, theobromine]). Some alkaloids (e.g., strychnine) are difficult to detect in antemortem blood. Postmortem samples should include liver, kidney, and urine. Refrigeration is adequate for preservation of alkaloids.

Analysis • Alkaloidal compounds of toxicologic importance to small animals include 4-aminopyridine, amphetamines, atropine, caffeine and other methylxanthines, cocaine, and strychnine. Screening analyses may be performed by TLC. Quantitation is best performed by HPLC, GC, GC-MS, or LC-MS.

Normal Values • For the alkaloids listed previously, normal values are not expected; their presence in the body indicates exposure to an exogenous source. For some

(e.g., strychnine), any amount supports a diagnosis of toxicosis.

Danger Values • Analysis confirms exposure, but toxic concentrations are not well established for most common alkaloids.

Artifacts • Many plant compounds and drugs contain nontoxic heterocyclic nitrogen compounds that react as alkaloids. Simple spot tests depending on colorimetric reactions measuring only a general alkaloidal reaction can incorrectly implicate toxicosis because of a false-positive test result. A minimum of TLC after appropriate solvent extraction is recommended and followed, if necessary, by an alternative confirmatory instrumental procedure.

Causes • Poisoning with alkaloids needs to be differentiated from other poisons (see Tables 17-1 and 17-2), other causes of CNS excitation, and other causes of vomiting (see Chapter 9). Diagnosis is usually confirmed by a combination of history and clinical signs in conjunction with demonstration of the alkaloid in stomach contents or body fluids.

Amitraz

Indications • Intoxication with amitraz should be considered in patients with vomiting, depression, ataxia, frequent defecation, or diarrhea, as well as in animals with intentional use or misuse or accidental exposure to amitraz via dips, tick collars, or some livestock sprays. Advanced systemic signs can include bradycardia, hypotension, and convulsions. Hypoglycemia is a characteristic clinical laboratory change.

Sample Collection • Blood is not a good sample because it has low amitraz concentrations, but urinary metabolites may allow diagnosis. Amitraz on hair or in vomitus can be analyzed when exposure is uncertain. Samples of vomitus, liver, kidney, skin, hair, brain, or lungs can be used in postmortem cases.

Analysis • Samples are typically analyzed by GC using a nitrogen-phosphorus detector to quantitate amitraz. HPLC may also be used, but a more extensive cleanup procedure may be needed to remove interferences.

Normal Values • Amitraz is not expected in normal tissues.

Danger Values • Not established.

Causes • Usual causes of amitraz intoxication are substantial therapeutic overdose or inadvertent exposure of a species (e.g., cats) for which amitraz is not recommended. Other toxins (see Table 17-2) and other causes of vomiting and diarrhea need to be eliminated (see Chapter 9). Clinical signs can appear similar to those of organophosphate or carbamate toxicosis; these may need to be ruled out by appropriate testing (e.g., acetylcholinesterase).

Anticoagulant Rodenticides

Indications • Hemorrhage, prolonged activated coagulation time (ACT), prolonged prothrombin time (PT) or prolonged activated partial thromboplastin time (aPTT) (or both), or a combination thereof suggests intoxication with vitamin K-responsive anticoagulant rodenticides (see Table 17-5). Abnormal results for proteins induced by vitamin K activity (PIVKA) have also been used to detect impaired coagulation from vitamin K antagonists.

Sample Collection • Whole blood (in EDTA or heparin), serum, or urine may be used for chemical analysis of coumarin-based rodenticides. The clinician should use a small-gauge needle for blood collection to minimize hemorrhage from the venipuncture site. Stomach contents or vomitus may contain little or no anticoagulant, because clinical signs occur after a 1- to 2-day latent period. Postmortem samples should include liver and kidney.

Analysis • Anticoagulant rodenticides of toxicologic importance to small animals include warfarin, pindone, and chlorophacinone (first generation) and bromadiolone, diphacinone, difethialone, and brodifacoum (second generation). Analytical techniques can range from initial screening with TLC to quantification with HPLC with UV or fluorescence detection using suspect baits, blood, serum, or urine from poisoned animals. In addition, LC-MS/MS can be used if available. Baits commonly contain 0.05% active ingredient. Blood concentration may be very low (ppb range) when highly potent rodenticides are used (e.g., bromadiolone, diphacinone). Liver and kidney from poisoned animals may contain both parent compound and hydroxylated metabolites.

Hydroxylated metabolites are excreted in the urine and may be present for 1 to 2 days after exposure ceases or for much longer (several weeks) if second-generation anticoagulants are involved. Brodifacoum residues may persist in tissues for up to 120 days.² Indirect evidence of vitamin K antagonism can be gained by clinical laboratory testing for nonfunctional vitamin K-dependent coagulation factors (PIVKA), but confirmatory diagnosis is most certain when the rodenticide or its metabolites are detected.

Normal Values • Anticoagulant rodenticides are not expected in blood or tissues; values less than 1 ng/ml (1 ppb) are not associated with clinical signs of toxicosis.

Danger Values • Serum or plasma concentrations vary widely depending on initial dose and duration of exposure and may range from 1 to 10 ng/ml during toxicosis by brodifacoum.¹ The liver appears to concentrate brodifacoum as much as 1000-fold over plasma or serum. Diphacinone and bromadiolone concentrations at necropsy may be less than 1 part per million (ppm) in blood or liver of clinically poisoned dogs.

Causes • In a patient with a bleeding problem, toxicity must be differentiated from other causes of factor deficiencies, as well as thrombocytopenia and disseminated

intravascular coagulation (see Table 17-5 and Chapter 5). Significant concentrations of anticoagulant rodenticides in conjunction with elevated ACT, PT, and aPTT with or without PIVKA, clinically confirms a diagnosis. Because most current rodenticides use the second-generation group, the clinician should assume that residues are present in urine for several weeks.

Aspirin

Indications • Intoxication with aspirin should be considered in patients with vomiting, fever, and laboratory findings consistent with metabolic acidosis (see Chapter 6). Cats are at greater risk than dogs. History of recent administration of aspirin to cats, especially if dose is excessive (i.e., >75 mg/kg for more than 7 days), suggests aspirin toxicosis.

Sample Collection • EDTA blood is analyzed for thrombocytopenia and Heinz bodies while plasma is analyzed for serum salicylate concentration. For tests performed within 24 hours, refrigeration is adequate preservation. Plasma held longer than 24 hours for salicylate analysis should be frozen. Salicylate metabolites may be present in liver and kidney, but toxic concentrations are not established.

Analysis • A qualitative presumptive urine test involves adding a few drops of 10% ferric chloride to 1 ml of urine. In the presence of salicylates, a purple color will form. HPLC, GC, GC-MS, or LC-MS/MS allows more definitive and quantitative testing of urine or plasma.

Normal Values • Normal values are not well established in animals; expected human therapeutic plasma concentrations are less than 50 mg/dl.

Danger Values • Serum or plasma salicylate concentrations indicate exposure, but toxic thresholds are not well established in animals. Human toxic blood concentration is 50 to 100 mg/dl; fatal intoxication in a cat has been associated with a blood concentration of 60 mg/dl.

Causes • Most aspirin toxicosis occurs in cats. A presumptive diagnosis is established in a cat with a history of aspirin administration and vomiting. Diagnosis can be confirmed by plasma salicylate determination.

Blue-Green Algae

Indications • Sudden onset of neurologic signs or acute vomiting followed by delayed hepatic damage or hepatic failure are consistent with blue-green algae poisoning from *Microcystis aeruginosa*. The toxicant microcystin is a potent hepatotoxin that disrupts the liver cytoskeleton, leading to acute hepatic necrosis. In dogs, acetaminophen poisoning, acute aflatoxicosis, iron toxicosis, sago palm (cycad palm), and xylitol poisoning are valid differential diagnoses.

Sample Collection • Potentially dangerous water can be examined for *Microcystis* spp. algae by microscopic identification. Analysis for microcystin toxin testing is

offered at the California Animal Health and Food Safety Laboratories at Davis, California (<http://cahfs.ucdavis.edu>) and Auburn University CyanoPros, (<http://www.cyanopros.com>).

Analysis • Microcystin toxins can be analyzed by ELISA kit, but LC-MS/MS is the method of choice; some of these toxins even require liquid chromatography–triple mass spectrometry (LC-MS/MS/MS).

Normal Values • Normal serum hepatic enzymes and electrolytes suggest no effect from microcystin.

Danger Values • Increased serum concentrations of alkaline phosphatase (ALP), AST, ALT, and bilirubin, as well as hyperkalemia and hypoglycemia are characteristic serum chemistry changes in poisoned dogs. Presence of microcystins in suspect water samples or stomach contents of dogs can confirm exposure to this potent algal toxin.

Histopathology • Hepatic lesions of hepatocyte swelling, enlarged liver, and microscopic evidence of centrilobular hepatic necrosis occur in animals that die from microcystin poisoning.

Artifacts • None reported.

Causes • *Microcystis* spp. most commonly are called blue-green algae, and some species of that genus produce a potent hepatotoxin known as microcystin. Toxic algal blooms occur on nitrogen- and phosphorus-enriched lakes and ponds during hot summers and/or at low water levels. Concentration of the dying algal bloom can lead to microcystin exposure, primarily in dogs that swim in or drink contaminated water.

Carbon Monoxide

Indications • Animals with signs of lethargy, decreased mentation, or sudden onset of somnolence and depression may have exposure to carbon monoxide (CO). Physical findings can include decreased reflexes, tachycardia, and tachypnea. Residual neurotoxicity can occur following prolonged CO exposure.

Sample Collection • Routine sampling of blood and serum is adequate for the range of clinical laboratory examinations important to CO poisoning diagnosis.

Analysis • Whole blood can be analyzed by microdiffusion method or head-space GC.

Danger Values • Normal venous color of blood is accompanied by normal CBC and serum chemistry profile. Blood with elevated carboxyhemoglobin (COHb) is colored red to pink as a result of the pigment properties of COHb. Whole unclotted blood can be tested for CO exposure by measuring COHb in blood. The assay is offered at some veterinary diagnostic laboratories and at many human hospital laboratories. COHb

concentrations greater than 20% are associated with depression and respiratory effects. Blood COHb above 50% indicates serious to acute poisoning that requires immediate attention.

Artifacts • Hemoglobin saturation analysis may be inaccurate when CO poisoning occurs. Pulse oximetry overestimates the amount of saturated hemoglobin because the procedure does not discriminate between COHb and oxyhemoglobin. Thus direct measurement of COHb is important if CO poisoning is suspected.

Causes • CO is a threat to pets from automobile exhaust in enclosed spaces, exhaust leakage during transport, defective space heaters in kennels, and acute exposure to smoke during fires. CO itself is colorless, odorless, and very close to the density of air.

Copper

Indications • Assay of hepatic tissue for copper is indicated when chronic or progressive hepatic damage occurs resulting in chronic hepatic failure sometimes leading to cirrhosis (see Chapter 9).

Essential Oils

Indications • Cats are more susceptible to essential oils than are dogs. Initial signs of poisoning can include acute hepatic failure, ataxia, tremors, hypothermia, and coma. Exposure is often from spills or other direct contact of the oils with skin.

Sample Collection • Blood and serum for determination of hepatic function and coagulation profile is recommended. Endoscopy can be helpful in evaluation of gastrointestinal (GI) damage if ingestion is the route of exposure.

Analysis • Routine methods for clinical chemistry and coagulation profile are appropriate for evaluation of hepatic function and coagulopathy. Assays for essential oils in serum or plasma are not commonly available, but if offered, would generally be by capillary GC with FID detection or capillary GC-MS.

Danger Values • The ALP, AST, ALT, creatine kinase (CK), total bilirubin, and coagulation profile are elevated.

Artifacts • None reported, but the pattern of enzyme changes and coagulation profile are similar to those of other hepatotoxic agents.

Causes • A wide variety of essential oils are extracted from plants and used in a variety of products, from aromas and flavors to medications and insecticides. Some essential oils are considered hazardous to animals, including bitter almond, melaleuca/tea tree oil, pennyroyal oil, and wormwood. Their use in homes results in inadvertent exposure of dogs and cats.

Ethylene Glycol

Indications • Intoxication with ethylene glycol (EG) should be considered in patients with known or suspected ingestion of EG. Ataxia, depression, and vomiting are consistent with EG intoxication; seizures may occur. Cats are more susceptible than dogs on a body weight basis.

Sample Collection • Samples collected in whole blood (in EDTA or heparin), serum or plasma, and urine are required initially. Electrolytes, acid-base status, osmolality, and anion gap should be determined promptly (see Chapter 6). Blood samples taken within 6 hours of ingestion are most likely to be positive for EG. After that acid intermediates are expected (e.g., glycolic acid). Kidney should be obtained at postmortem for histopathologic detection of oxalate crystals; kidney and urine may be saved for possible analysis of EG or acidic metabolites.

Analysis • EG in serum or blood has been detected using a commercial test kit (REACT Ethylene Glycol Test Kit; PRN Pharmacal Inc., Pensacola, FL). Results are best if analysis is performed less than 24 to 36 hours post-ingestion. Availability of rapid tests for EG has been variable in recent years, due to lack of specificity or potential nonspecificity of the assay. Urine, if available, contains EG early (i.e., <6 hours post-ingestion), followed by oxalates and calcium oxalate crystals, which can be viewed by light microscopy using polarized light. Calcium oxalate crystal-luria (see Figure 7-7) appears 3 to 6 hours post-ingestion and, although not infallible, is useful for diagnosis. Serum calcium may be depressed after 4 hours because of complexing with oxalate metabolites of EG. Osmolality, osmolal gap, and anion gap are usually increased, but these may be affected by other alcohols or by metabolic acidosis; severe metabolic acidosis is expected (see Chapter 6). EG is best analyzed by GC or GC-MS. Besides serum and urine, kidney is also a good specimen for analysis.

Normal Values • Trace amounts of EG or oxalate crystals.

Danger Values • EG values greater than 20 to 50 mg/dl indicate exposure; toxic concentrations vary widely, depending on initial dose and time since exposure. High anion gap and a severe metabolic acidosis suggest serious EG toxicosis.

Artifacts • Ethanol or methanol may also abnormally increase serum osmolality but should not cause oxalate crystals or high EG concentrations. EG kits cross-react with propylene glycol, formalin, glycerol, excessive serum lactate dehydrogenase (LDH), and some drugs (e.g., pentobarbital, diazepam).

Causes • EG toxicity needs to be differentiated from toxic (see Tables 17-1 and 17-6) and other causes of acute CNS depression, metabolic acidosis, and oliguric renal failure. Rapid progression (i.e., 1 to 3 days) from CNS signs to oliguric renal failure with calcium oxalate crystals and metabolic acidosis with increased anion gap (see Chapter 6) allow a presumptive diagnosis of EG toxicity.

CNS signs are transient and may be missed. Unless EG toxicity is treated within hours of ingestion, prognosis is poor.

Grapes/Raisins

Indications • Acute onset of vomiting and depression, followed by oliguric or anuric renal failure with hyperkalemia and metabolic acidosis are the major features of grape/raisin toxicosis. Thus far, only dogs have been confirmed susceptible to this toxicosis. Major differentials (nonsteroidal anti-inflammatory drug [NSAID] toxicosis, aminoglycoside poisoning, EG ingestion, and vitamin D₃ [cholecalciferol] poisoning) require prompt determination of the true cause to best support life-saving therapy.

Sample Collection • Presence of grapes or raisins in vomitus should suggest potential poisoning. Since the toxic principle is unknown, eliminating the leading differential diagnoses is the only route for diagnostic confirmation.

Analysis • No assays are available for the toxic principle, which is unknown. Diagnosis is supported by appropriate clinical chemistry results.

Normal Values • Electrolyte values within normal limits, lack of azotemia, and normal urine output and specific gravity are indications that grape/raisin toxicosis is not occurring.

Danger Values • Elevations in BUN, creatinine, ALP, ALT, and amylase and lipase are accompanied by hyperkalemia, hyperphosphatemia, hypercalcemia, isosthenuria, proteinuria, and glucosuria.

Artifacts • Other renal toxicants can have similar patterns of clinical chemistry changes.

Causes • *Vitis* spp. (grapes or raisins) contain unknown toxins that cause acute renal failure associated with hypercalcemia, hyperphosphatemia, hyperkalemia, and increased hepatic enzymes.

Iron

Indications • Iron toxicosis presents in four clinical stages. Stage 1 (0 to 6 hours post-ingestion) is primarily a GI syndrome with vomiting, diarrhea, colic, and GI hemorrhage. Stage 2 (6 to 24 hours) is a period of reduced clinical signs and apparent recovery, but is followed by stage 3, persisting up to 4 days with return of vomiting, diarrhea, GI hemorrhage, hypovolemia, and acidosis. If the animal survives this stage, stage 4 may develop with GI scarring and stricture as a result of severe GI mucosal damage.

Sample Collection • Blood or serum.

Analysis • Testing for iron toxicosis can use flame AAS, GF-AAS, ICP-ES, or ICP-MS. Total serum iron and total iron-binding capacity (TIBC) are compared to assess if iron transport capabilities have been exceeded (Chapter 3).

Normal Values • Normal serum iron in dogs ranges from 95 to 125 µg/dL. Normal TIBC for dogs is 3 to 4 times the serum iron concentration, and 450 µg/dl is the normal upper limit (Chapter 3).

Danger Values • When total serum iron exceeds the TIBC, this indication of free iron suggests the oxidative effects of unbound iron will exert toxic and oxidant effects on the GI tract and liver.

Artifacts • Hemolyzed serum may result in an erroneous high value. There are no significant artifacts in the GF-AAS procedure for serum iron, but total iron in serum may be higher after hemolysis because GF-AAS will detect heme iron that has escaped from hemolyzed red cells.

Causes • In small animals, primarily dogs, iron toxicosis often results from inadvertent ingestion of large numbers of iron supplement tablets. Results are a combination of severe GI damage, hepatic damage, shock, and potential GI scarring in survivors.

Lead

Indications • Lead poisoning should be considered in dogs with periodic episodes of vomiting, with or without mild anemia and basophilic stippling, and cats with anorexia and occasional vomiting. Most lead-poisoned animals also experience seizures and behavioral changes (e.g., hysteria, depression) and occasionally ataxia and facial tremors.

Sample Collection • Whole blood collected in EDTA or heparin is the preferred antemortem sample and can be used to make blood smears looking for basophilic stippling and nucleated RBCs, to test for porphyrins (plasma), or for analysis for lead (lead is primarily in erythrocytes). Lead is stable in blood for long periods, but the sample should be refrigerated to preserve RBC integrity. Urine may be helpful (porphyrins are elevated). Liver and kidney are recommended for postmortem analysis. Recently one or more rapid and simple blood lead assays have become available for emergency use, providing a positive result when the blood lead is above a clinically significant value. The Lead Care II system is an instrument of minimal cost (\$2,000–\$3,000) that can be used for blood lead analysis; it was recently compared to GF-AAS and shown to give comparable results for diagnosis although for precise lead concentration GF-AAS is still the gold standard.

Analysis • Blood lead is usually detected by GF-AAS, ICP-ES, or ICP-MS; in tissues one can also use flame AAS.

Normal Values • Less than 0.05 ppm in whole blood or less than 3 ppm in liver or kidney is considered nontoxic exposure. Normal range of urinary or plasma porphyrins is not well established in small animals. Normal human protoporphyrin values are less than 50 mg/dl. If samples are submitted for analysis, the clinician should include specimens from a normal, unexposed animal for comparison.

Danger Values • Blood lead values greater than 0.3 ppm suggest excessive exposure, and greater than 0.4 ppm are usually considered confirmatory of toxicosis. Hepatic or renal lead values greater than 10 ppm support a diagnosis of lead toxicosis.

Artifacts • Some laboratories that use organic chelate extraction of lead rather than acid digestion prefer heparinized whole blood. Above a minimum value, lead concentrations are not well correlated with severity of clinical signs but are a reflection of body burden. Values as little as 50% of the danger values (i.e., 0.15 ppm) could indicate toxicosis if other parameters (i.e., clinical signs, basophilic stippling, nucleated RBCs, elevated urinary or plasma porphyrins) are consistent with toxicosis.

Causes • Basophilic stippling is neither sensitive nor specific for lead poisoning. When basophilic stippling is found in conjunction with nucleated RBCs and absence of reticulocytosis, a lead assay is indicated. Lead poisoning must be differentiated from toxic (see Table 17-1) and other causes of CNS stimulation. Poisoning is most common in young dogs (i.e., <1 year) with access to old housing, peeling paint, and lead objects. Blood lead concentrations establish exposure and usually correlate with toxicosis but not degree of severity. To detect prolonged low-level exposure with a relatively low blood lead concentration, the clinician should collect a 24-hour urine sample, then inject calcium disodium EDTA (75 mg/kg intramuscularly [IM]), and obtain a second 24-hour urine sample during the 24-hour period following EDTA. Lead-poisoned animals should demonstrate greater than 10-fold increase in urine lead.

MDR1 Gene Mutation

Indications • Certain toxicants and a number of drugs used in veterinary medicine can be unusually toxic to specific breeds of dogs. Susceptibility is influenced by the *MDR1* gene that encodes P-glycoprotein, a drug transport pump that limits drug absorption across the blood-brain barrier. Herding breeds of dogs are especially susceptible. These and others at high risk (>50% *MCR1* mutation) include Australian shepherds (standard and minis), collies, long-haired whippets, all of which have a mutation in the *MDR1* gene that impairs the normal capacity to limit drug absorption and distribution and excretion. Other breeds at lower risk are border collies, English shepherds, German shepherds, McNabs, Old English sheepdogs, Shetland sheepdogs, and silken windhounds. Drugs affected by the mutation include acepromazine, butorphanol, doxorubicin, erythromycin, ivermectin, loperamide, milbemycin, moxidectin, rifampin, selamectin, vinblastine, and vincristine.

Information • Contact the laboratory at www.vetmed.wsu.edu/vcpl or by phone (509-335-3745).

Sample Collection • The laboratory requires 2 ml of blood in an EDTA tube along with a submission form; this should be sent by overnight service on ice. A cheek brush sample is also offered. The clinician should contact the laboratory or check the online instructions.

Analysis • Samples should be submitted to: Veterinary Clinical Pharmacology Laboratory, PO Box 609 (U.S. Mail) or 17 VTH (FedEx or UPS), Pullman, WA 99163-0609; Phone: 509-335-3745; FAX: 509-335-6309.

Danger Values • The laboratory can provide interpretation of testing.

Artifacts • Contact the laboratory for any questions about test results.

Metaldehyde

Common Indications • Intoxication with metaldehyde should be considered in patients with acute onset of salivation, tremors, ataxia, seizures, nystagmus, and vomiting in conjunction with exposure to snail baits or solid fuels (i.e., solid blocks used for camp stoves, also known as “canned heat”).

Sample Collection • Samples can be collected in whole blood, serum, and urine and should be kept frozen until analysis. Postmortem samples (i.e., gastric contents, urine) establish exposure; toxic concentrations are not established.

Analysis • A quick test for metaldehyde is to place the bait in a test tube and warm it slowly. Metaldehyde presents sublimates, then forms a substantial “snowfall” of crystals. Metaldehyde is metabolized to acetaldehyde, which can be detected by GC or GC-MS in serum or urine during acute poisoning. Acetaldehyde produces metabolic acidosis (see Chapter 6).

Normal Values • Metaldehyde is not expected in blood of normal animals.

Danger Values • Not documented.

Artifacts • Acetaldehyde is also a metabolite of ethanol. Acetaldehyde may form during analytical procedures unless blood proteins are precipitated before analysis.

Causes • Metaldehyde toxicosis is somewhat seasonal and geographically influenced. Mild, moist climates (e.g., West Coast, southeastern United States) are expected regions of metaldehyde exposure. Careful history correlated with clinical signs and detectable metaldehyde may allow a presumptive diagnosis. Metaldehyde must be differentiated from toxic (see Table 17-1) and other causes of CNS excitation.

Nonsteroidal Anti-inflammatory Drugs

Indications • A history of probable NSAID overtreatment or prolonged therapy suggests a need to evaluate potential adverse effects. A known massive ingestion, or a patient presenting with a history of continuing vomiting (often hemorrhagic), diarrhea, melena, and/or advancing renal impairment with oliguria or anuria should be cause for evaluating NSAID intake and effects.

Sample Collection • Whole blood and serum are useful for CBC, clinical chemistry profile, and urinalysis. Daily monitoring of packed cell volume is recommended.

Analysis • Analysis is typically done by HPLC.

Danger Values • Decreased packed cell volume (PCV), thrombocytopenia, azotemia, oliguria or anuria, and gastric or intestinal bleeding are all causes for concern that renal or GI damage are continuing. NSAID values for poisoned dogs are not well established, but serum ibuprofen concentrations of dogs with toxicosis may be greater than 100 µg/ml in dogs. Drug concentrations can drop rapidly in the first 48 hours of effective therapy.

Artifacts • None reported.

Causes • NSAIDs are prostaglandin inhibitors, resulting in a beneficial effect defined by reduction of inflammation. However, adverse effects from prolonged use or overdosage can result in impaired blood flow to kidneys and GI tract and compromised gastric bicarbonate buffer secretion. Progressive renal failure and gastric ulceration can result.

Organophosphate and Carbamate Insecticides

Indications • Intoxications with organophosphates (OPs) or carbamate should be considered in patients with muscarinic signs (i.e., salivation, gastrointestinal hypermotility, miosis), nicotinic signs (i.e., muscle tremors, weakness), and CNS excitation or depression, especially if previously treated with OP or carbamate compounds.

Sample Collection • For acetylcholinesterase (AChE) determination, the clinician should collect whole blood in EDTA or heparin. Samples should be refrigerated, not frozen. AChE is stable for at least 3 days when refrigerated; freezing may destroy or reduce the activity. Early sampling is important for carbamates because AChE may spontaneously reverse as soon as 1 to 3 hours after exposure. Sampling time is less critical with OP compounds, because they bind strongly after a period of time (known as aging). Because blood may not contain adequate OP or carbamate residues to evaluate diagnosis, the clinician should collect urine, vomitus, hair, and potential baits or sprays for analysis as well. Fur or hair may be submitted if exposure is by dips or spraying. For dead animals, the clinician should submit the whole brain or at least the caudate nucleus (below the anterior ventral wall of the lateral ventricles). **Caution:** Some OP and carbamate insecticides are highly toxic; clinicians and attending staff should avoid self-exposure by wearing gloves or other protective clothing as well as a mask and eye protection when examining or bathing poisoned animals.

Analysis • Direct chemical analysis of OP or carbamate compounds is performed on baits, pesticides, stomach contents, and liver by GC with TSD or FPD, or GC-MS for OPs. Some carbamates can be analyzed by GC but have a tendency to break down. HPLC or LC-MS is a better technique. Serum pseudocholinesterase determination is

not used in veterinary medicine. Although residues of OPs and carbamates may be present in blood or tissues, very low values and rapid metabolism often hinder detection. Most OPs and carbamates are rapidly metabolized and may be undetectable in blood or tissues within minutes to hours after exposure. Determination of AChE activity is commonly used to detect activity of OP compounds. Several laboratory methods are used.

Normal Values • Different methods of analysis for AChE are used. The clinician should ask the laboratory for a range of normal values and to determine the percentage of inhibition. Treatment for parasites with OP compounds may reduce AChE by up to 50% within the first 1 to 7 days after treatment.

Danger Values • If whole-blood AChE is depressed by more than 50%, excessive OP exposure is indicated. Significant OP or carbamate concentrations vary with the agent suspected. Usually, greater than 50% inhibition of AChE signifies overexposure, and greater than 75% to 80% inhibition supports a tentative diagnosis of toxicosis.

Artifacts • AChE values are highly variable. Blood values may be elevated by hepatic disease. In OP poisoning, cholinesterase depression is persistent; in carbamate toxicosis, cholinesterase values quickly return to normal.

Causes • Typical clinical signs, depression of cholinesterase, and detection of pesticide are usually adequate for diagnosis. Other toxins (see [Tables 17-1 and 17-2](#)) and other causes of CNS excitation or depression, vomiting, and diarrhea (see Chapter 9) need to be considered. The triad of muscarinic, nicotinic, and CNS signs is similar to pyrethrin intoxication (discussed next).

Pyrethrin and Pyrethroid Insecticides

Indications • Intoxication with pyrethrin and pyrethroid insecticide should be considered in animals with tremors, salivation, vomiting, or CNS excitation or depression after application of dips or sprays. Toxicosis is rare unless oral or respiratory exposure has taken place.

Sample Collection • Antemortem animal samples are not useful because of analytical difficulties and interpretation. Bait or sprays can be analyzed for comparison with the stated label concentration. Hair or fur may be used to establish exposure. Analysis of gastric contents confirms oral exposure, and brain pyrethrins/pyrethroids may occur after exposure, but concentrations are not correlated with severity of response. Refrigeration or freezing is adequate preservation.

Analysis • Analysis is accomplished by GC-ECD of concentrated sources such as sprays, dips, hair or fur, or stomach contents. Tissues from exposed animals usually contain very low concentrations. Analysis of brain has been used to confirm exposure, but quantification correlating with toxicosis is not reliable. AChE is not inhibited by pyrethrins, so AChE determination can help eliminate OP toxicosis.

Normal Values • Pyrethrins/pyrethroids are not expected in tissues of normal animals.

Danger Values • Not well documented. Presence of pyrethrins/pyrethroids indicates exposure, which must be correlated with history and clinical evidence. Concentrations of several hundred ppm could be present in the stomach or on hair and only indicate exposure, whereas more than 100 ppb in brain could suggest toxicosis.

Causes • Because of the safety of these products with normal use, pyrethrin or pyrethroid toxicosis is more often suspected than proven. Most reported poisonings are in cats. Analyses for other toxicants (e.g., OPs, carbamates) are used to help rule them out and establish exposure to pyrethrins.

Sago Palm (*Cycas* spp.)

Indications • Affected dogs develop hepatic necrosis within a few days post-ingestion; early signs prior to hepatic damage include vomiting and diarrhea. Neurologic signs ranging from ataxia to seizures can appear secondary to severe hepatic disease. This is a serious toxicosis that can be fatal if not treated promptly.

Sample Collection • Routine sampling of blood and serum is adequate for the range of clinical laboratory examinations important to sago palm diagnosis.

Analysis • Routine assays for the toxic principle(s) of sago palm are not generally available. Diagnosis is supported by clinical chemistry assays as described.

Danger Values • Characteristic changes reflect hepatic function and altered hematologic values. There may be leukocytosis (primarily neutrophilia), increased PCV, decreased total protein, thrombocytopenia, and increased ACT, PT, and partial thromboplastin time (PTT). Increases in ALT, AST, ALP, bilirubin, and bile acids reflect serious hepatic disease. Azotemia may occur as well as hypoalbuminemia and hypoglycemia.

Artifacts • Artifacts in testing are not known, but the clinical laboratory changes may occur with other common and severe hepatotoxins.

Causes • Sago palm or cycad palm (*Cycas* spp.) is a relatively common ornamental plant in subtropical climates (e.g., Florida) or where grown indoors as a container houseplant. All parts of the plant are toxic, with greatest toxicant concentration in the seeds. Consumption by dogs is most likely, and effects result from hepatic toxicosis with related secondary signs. The toxins are glycosides known as cycasin and methylazomethanol.

Vitamin D/Cholecalciferol

Indications • Intoxication with vitamin D should be considered in animals exposed to compounds containing vitamin D₃ (i.e., cholecalciferol). Common trade names include Quintox, Rampage, and Ortho Rat-B-Gone. Bait usually contain 0.075% cholecalciferol. Clinical signs

occur after a 1- to 2-day latent period and include anorexia, lethargy, weakness, and vomiting. Similar signs of renal failure and hypercalcemia in dogs are caused by excess consumption of grapes or raisins.

Sample Collection • Because of the lag period between consumption and onset of clinical signs, time of collection of stomach contents is critical to interpretation (see Analysis). Serum or plasma (EDTA or heparin anticoagulant) can be analyzed. The sample should be refrigerated before analysis and frozen if analysis will be delayed more than 24 hours. Marked hypercalcemia (i.e., >14 mg/dl; see Chapter 8) is highly suggestive of toxicosis, and testing for hypercalcemia is easier than analysis for cholecalciferol or its metabolites. At postmortem, liver and kidney should be collected for potential analysis of vitamin D metabolites. In addition, renal calcium concentration is elevated 10 to 20 times normal and may be a useful postmortem sample to support vitamin D toxicosis.

Analysis • Baits can be tested for vitamin D₃. Gastric contents or vomitus may contain small amounts (i.e., <0.01%) of cholecalciferol. Because of the latent period, the stomach has often emptied the toxic material by the time clinical effects occur. Although liver and kidney contain vitamin D metabolites (e.g., 1,25-dihydroxycholecalciferol), testing is specialized and mainly performed by HPLC or LC-MS/MS and not routinely offered by clinical laboratories. Histopathologic examination with special stains for calcium may also aid in detection of renal or vascular calcification. Postmortem analysis reveals elevated renal calcium values.

Normal Values • Serum 1,25-dihydroxycholecalciferol values less than 30 pg/ml are considered normal. Normal canine renal calcium values are 50 to 200 ppm on a wet weight basis.

Danger Values • Serum 1,25-dihydroxycholecalciferol values greater than 30 pg/ml indicate potential toxicosis, and values greater than 50 pg/ml are toxic. Postmortem renal calcium concentrations greater than 2000 ppm and less than 8000 ppm support a diagnosis of cholecalciferol toxicosis. Values above 8000 ppm indicate potential EG toxicosis.

Artifacts • Hyperactivity of parathyroid function or tumors associated with hypercalcemia (e.g., lymphosarcoma) can produce serum calcium values similar to those of cholecalciferol toxicosis (see Box 8-1).

Causes • A tentative diagnosis can be established from history, clinical signs, hypercalcemia, and azotemia. Other common causes of hypercalcemia are discussed in Chapter 8.

Xylitol

Indications • Early signs usually begin with vomiting and are followed quickly (<1 hour post-ingestion) by hypoglycemia that is manifest as depression, ataxia, weakness, and tremors or seizures. Hepatic damage occurs

later, approximately 12 hours to 72 hours after xylitol consumption. Signs of hepatic damage are expected, including vomiting, diarrhea, melena, icterus, hemorrhage and possibly hepatic encephalopathy.

Sample Collection • Routine sampling of blood and serum is adequate for the range of clinical laboratory examinations important to xylitol diagnosis.

Analysis • Xylitol can be analyzed in serum or urine by GC or GC-MS but must be derivatized.

Danger Values • CBC, clinical chemistry profile, clotting function, and hepatic morphology by abdominal ultrasound and hepatic biopsy are the principal tests that may be altered. Marked hypoglycemia occurs in the first 12 hours; later changes include neutrophilic leukocytosis, thrombocytopenia, increased PCV, and elevated ALT, AST, ALP, and bilirubin. Low serum potassium, variable serum phosphate, and hypercalcemia are common. Coagulation parameters are elevated and fibrin degradation products are elevated. Hepatic biopsy reveals microscopic degenerative and necrotic changes.

Artifacts • Diagnosis is based on clinical laboratory tests, and results can be similar to those with other causes of severe acute hepatic disease or toxicosis.

Causes • Xylitol is present naturally at low levels in fruits and vegetables. It is also used as an alternative sweetener/sugar substitute in chewing gum and candy as well as in some baked food products. Dogs readily ingest foods sweetened with xylitol, and the excess amount from high ingestion causes hypoglycemia as a result of insulin secretion and hepatic necrosis by a currently unconfirmed mechanism. Additional effects are likely secondary to severe hepatic damage or necrosis.

Zinc

Indications • Intoxication with zinc should be considered in patients with acute vomiting, hematuria, hemoglobinuria, icterus, renal failure, and hepatic failure. Radiography showing radiopaque objects (e.g., pennies) in the gastrointestinal tract is an indication for further testing. For dogs being treated with dietary zinc to aid in treatment/prevention of copper hepatotoxicosis, measuring serum zinc may be a useful way to monitor the zinc status.

Sample Collection • Either whole blood (i.e., EDTA, heparin anticoagulant) or serum may be used for analysis. The clinician should consult a laboratory to determine preferred specimens. Trace element-free tubes are preferred (i.e., Vacutainer, royal-blue stopper). Refrigeration is adequate preservation. Postmortem samples include liver and kidney.

Analysis • Zinc analysis is performed by flame AAS, GF-AAS, ICP-ES, or ICP-MS.

Normal Values • Less than 2.0 mg/ml in serum. Normal hepatic zinc is 30 to 70 ppm (wet weight basis).

Danger Values • During toxicosis, serum zinc may range from 3 to 10 µg/ml (ppm). Hepatic and renal zinc concentrations greater than 200 ppm (sixfold to eightfold increase over normal) are likely during toxicosis.

Artifacts • Plastic syringes with rubber grommets may contain zinc and contaminate a sample.

Causes • Most reported cases are from ingestion of zinc-containing objects (e.g., pennies). Other causes of hemolytic anemia (see Chapter 3), renal failure (see Chapter 7), and vomiting (see Table 17-2 and Chapter 9) need to be eliminated. Radiography is indicated in dogs with vague signs of vomiting, hepatic or renal disease, and hemolytic anemia. Vomiting, laboratory indicators of hepatic damage (e.g., increased ALP and ALT), azotemia, and intravascular hemolysis (especially if an intragastric metal density is observed on abdominal radiographs) are highly suggestive of zinc toxicosis.

References and Suggested Readings

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Therapeutic Drug Monitoring

Dawn Merton Boothe

Fixed dosing regimens provided on drug labels are designed to generate plasma drug concentrations (PDCs) within a therapeutic range (Figure 18-1). PDCs are intended to remain above a minimum effective concentration ($C_{\min}$) to avoid therapeutic failure while remaining below the maximum concentration ($C_{\max}$) and minimizing side effects. Fixed dosing regimens are based on pharmacokinetic studies conducted in a small sample population of normal adults. These regimens, however, are generally administered to unhealthy animals for which drug absorption, distribution, metabolism, or excretion (or a combination of these) have been altered by physiologic factors (e.g., age, gender), pathologic factors (leading to renal or hepatic impairment), or pharmacologic factors (e.g., drug interactions). Further, many drugs used in animals are human drugs based on anecdotal doses. As a result, PDC may be higher or lower than expected, increasing risk of toxicity or therapeutic failure, respectively. In these instances, individual monitoring and adjustment of doses using therapeutic drug monitoring (TDM) can optimize drug efficacy and safety. Drugs for which TDM has proven useful in veterinary medicine include several anticonvulsants (e.g., selected benzodiazepines, phenobarbital, primidone, levetiracetam, potassium bromide, zonisamide, gabapentin); antimicrobials (e.g., aminoglycosides: gentamicin, amikacin); cardioactive drugs (e.g., digoxin, procainamide, lidocaine); theophylline; thyroid hormones (for thyroid supplementation); and immunomodulators (e.g., cyclosporine) (Table 18-1).

INDICATIONS

“Start-up, checkup, and what’s up” is a helpful mnemonic for indications. TDM is indicated to establish the patient’s therapeutic range once response has been realized or fails to be realized despite dose increases beyond that recommended (“start-up”); in order to be assured that concentrations have not changed in response to patient, drug, diet, or disease factors (“checkup”); and in response to clinical signs indicative of toxicity or therapeutic failure (“what’s up”). Seven specific situations when TDM may be particularly useful are (1) there is a poorly defined or

difficult-to-detect clinical end point of drug therapy (e.g., immunomodulators); (2) the end point, if manifested, puts the patient at risk (e.g., anticonvulsant therapy); (3) the therapeutic index is narrow, indicating little difference exists between effective and toxic PDCs (e.g., digoxin, theophylline); (4) marked interindividual pharmacokinetic variability exists, making it difficult to predict PDCs (e.g., phenobarbital, cyclosporine); (5) pharmacokinetics are nonlinear, leading to a rapid accumulation to potentially toxic concentrations or to a rapid decline to potentially subtherapeutic concentrations (e.g., phenobarbital [in cats]); (6) drug interactions may potentiate toxicity (e.g., cyclosporine and macrolides, enrofloxacin-induced theophylline toxicity, chloramphenicol- or imidazole-induced phenobarbital toxicity); and (7) there is a need to identify owner noncompliance or poorly compounded drugs as a cause of therapeutic failure or adverse drug reaction, or when overdose may have occurred.

ANALYSIS AND LABORATORY AVAILABILITY

Generally, PDCs are measured with assays involving specific binding of antibodies to the drug of interest. Antibody-based methods are easily automated and are usually more rapid and cost-effective than chromatographic and spectrophotometric assays, the latter being necessary for a limited number of drugs. Although automated assays are also available for bromide, the gold chloride method remains the accepted standard for validation purposes, and a variety of assays can be used to accurately measure PDCs of most drugs. It is imperative that each assay is validated for use in the species of interest and that results generated by the laboratory are accurate and reproducible.

TDM is offered at most veterinary colleges and many diagnostic laboratories in the United States. However, the range of services offered and methods of analysis used vary widely. Before one submits samples to a particular laboratory, information should be sought regarding procedures for sample handling, delivery, and quality assurance practices. When submitting samples to facilities

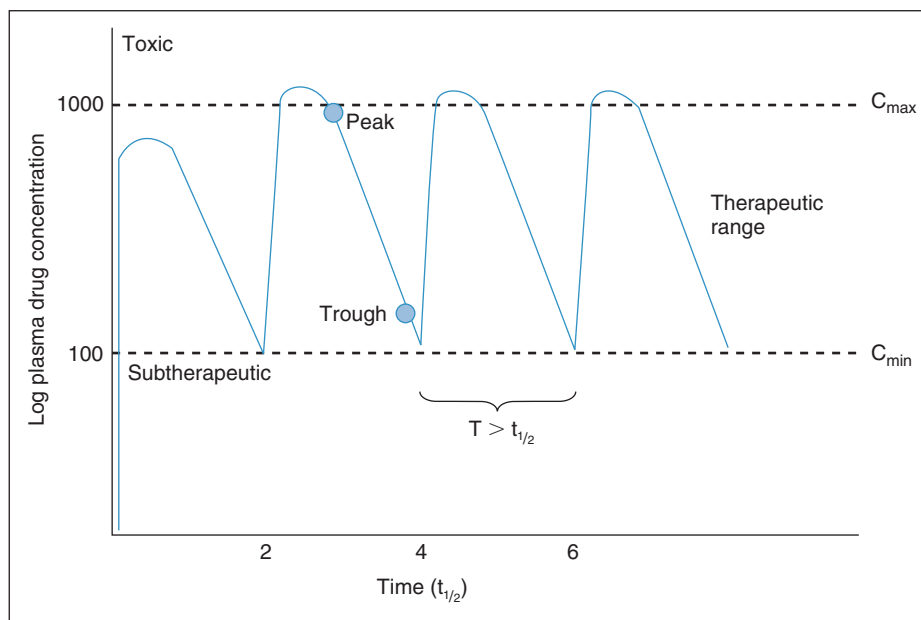


FIGURE 18-1 Plasma drug concentrations (PDCs) after multiple administrations of a drug with a half-life ($t_{1/2}$) that is shorter than the dosing interval. In this example, the dose is administered every four half-lives. Because most (94%) of each dose is eliminated before the next dose, PDCs fluctuate markedly and a “steady state” is never really achieved. Both toxic and subtherapeutic drug concentrations can occur during a single dosing interval; both peak and trough samples should be collected for such drugs.

usually serving human patients, one must be cautious not to confuse recommended therapeutic ranges in humans with those in animals (e.g., clorazepate, bromide, procainamide). Among the more critical considerations is the availability of recommendations by a clinical pharmacologist.

ARTIFACTS

Assuming proper quality assurance practices are used, false assay results arising from errors related to drug analysis are rare. A notable exception may be for drugs that are metabolized. If the drug is a prodrug (e.g., clorazepate) or the metabolites are active (e.g., diazepam), then the assay ideally measures all active compounds. If the metabolites are inactive or only weakly active, the assay should be specific for the parent compound only (e.g., phenobarbital) or the therapeutic range of the assay should reflect the metabolites. Thus the therapeutic range of a drug may vary depending on the assay performed. Erroneous PDC data are more likely to be caused by inappropriate sample collection. Serum separator tubes should be avoided because silicon gel in the tubes can bind to and remove drug from the sample, leading to falsely decreased values. Other examples of artifacts caused by collection tubes include binding of aminoglycosides to glass tubes and binding of digoxin to red stoppers. Hemolysis of blood samples and lipemia should be avoided, but their impact often is negligible. Validation procedures of automated methods, in general, should determine the expected impact of lipemia or hemolysis on accuracy.

IMPLEMENTING THERAPEUTIC DRUG MONITORING

Handling Procedures and Decisions

In general, serum is monitored; however, most methods can measure drug concentrations in plasma. Cyclosporine should be measured in whole blood. Other deviations from these generalities may occur and should be checked prior to drawing and submitting the sample. If samples are to be assayed immediately, special handling procedures (e.g., refrigeration, freezing) often are not necessary. Before TDM is conducted, several decisions should be addressed. Three of these are (1) when TDM should be initiated, (2) the number of samples to be collected, and (3) the timing of sample collection. Because of variability among laboratories, the laboratory of submission should be contacted regarding sample submission.

Steady State

When therapy is initiated, repeated and regular administration of successive doses may result in accumulation of drug. The longer the half-life compared with the dosing interval, the greater the amount of drug accumulation. Eventually the amount of drug eliminated during each dosing interval equals the amount administered with each dose. At this time, steady-state equilibrium has been reached; peak (i.e., the highest blood concentration of the drug) and trough (i.e., the lowest blood concentration of the drug) blood concentrations remain constant across multiple dosing intervals as long as neither the dose nor interval is changed. Ideally, TDM and clinical response to

TABLE 18-1. THERAPEUTIC DRUG MONITORING DATA FOR DRUGS MONITORED IN NORMAL, HEALTHY, SMALL ANIMALS

DRUG	INTERVAL (hr)	THERAPEUTIC RANGE*	ELIMINATION HALF-LIFE†	TIME TO STEADY STATE	SAMPLE COLLECTION PEAK	SAMPLE COLLECTION TROUGH
Amikacin	24	2–25 µg/ml ¹	1–2 hr	<1 day	1 hr (plastic only)	Two half-lives (3–6 hr) ²
Aspirin						
Dog	8–12	50–100 µg/ml	8 hr	40 hr	2–4 hr	BND
Cat	72	50–100 µg/ml	38 hr	8 days		
Benzodiazepines						
Bromide	12–24	100–200 ng/ml ³	8 hr	1 day	2–5 hr	BND ²
		1.0–3.5 mg/ml ⁴	24 days	2–3 months		BND
		(1.4–3.9 mol/L) (with or without other anticonvulsants)				
Cyclosporine	12	12-hr trough above ⁵	6 hr	1 day	2 hr	BND
	12	400–600 ng/ml; for renal transplantation: 750 ng/ml for induction followed by 350–400 ng/ml for maintenance; for perianal fistulae: 100–600 ng/ml; for inflammatory bowel disease and other chronic inflammatory diseases, 250 ng/ml				
		2-hr Peak: 800–1400 ng/ml				
Digoxin						
Dog	12	0.9–3.0 ng/ml	31 hr	7 days	Toxicity: 2–5 hr ⁶ (glass only)	Efficacy: BND ⁵
Cat	12–24	0.9–2.0 ng/ml	32 hr	7 days		
Gabapentin	8	12–21 µg/ml (70–120 µmol/L)	3 hr	<1 day	2 hr ⁷	BND; 8 hr if 12-hr interval

BND, Before next dose; C, cat; D, dog.

*Therapeutic ranges are extrapolated from human patients unless noted otherwise. Ranges may also vary with the laboratory findings and specifically with the instrumentation used to assay the drug of interest. Values in this table may be superseded if the values for the instrument have been appropriately validated. Because samples sizes and assay methodologies vary, the specific laboratory that performs the assay should be contacted regarding sample volume, proper collection tubes, need of refrigeration, and other sample handling specifics, as well as “normal” ranges.

†Half-lives listed here are approximate and are likely to vary among patients. Half-life indicates the amount of fluctuation during a dosing interval and the time to steady-state.

¹“Target” peak concentration for aminoglycosides depend on the infecting organism and specifically on the minimum inhibitory concentration (MIC) of the infecting organism. The target peak concentration should be 8 to 10 times the MIC. Trough concentration should be equal to or below that recommended to minimize toxicity.

²For drugs with a very short half-life and at low concentrations, trough sample may no longer have detectable drug. Wait one or two predicted elimination drug half-lives between peak and trough sample collections. Such drugs do not reach a true “Steady-state.”

³A therapeutic level of 600 ng/ml listed in humans. Assay should measure all benzodiazepines (parent and active metabolites) relative to dosing interval.

⁴If loading, single sample immediately post-load and 3 weeks later. If not loading, single sample at 3 to 4 weeks. For either, collect single sample at 3 months for new baseline.

⁵The therapeutic range varies with the methodology. Use of conversion factors to change results determined by one method to those expected if determined by another method is less ideal than having laboratory-established ranges for each methodology. Laboratory should be contacted for specific range for their assay. Ranges are largely established in people; data are limited in dogs. Ranges are based on a monoclonal immunoassay that detects the parent compound. Trough of 100 ng/ml may be acceptable for some indications, particularly chronic inflammatory disease. Concentrations also may vary with assay.

⁶Both peak and trough recommended because of the potential for a short half-life particularly in those patients responding to therapy; single peak acceptable if toxicity is a concern.

⁷Peak and trough recommended if seizures are difficult to control. Two-hour peak concentrations recommended by our laboratory.

Continued

TABLE 18-1. THERAPEUTIC DRUG MONITORING DATA FOR DRUGS MONITORED IN NORMAL, HEALTHY, SMALL ANIMALS—CONT'D

DRUG	INTERVAL (hr)	THERAPEUTIC RANGE*	ELIMINATION HALF-LIFE†	TIME TO STEADY STATE	SAMPLE COLLECTION PEAK	SAMPLE COLLECTION TROUGH
Gentamicin Dog	12–24	0.5–1.5 µg/ml ¹	0.9–1.3 hr	<1 day	1 hr (plastic only)	Two half-lives (3–6 hr) ²
Cat	12–24	5.0–8.0 µg/ml				
Levetiracetam	8–12	6–21 µg/ml (32–120 µmol/L)	3–4 hr	<1 day	2 hr ⁷	BND; 8 hr if 12-hr interval
Phenobarbital (dog)	12	20–45 µg/ml (86–194 µmol/L)	32–75 hr	14–16 days	2–4 hr ⁷	BND
Primidone						
Dog	12–24	Based on phenobarbital	6.1 hr	14–16 days	2–4 hr ⁷	BND
Cat	12–24					
Procainamide (dog)	12	25–50 µg/ml ⁸	2.9 hr	<1 day (15 hr)	2–4 hr	BND
Theophylline						
Dog	8–12; slow release: 12	10–20 µg/ml	5.7 hr	29 hr	1–2 hr ⁹	BND
Cat	12–24; slow release: 24	10–20 µg/ml	7.9 hr	40 hr		
Thyroid hormones	12–24	0.8–1.5 ng/ml (D) ¹⁰	5–6 hr (D)	<24 hr ¹¹	4–5 hr	BND
	12–24	0.8–1.5 ng/ml (C) ¹⁰				
	12–24	1.5–3.5 µg/dl (D)	12–15 (D)	48–72 hr ¹¹		
	12–24	1.5–5.0 µg/dl (C) ¹²				
Zonisamide	12	10–40 µg/ml ¹³ (35–180 µmol/L)	16–20 (D) ¹² 30 (C)	Up to 14 days	2 hr	BND

⁸As suggested in Papich MG, Davis LE, Davis CA: Procainamide in the dog: antiarrhythmic plasma concentrations after intravenous administration. *J Vet Pharmacol Ther* 9:359, 1986.
⁹For slow-release preparations, one sample may be sufficient.
¹⁰Values for ranges of thyroid hormones reflect a radioimmunoassay. Values are likely to be different for each laboratory. Contact the laboratory, or if doing in house, establish your own normal ranges. Overlap between normal and abnormal is great, regardless of the laboratory, and interpretation should be based on clinical signs.
¹¹Monitoring should not take place until the body has had a chance to physiologically adapt to drug therapy (i.e., 4 to 6 weeks after therapy is implemented).
¹²Monitoring can occur at any time for cats in which hyperthyroidism is being managed.
¹³Human therapeutic range may not be applicable to dogs or cats. Higher concentrations may be indicated in dogs. Half-life may be much longer (>150 hours) in some dogs.

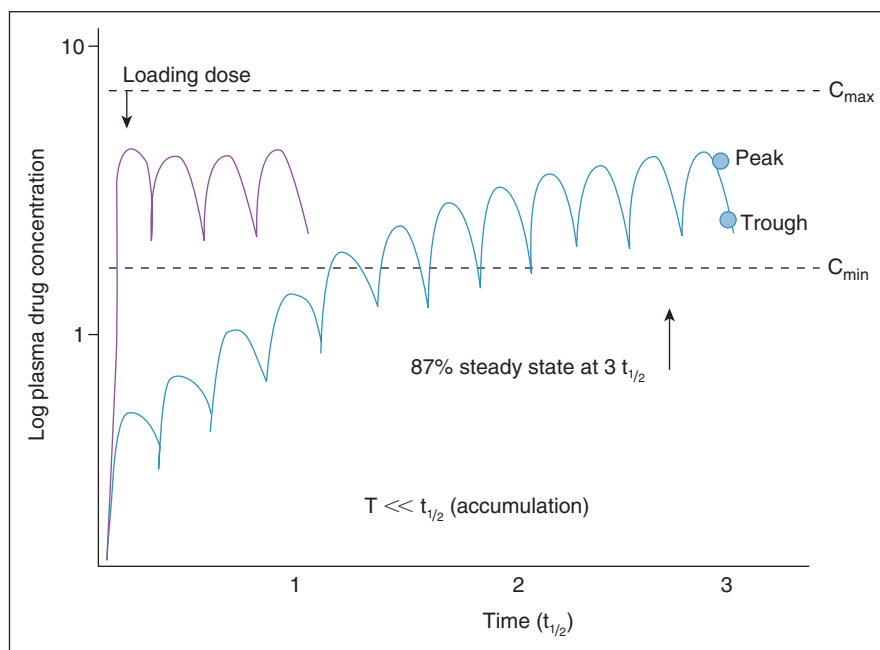


FIGURE 18-2 Plasma drug concentrations (PDCs) after multiple administrations of a drug with a half-life ($t_{1/2}$) that is longer than the dosing interval. In this example, the dose is given approximately twice every drug half-life. Because little of each dose is eliminated during the dosing interval, little fluctuation occurs and a single sample can be collected for monitoring. To rapidly achieve a pharmacologic effect, a loading dose might be given. A steady-state equilibrium will still occur, however, and PDCs may increase or decrease if the maintenance dose does not maintain what the loading dose achieved.

therapy should be implemented at steady state to assess maximum response. Time taken for steady state to be reached depends only on the rate of drug elimination (i.e., drug elimination half-life), irrespective of dose or interval. With multiple dosing of drugs that accumulate, PDC reaches 50% of steady-state concentrations at one half-life ($t_{1/2}$), 75% by two half-lives, 87.5% at three half-lives, and so forth (Figure 18-2). Generally, TDM should not be implemented until three to five half-lives have elapsed since initiation of drug therapy at the same dosing regimen. For example, for phenobarbital with a $t_{1/2}$ of 72 hours, TDM should not be implemented until approximately 15 days after initiation of therapy. If any aspect of the dosing regimen is changed, the same time period (i.e., five half-lives) must elapse for steady-state plasma concentrations to be re-established. An exception might be made for bromide, for which a sample might be taken at one half-life (i.e., 3 weeks) after a dose is begun to proactively assess a dosing regimen; at one half-life, drug concentrations should approximate the steady-state concentration. Times taken to achieve steady state (as well as other pharmacokinetic and dosing information) are listed in Table 18-1. However, these times are based on average half-lives determined from sample populations. Some drugs (e.g., zonisamide, cyclosporine) have half-lives that vary dramatically between individuals. Drug interactions may cause similar variations. Time to steady state of a drug in an individual patient can be determined by measuring the half-life of a drug in that patient (i.e., by collecting both a peak and trough sample).

For drugs with very short $t_{1/2}$ values compared with the dosing interval (e.g., gentamicin $t_{1/2} = 0.9$ to 1.3 hours;

dosing interval 12 to 24 hours), steady state is irrelevant because the drug does not accumulate with repeated dosing. As such, TDM can be initiated immediately (i.e., after the first dose or the first few days after a dosing change).

Loading Dose

When the clinical situation necessitates immediate attainment of therapeutic drug concentrations (e.g., a seizing patient being treated with bromide), PDC predicted at steady state can be achieved more rapidly by administration of a single loading dose followed by recommended maintenance doses (see Figure 18-2). Loading doses are based on the volume of tissue that dilutes the drug; bioavailability of the drug also affects loading dose for those drugs not administered intravenously (IV). Despite the fact that predicted steady-state PDCs have been achieved by loading, PDCs are not yet at steady state and will not be until equilibrium has been reached at three to five half-lives of the drug at maintenance dose in the patient. PDC achieved after loading may increase or decrease if the maintenance dose is more or less, respectively, than that eliminated during each dosing interval. For bromide in particular, TDM should occur within 1 to 3 days after loading to confirm that targeted concentrations (i.e., predicted steady-state concentrations) have been achieved and again one drug half-life later (i.e., 3 to 4 weeks for bromide) to ensure that the maintenance dose is maintaining PDC achieved with loading. Although use of a loading dose decreases the time taken for maximum

response to occur (by avoiding slow accumulation to steady state), hazards of adverse reactions are much greater. Thus loading doses are not advised for drugs characterized by a narrow therapeutic index and that tend to cause undesirable adverse reactions (e.g., digoxin).

Number of Samples

The relationship between drug half-life and dosing interval (as well as the intent of monitoring) determines the number of samples to be collected. If the dosing interval is longer than drug half-life (e.g., diazepam, levetiracetam, gabapentin, most antibiotics, cyclosporine), the PDC fluctuates widely during each dosing interval (see Figure 18-1). Assuming that both efficacy and safety are of interest, one should collect two samples to coincide with peak and trough concentrations. For drugs with a long half-life compared with dosing interval, drug concentrations do not change much during each dosing interval, and a single sample generally reflects PDC throughout the dosing interval. The need for one or two samples varies for some drugs. For digoxin, collection of both a peak and trough sample might be considered because of the narrow therapeutic index of this drug and marked variability in half-life that can occur in patients receiving cardiovascular drugs. A single trough sample is often sufficient for phenobarbital; however, because elimination half-lives of less than 24 hours have been measured, clinicians should collect both a peak and trough sample in patients in which seizure control is difficult. Although cyclosporine has a short half-life, recommendations extrapolated from humans may be based on either a peak or trough sample, or both.

Timing of Sample Collection

Timing for single sample collection depends on the intent of TDM. If efficacy is of concern and only one sampling time is indicated, assessment of trough concentrations is recommended because this concentration is always the lowest during the dosing interval and thus can consistently be compared with subsequent TDM. In contrast, peak concentrations do not occur consistently at the same time and are difficult to predict, particularly with orally administered drugs. One can easily determine the trough concentration by collecting the sample immediately before administration of the next dose. Ideally, trough PDC will not drop below recommended C_{min} . For drugs with a short half-life compared with the dosing interval (e.g., the aminoglycosides) and for which both a peak and trough sample will be submitted, collection of a trough sample may result in nondetectable concentrations. Collection of the second sample at two to three half-lives (rather than a true trough sample) after dosing would be more prudent for such drugs, but only if a peak sample also is collected such that half-life can be calculated and a trough concentration predicted. For toxicity, a single peak sample should be determined.

Timing of samples used to determine peak concentrations is more difficult to estimate because these samples should only be collected when drug absorption and distribution are complete. In particular, oral absorption of drugs is variable and is influenced by feeding (fasting is

generally indicated). Generally, peak PDCs occur 2 to 4 hours after oral administration, although drugs that are absorbed slowly take longer to achieve peak PDC (e.g., 5 hours for phenobarbital). For intramuscular (IM) and subcutaneous (SC) administrations, absorption occurs more rapidly (i.e., 30 to 60 minutes). Absorption times are not relevant to IV-administered drugs, but distribution may take 1 to 2 hours. Thus peak PDCs are generally measured 1 to 2 hours after parenteral drug administration. Peak and trough samples are generally collected during a single dosing interval. A less ideal method is collection of a trough sample, dosing of the patient, followed by collection of a peak sample.

MODIFYING DOSE REGIMENS

Precise adjustment of dose regimens requires generation of a pharmacokinetic profile and calculation of pharmacokinetic parameters, such as clearance values and volumes of distribution, which can be determined only after IV administration of drug unless bioavailability of the drug is known in the patient. If this degree of sophistication is necessary, a specialist in clinical pharmacology should be consulted. A modified profile can be generated from data collected with two (peak and trough) samples. For situations in which precise dose adjustment is not essential and the patient is at steady state, a relatively simple approach is to use the direct proportionality between dose and PDC. For example, if the dose is doubled, the resulting PDC will also be doubled. Conversely, if the dose is halved, the resulting PDC will be halved. The following dose adjustment equation describes this relationship:

$$\text{New dose} = \frac{\text{Old dose} \times \text{Target PDC}}{\text{Measured PDC}}$$

With the therapeutic ranges listed in Table 18-1 and PDC results derived from TDM, a new adjusted dose can be calculated that will achieve a desired target PDC. For example, if the measured peak PDC after administration of 8 mg/kg of zonisamide to a dog is 23 $\mu\text{g/ml}$ and a decision is made to increase the dose to achieve a target peak PDC of 35 ng/ml , the new dose can be estimated as follows:

$$\begin{aligned}\text{New dose} &= \frac{8 \text{ mg/kg} \times 35 \text{ } \mu\text{g/ml}}{23 \text{ } \mu\text{g/ml}} \\ &= 12 \text{ mg/kg}\end{aligned}$$

Increasing the dose for drugs with a short half-life may result in PDCs that are both too high and too low during a dosing interval. The decision to modify the dose versus interval should take into account client convenience and desired fluctuation in PDC during the dosing interval. Dosing interval also can be changed proportionately, although calculation of elimination half-life provides a more accurate method on which changes in dosing interval can be based. Elimination half-life of a drug can be calculated as $t_{1/2} = 0.693/k_{el}$, where k_{el} is the slope of the line drawn between the two TDM points

(peak or C_1 , t_2 ; and trough, C_2 , t_2): $k_{el} = \ln [C_1/C_2]$ divided by $t_2 - t_1$. The natural log ($\ln$) for concentrations must be used because drug elimination is first-order. A clinical pharmacologist can be consulted for assistance in design of a dosing regimen.

Clinicians should never base decisions to modify a dosing regimen solely on PDCs and their relationship to the therapeutic range. The therapeutic ranges that accompany TDM results are not the same as “normals” that accompany clinical laboratory tests. A therapeutic range reflects the C_{min} and C_{max} between which a large percentage (e.g., 95%) of the target population responds to drug therapy. Some animals, however, respond outside the therapeutic range (below C_{min} or above C_{max}), whereas other animals may become “toxic” within the therapeutic range. Clinical response always must be considered when doses are adjusted. This is particularly important for drugs with effective therapeutic and toxic PDC ranges that overlap (e.g., digoxin, thyroid hormones). Whenever doses are changed, TDM should be continued to confirm that target PDCs for the patient have been achieved.

While TDM will determine if the patient has achieved a desired therapeutic range, it cannot be used to determine therapeutic success. When PDCs are within recommended therapeutic ranges but the animal fails to respond satisfactorily or has responded but a decrease in dose is desired, minor, “stair-step” adjustments in dose are recommended. The size of each increment depends on the therapeutic range and safety of the drug. If a patient’s response to drug is insufficient, doses are increased proportionately to the desired increase in PDC until either desired response is achieved or maximum limit of the range is reached and risk of adverse effects precludes further increase in dose. For example, phenobarbital (therapeutic range: 15 to 45 $\mu\text{g}/\text{ml}$) might be increased by 5- $\mu\text{g}/\text{ml}$ and bromide (therapeutic range: 1.0 to 3.5 mg/ml) by 0.5- mg/ml increments. Doses for each drug are increased by about 25% to achieve incremental increase. Likewise, if PDCs are close to or exceed the maximum of the range (raising concerns that side effects or toxicity could result), stepwise decreases can be used to establish minimum effective concentration necessary to control clinical signs while avoiding toxicity (e.g., anti-convulsant therapy). For each dose change, response should not be evaluated until a new steady state has been reached.

THERAPEUTIC MONITORING OF SELECTED DRUGS

Aminoglycosides (Amikacin, Gentamicin)

Indications • TDM is used to monitor aminoglycoside PDCs in life-threatening, serious, or chronic infections caused by susceptible bacteria.

Sample Collection • Generally, both C_{max} (to verify efficacy) and C_{min} (to verify safety) are indicated. Current dosing recommendations are for 24-hour dosing intervals. Ideally, C_{max} should be 8 to 10 times the minimum inhibitory concentration of the drug for the infecting organism (based on culture and susceptibility data).

C_{min} concentrations should be less than 2 $\mu\text{g}/\text{ml}$. Because elimination half-life of aminoglycosides is short (i.e., 1 to 3 hours), trough concentrations should be collected at 4 to 6 hours after peak concentration rather than true trough (i.e., “before next dose” [BND]) because concentrations are not likely to be detectable just before the next dose.

Artifacts • Aminoglycosides are bound to glass. Samples intended for TDM should not be collected in glass tubes or should be immediately transferred to plastic tubes upon collection.

Dosing Modification • Doses can be proportionately increased or decreased based on C_{max} . Intervals should be prolonged in increments of one drug half-life if C_{min} is above recommended trough concentration (see Table 18-1).

Benzodiazepines (Diazepam, Clorazepate)

Indications • TDM is indicated to assure PDCs do not drop below C_{min} in patients receiving the drug for long-term seizure control. Diazepam is generally the drug measured in cats and clorazepate in dogs. Because each is metabolized to active metabolites, both parent drug and metabolites are monitored.

Sample Collection • The elimination half-life of the benzodiazepines is short, and PDCs fluctuate significantly during an 8-hour or 12-hour dosing interval. Two samples are recommended (see Table 18-1).

Dosing Modification • The interval should be decreased if magnitude of PDC fluctuation during dosing interval is unacceptable. The dose should be changed proportionately as indicated by PDCs. Both dose and interval may be modified simultaneously.

Bromide

Indications • TDM is indicated in any epileptic patient receiving the drug. Regardless of the salt used, bromide is the active ingredient measured.

Sample Collection • The half-life of bromide is very long compared with dosing interval; hence, single samples are indicated for TDM. Trough concentrations are recommended, although any time during the dosing interval is acceptable. Concentrations should be measured at baseline steady state, at 3- to 6- or 12-month intervals, and any time the animal has a seizure. Proactive monitoring should occur after a loading dose (to determine what the loading dose achieved) and again at 3 weeks post-load (to assure the maintenance dose is maintaining concentrations). If a loading dose is not administered, proactive monitoring might occur 3 weeks into therapy with the maintenance dose. Proactive monitoring (at one half-life) should also be implemented in patients for which chloride intake or clearance might change. A clinical pharmacologist is strongly recommended for consultation when monitoring bromide.

Artifacts • An automated method has been validated for bromide assay by some laboratories; the gold chloride method remains the accepted standard technique. Bromide can cause chloride concentrations to be artifactually increased.

Cyclosporine

Indications • TDM is indicated in patients undergoing organ transplantation and those being treated for immune-mediated diseases and selected chronic inflammatory and allergic diseases (e.g., asthma, inflammatory bowel disease, perianal fistulae). Although the drug is used extensively in dogs and cats, information regarding therapeutic ranges is limited. Cyclosporine appears to be better tolerated in dogs or cats compared to humans; a toxic concentration has not been well established nor have therapeutic ranges necessary to mount an appropriate response. Monitoring to assure concentrations are not extremely high or are within human suggested ranges, establishing the patient's therapeutic range once response has occurred, and assuring concentrations are maintained is reasonable and recommended. Cyclosporine is subject to many drug interactions including those involving P-glycoprotein and drug-metabolizing enzymes. As such, preventative monitoring should be implemented in patients receiving other drugs that might interact with cyclosporine. Because human generic products vary widely in animals, monitoring should be implemented each time a product is changed. Because different methods yield different results, the laboratory should be called for therapeutic ranges specific for that laboratory. Using correction factors to adjust results obtained by one method to those that would have been obtained by another method are less useful than having therapeutic ranges established for each laboratory's assay.

Sample Collection • In critical patients, both peak and trough concentrations should be collected (see Table 18-1). For most conditions, recommendations are based on trough (but not mid-interval) samples. Because the drug distributes to red blood cells (RBCs), whole blood generally should be collected. In critical patients, monitoring might occur weekly to every 3 weeks during induction and then monthly to every 3 to 6 months thereafter. In the case of potential drug interactions, monitoring both peak and trough such that half-life can be calculated should occur just before and 1 to 2 weeks after the interacting drug is begun or discontinued.

Dosing Modification • When dosing at 12-hour intervals, the dose generally should be modified. If an animal is a poor responder and is being dosed at 24-hour intervals, a 12-hour dosing interval should be considered.

Digoxin

Indications • TDM is indicated in patients suspected of digoxin toxicity or in patients with inadequate response.

Sample Collection • Both a peak and trough sample may be necessary to design the safest and most effective dosing regimen. This is particularly true in patients with

an inadequate response or those in which disposition of digoxin is likely to be abnormal because of changes in renal and hepatic function induced by either disease or drug therapy. If toxicity is of concern, a single peak sample may be sufficient. Absorption and distribution of digoxin can vary with product used and patient, and it can take up to 8 hours. Peak concentrations at 3 to 5 hours are recommended (see Table 18-1). The half-life of digoxin also varies, being as short as 12 hours in some animals despite a 36-hour half-life reported in normal animals. If a single sample is to be collected to evaluate efficacy, a trough sample should be collected. **Because cardiac disease can cause marked disposition changes, TDM should be implemented before and after response to afterload therapy, diuretic therapy, or both because disposition is likely to change as the disease responds to therapy.**

Artifacts • Red stoppers on collection tubes may bind drug, decreasing concentrations.

Dosing Modification • If a single sample has been collected, the dose of digoxin should be changed proportionately. If both peak and trough samples are collected, a pharmacokinetic profile (see earlier) should be generated for the patient and an interval appropriate and convenient should be established along with a new dose.

Gabapentin

Indications • TDM is indicated in any animal receiving gabapentin for seizure control; treatment for analgesia also might be considered.

Sample Collection • The half-life of gabapentin is sufficiently short that PDC may fluctuate markedly during a dosing interval. Both peak and trough samples are recommended at baseline and in the event of therapeutic failure. For efficacy and to assure concentrations remain above C_{min} during a dosing interval, a true trough is recommended for single samples. Neither a peak nor a mid-interval sample can predict concentrations throughout a dosing interval. If the patient remains seizure-free, a single trough sample is sufficient at 6- to 12-month intervals. However, if the patient has a "break-through" seizure, both peak and trough samples are recommended.

Dosing Modification • The dose of gabapentin may be changed proportionately. However, a shortening of the dosing interval may be indicated in patients with a short half-life.

Levetiracetam

Indications • TDM is indicated in any animal receiving levetiracetam for seizure control.

Sample Collection • The half-life of levetiracetam is markedly variable, but it is very short in most animals receiving the regular-release product. PDC may fluctuate markedly during a dosing interval. Both peak and trough

samples are recommended at baseline and in the event of therapeutic failure. For efficacy and to assure concentrations remain above $C_{\min}$ during a dosing interval, a true trough is recommended for single samples. Neither a peak nor a mid-interval sample can predict concentrations throughout a dosing interval. If slow-release product is administered, a peak and trough are recommended as therapy is begun to establish proper dosing interval. If the patient remains seizure-free, a single trough sample is sufficient at 6- to 12-month intervals. However, if the patient has a “breakthrough” seizure, both peak and trough samples are recommended.

Dosing Modification • The dose of levetiracetam may be changed proportionately. However, a shortening of the dosing interval may be indicated in patients with a short half-life.

Phenobarbital and Primidone

Indications • TDM is indicated in any animal receiving either of these drugs for seizure control. Primidone is converted to phenobarbital, which is measured rather than primidone. Disposition of both drugs varies markedly among animals. In addition, both drugs cause induction of drug-metabolizing enzymes, leading to greater variability. Finally, side effects (i.e., grogginess, hepatotoxicity) of the drug are of sufficient concern that samples should be monitored for safety.

Sample Collection • The half-life of phenobarbital can be short enough to necessitate both peak and trough samples, or it can be long enough to allow only a trough sample collection. A trough sample is recommended at baseline and, in anticipation of induction, 1 to 3 months later (see Table 18-1). If the patient remains seizure-free, a single trough sample is sufficient at 6- to 12-month intervals. However, if the patient has a “breakthrough” seizure, both peak and trough samples may be indicated.

Dosing Modification • The dose of phenobarbital can be changed proportionately.

Procainamide

Indications • Although not routine, procainamide could be monitored in patients responding inadequately to long-term control of cardiac arrhythmias.

NOTE: Efficacy of procainamide in people reflects an active metabolite that is minimally formed in dogs. Recommended concentrations reflect both procainamide and its acetylated metabolite.

Sample Collection • Because half-life of procainamide is sufficiently short and the potential of adverse drug interactions sufficiently great, both peak and trough samples should be collected (see Table 18-1). A single trough sample can also be used to verify efficacy.

Theophylline

Indications • TDM is indicated in patients receiving the drug as a bronchodilator that have not experienced sufficient response and those suspected of reacting adversely to theophylline.

Sample Collection • The half-life of regular theophylline is short enough that it is best to collect both peak and trough samples (see Table 18-1). For efficacy, a single trough sample is acceptable. For safety (i.e., detection of adverse reactions), a single peak sample can be collected. For slow-release preparations, absorption is slow enough that a single trough sample is sufficient.

Dosing Modification • The dose of theophylline can be changed proportionately. Intervals can be modified if both peak and trough data are available.

Thyroid Hormones

Indications • TDM is indicated in any animal receiving thyroid supplementation (usually dogs) or animals with hyperthyroidism that are being medically managed (usually cats). Because thyroxine (T_4) is the circulating hormone of interest (triiodothyronine [T_3] being located primarily intracellularly), it is generally the hormone measured. The majority of hormone is bound to proteins and thus is pharmacologically inactive; therefore free T_4 (fT_4) may be the preferred hormone to be tested. Equilibrium dialysis is the most accurate method to measure fT_4 .

Sample Collection • Although concentrations of hormones may achieve steady state rapidly, the body may not equilibrate immediately. Physiologic equilibrium to thyroid hormones may take 4 to 6 weeks, and monitoring should not take place until equilibrium is likely to have occurred (see Table 18-1). Likewise, response to drugs intended to control hyperthyroidism may take 3 to 4 weeks.

Analysis • Methods that have been validated in the species of interest are preferred.

Dosing Modification • Doses should be altered proportionately.

Zonisamide

Indications • TDM is indicated in any animal receiving zonisamide for seizure control.

Sample Collection • Half-life of zonisamide varies from as little as 15 hours to as many as 150 hours in dogs. However, for most animals, half-life is sufficiently long that PDCs do not fluctuate markedly during a dosing interval. A single trough sample should be sufficient for monitoring in most patients. For patients at risk for life-threatening seizures or in patients who do not respond well, or in patients also receiving phenobarbital, both a peak and trough sample might be recommended at baseline and in the event of therapeutic failure. If the patient remains seizure-free, a single trough sample is sufficient at

6- to 12-month intervals. Therapeutic range for zonisamide has yet to be established in dogs but may exceed maximum recommended concentration in humans. Zonisamide at high PDC may suppress thyroid hormone synthesis, and thyroid monitoring may be indicated.

Dosing Modification • The dose of zonisamide may be changed proportionately.

Suggested Readings

- Boothe DM: Principles of drug therapy. In *Small animal clinical pharmacology and therapeutics*, ed 2, St. Louis, 2011, Elsevier.
- Boothe DM: Factors affecting drug disposition. In *Small animal clinical pharmacology and therapeutics*, ed 2, St. Louis, 2011, Elsevier.
- Boothe DM: Therapeutic drug monitoring. In *Small animal clinical pharmacology and therapeutics*, ed 2, St. Louis, 2011, Elsevier.

Appendix I:

Listing of Selected Referral and Commerical Laboratories

Harold Tvedten and Michael D. Willard

LISTING OF SELECTED REFERRAL AND COMMERCIAL LABORATORIES

Appendix I in previous editions included contact information for various laboratories and details on submission procedures, prices, etc. In the current edition we list website addresses for laboratories that have information on their websites about sample preparation, submission procedures and restrictions, prices, etc. The advantage of contacting the appropriate websites is that, unlike a book, information is updated frequently. However, the URLs for laboratory websites will change from time to time; the reader must be prepared to use various search engines if the web address changes. The reader can also try the shorter company address if the longer address does not work. This listing is not complete nor does it endorse any or all of the laboratories or indicate that they provide better service than laboratories not listed. The current listing is simply to provide some practical information for veterinarians trying to obtain specialized testing. Because most of the readers and authors are from North America, this list includes mainly laboratories in the United States and Canada.

Full-Service Laboratories as per the American Association of Veterinary Laboratory Diagnosticians (AAVLD)

<http://www.aavld.org>

The website above provides a listing of laboratories accredited by the AAVLD including contact information for many diagnostic laboratories in the United States and Canada. These are usually full-service laboratories with endocrinology, microbiology, toxicology, clinical pathology, nutritional, and other types of testing. These laboratories have websites that may be accessed to see how to prepare samples for testing and current prices.

Listing of National Animal Health Laboratories

<http://eden.lsu.edu/Topics/AgDisasters/BSE/Pages/NationalAnimalHealthLaboratoryNetwork.aspx>

Centers for Disease Control and Prevention (CDC)

<http://www.cdc.gov/>

The CDC provides information on infectious disease threats for animals and people and often is the source of specific diagnosis of new or unusual infectious agents.

SELECTED STATE DIAGNOSTIC LABORATORIES IN THE UNITED STATES AND CANADA

United States

Alabama	http://labs.alabama.gov/
Arizona	http://microvet.arizona.edu/
California	http://cahfs.ucdavis.edu/
Colorado	http://www.dlab.colostate.edu/
Connecticut	http://cvmdl.uconn.edu/
Florida	http://www.doacs.state.fl.us/ai/labs/labs_main.shtml
Georgia	http://www.vet.uga.edu/dlab/
Hawaii	http://hawaii.gov/hdoa/ai/vlab
Illinois	http://vetmed.illinois.edu/vdl/

Continued

SELECTED STATE DIAGNOSTIC LABORATORIES IN THE UNITED STATES AND CANADA—cont'd

Indiana	http://www.addl.purdue.edu/
Iowa	http://vetmed.iastate.edu/diagnostic-lab
Kansas	http://www.vet.k-state.edu/depts/dmp/service/index.htm http://www.vet.ksu.edu/depts/dmp/service/index.htm
Kentucky	http://www.ca.uky.edu/gluck/ServLDDC.asp
Louisiana	http://laddl.lsu.edu/
Maine	http://www.umaine.edu/vetlab/
Maryland	http://www.mda.state.md.us/animal_health/regional_animal_health_offices.php
Michigan	http://animalhealth.msu.edu/
Minnesota	http://www.vdl.umn.edu/
Mississippi	http://www.mbah.state.ms.us/links/index.html
Missouri	http://mda.mo.gov/animals/health/diagnosticlabs.php
Montana	http://liv.mt.gov/lab/default.mcpix
Nebraska	http://nvdl.unl.edu/
New Hampshire	http://www.colsa.unh.edu/facilities/new-hampshire-veterinary-diagnostic-laboratory
New Jersey	http://www.state.nj.us/agriculture/divisions/ah/prog/lab.html
New Mexico	http://128.123.206.6/animal-and-plant-protection/veterinary-diagnostic-services
New York	http://ahdc.vet.cornell.edu/
North Carolina	http://www.ncagr.gov/vet/ncvdl/
North Dakota	http://www.vdl.ndsu.edu/
Ohio	http://www.agri.ohio.gov/addl/
Oklahoma	http://www.cvm.okstate.edu/index.php?option=com_content&view=category&layout=blog&id=17&Itemid=158 http://www.cvhs.okstate.edu/index.php?option=com_content&view=section&id=4&Itemid=64
Oregon	http://oregonstate.edu/vetmed/diagnostic
Pennsylvania	http://www.padls.org/
South Carolina	http://www.clemson.edu/public/lph/lab/
South Dakota	http://www.sdstate.edu/vs/adrdl/index.cfm
Tennessee	http://www.tennessee.gov/agriculture/regulatory/kord.html
Texas	http://tvmdl.tamu.edu/
Utah	http://www.usu.edu/uvid/
Washington	http://www.vetmed.wsu.edu/depts_waddl/
Wisconsin	http://www.wvdl.wisc.edu/
Wyoming	http://wyovet.uwyo.edu/
Canada	
British Columbia	http://www.agf.gov.bc.ca/ahc/index.htm
Guelph	http://www.labservices.uoguelph.ca/units/ahl/

SELECTED GENERAL LABORATORIES PERFORMING ROUTINE AND SOME SPECIALIZED TESTING

Antech Diagnostics
<http://www.antechediagnostics.com/>

Marshfield Laboratories
<https://www.marshfieldlabs.org/veterinary/>

IDEXX Diagnostics
<http://www.idexx.com/>

Heska Laboratories
<http://heska.com/>

SELECTED LABORATORIES FOR INFECTIOUS DISEASES**Tick-Borne Infectious Agents**

Vector Borne Disease Diagnostic Laboratory, College of Veterinary Medicine, North Carolina State University

<http://www.cvm.ncsu.edu/vhc/csds/ticklab.html>

Arthropod borne and Infectious Diseases Laboratory, Colorado State University

<http://www.cvmb.colostate.edu/cvmb/aidl.htm>

Serologic/Molecular Tests for Various Pathogens

Auburn University

<http://www.vetmed.auburn.edu/molecular-diagnostics/assays>

Trypanosoma cruzi Titer

Texas Veterinary Medical Diagnostic Laboratory, Texas A & M University

<http://tvmdl.tamu.edu/>

Fungal Antigens

MiraVista Diagnostics

<http://www.miravistalabs.com/>

Coccidioidomycosis Titer

Coccidioidomycosis Serology Laboratory, University of California at Davis

<http://www.ucdmc.ucdavis.edu/medmicro/cocci.html>

Tularemia Titer

National Veterinary Services Laboratory, U.S. Department of Agriculture, Ames, Iowa

http://www.aphis.usda.gov/animal_health/lab_info_services/downloads/AmesDiagnosticTestingCatalog.pdf
http://www.aphis.usda.gov/animal_health/lab_info_services/

Serology for Pythiosis

This website is not for a laboratory; it is for Dr. Grooters, who runs the lab.

<http://www.vetmed.lsu.edu/vcs/showfacultybio.asp?facultyID=20>

Various Serologic Tests

ARUP Laboratories (C 6 peptide antibodies)

<http://www.aruplab.com/guides/ug/tests/0051044.jsp>

National Veterinary Laboratory, Inc

<http://www.natvetlab.com/>

Galaxy Diagnostic, Inc

<http://www.galaxydx.com/web/>

Protatek Reference Laboratory

<http://www.protatek.com/reflab.html>

<http://www.protatek.com/>

SELECTED LABORATORIES FOR ENDOCRINE TESTS*

Endocrine Diagnostic Service, Auburn University

<http://www.vetmed.auburn.edu/endocrine-diagnostics>

Animal Health Diagnostic Center Endocrinology Laboratory, Cornell University

<http://ahdc.vet.cornell.edu/sects/Endo/>

Animal Health Diagnostic Laboratory, Michigan State University

<http://www.animalhealth.msu.edu/>

College of Veterinary Medicine Diagnostic Services, University of Tennessee

<http://www.vet.utk.edu/diagnostic/>

Veterinary Diagnostic Laboratory: Endocrinology Laboratory, University of Minnesota

<http://www.vdl.umn.edu>

*This list is derived from (<http://www.compendo.org/testing.html>).

SELECTED URINARY STONE ANALYSIS LABORATORIES

Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California at Davis

<http://www.vetmed.ucdavis.edu/usal/index.cfm>

Minnesota Urolith Center, Department of Small Animal Clinical Sciences, University of Minnesota

<http://www.cvm.umn.edu/depts/minnesotaurolithcenter/home.html>

SELECTED LABORATORIES FOR VARIOUS SPECIALIZED TESTS**Hemostasis Testing**

Coagulation Section—Animal Health Diagnostic Center,
Cornell University

<http://ahdc.vet.cornell.edu/sects/Coag/staff/contact.cfm>

Taurine Quantitation

Amino Acid Laboratory, Department of Molecular
Biosciences, School of Veterinary Medicine, University of
California at Davis

<http://www.vetmed.ucdavis.edu/VMB/aal/index.cfm>

Carnitine Quantitation

School of Medicine, University of California at San Diego

<http://vetneuromuscular.ucsd.edu/index.html>

Myasthenia Gravis (Acetylcholine Receptor Antibody)

Comparative Neuromuscular Laboratory, University of
California at San Diego

<http://vetneuromuscular.ucsd.edu/>

Metabolic Screening/Genetic Screening

Veterinary Hospital of University of Pennsylvania

<http://research.vet.upenn.edu/Default.aspx?alias=research.vet.upenn.edu/penngen>

MDR-1 Gene Testing

Veterinary Clinical Pharmacology Laboratory, Washington
State University

<http://www.vetmed.wsu.edu/depts-vcpl/test.aspx>

LISTING OF OTHER AVAILABLE TESTS**Canine Genetic Diseases**

American Kennel Club

<http://www.akcchf.org/search/search.jsp> (must search
under "tests for genetic diseases")

Therapeutic Drug Monitoring

Clinical Pharmacology Laboratory, Auburn University,
Auburn, Alabama

<http://www.vetmed.auburn.edu/clinical-pharmacology-lab>

Blood Typing

Veterinary Hospital of University of Pennsylvania

<http://research.vet.upenn.edu/Default.aspx?alias=research.vet.upenn.edu/penngen>

Cardiac Disease

NT-proBNP (IDEXX Diagnostics)

<http://www.idexx.com/>

SELECTED RESOURCES FOR TOXICOLOGIC TESTS**Selected Laboratories**

California

<http://cahfs.ucdavis.edu/>

Eurofins Laboratory

<http://www.eurofinsus.com/>

Animal Health Diagnostic Laboratory, Michigan State University

<http://www.animalhealth.msu.edu/>

Online Sources of Help for Intoxications

ASPCA Animal Poison Control Hotline

<http://www.asPCA.org/pet-care/poison-control/>

Pet Poison Hotline

<http://www.petpoisonhelpline.com>

Adverse Drug Effect Reporting

<http://www.fda.gov/AnimalVeterinary/SafetyHealth/ReportProblem/ucm055305.htm>

Agency for Toxic Substances and Disease Registry

<http://www.atsdr.cdc.gov/>

Appendix II:

Listing of Selected Reference Values

Harold Tvedten

LISTING OF SELECTED REFERENCE VALUES

Selected reference values are provided here for general guidance in interpretation of laboratory results. References to literature values or other sources for the information are given in respective tables. *It is essential to have reference values established locally for the instruments and methods used locally in order to have optimal interpretation of results from those instruments.* It is difficult to find 120 “normal” and representative dogs and cats each time a new instrument is purchased, but at least 20 animals should be used to compare a new method’s results to well-established reference values, which used at least 120 normal animals.

Hematology values such as hemoglobin concentration and white blood cell (WBC) count are considered somewhat “universal,” but there are definite differences in results from different types of instruments—and even within a type and model of instrument—related to calibration of the instrument and which software version is being used. For example, different hematology instruments use different stains and methods for determining

reticulocytes, and some reference values vary distinctly among instrument types. The Sysmex XT-2000iV detects more reticulocytes than the Advia 120 (see first table), and the LaserCyte detects fewer reticulocytes.

References for automated hematology results for dogs and cats from the Siemens Advia 120 are provided in the first two tables based on modified literature results from Moritz et al.,¹ but these will vary from results of other instruments. Note that differential leukocyte counts from automated instruments do not enumerate nonsegmented neutrophils; thus a manual differential leukocyte count is required to demonstrate a left shift. Neither the Advia 2120, Sysmex XT-2000iV, LaserCyte, nor Cell-Dyn instruments correctly detect basophils in the dog and cat; therefore no automated results for basophils are listed. Similarly, hemostasis and clinical chemistry results will vary with instruments, methods, and even different batches of reagents purchased at different times.

Reference

1. Moritz A, Fickenscher Y, Meyer K, et al: Canine and feline hematology reference values for the Advia 120 hematology system. *Vet Clin Pathol* 33:32, 2004.

CANINE AUTOMATED HEMATOLOGY REFERENCE VALUES FOR ADVIA 120 AND SYSMEX XT-2000iV INSTRUMENTS

PARAMETER*	UNIT	Advia 120		Sysmex XT-2000iV	
		MEAN	2.5 th –97.5 th PERCENTILES	MEAN	2.5 th –97.5 th PERCENTILES
RBC	10 ¹² /L	7.56	5.68–9.08	6.3	5.1–7.6
Hb	g/dL	17.07	13.77–20.38	15.8	12.4–19.2
HCT	L/L	0.52	0.42–0.62	0.43	0.35–0.52
MCV	fL	69	63–75	66	60–71
MCH	pg	23	20–25	24	22–26
MCHC	g/dL	32.9	31.6–34.4	36.6	34.4–38.1
CHCM	g/dL	31.3	29.7–32.8	—	—
RDW	%	12.6	12.0–13.2	16.2	13.2–19.1
WBC	10 ⁹ /L	10.55	5.84–20.26	11.0	5.6–20.4
LUC	%	1.0	0.6–1.7	—	—

Continued

CANINE AUTOMATED HEMATOLOGY REFERENCE VALUES FOR ADVIA 120 AND SYSMEX XT-2000iV INSTRUMENTS—CONT'D

PARAMETER*	UNIT	Advia 120		Sysmex XT-2000iV	
		MEAN	2.5 th –97.5 th PERCENTILES	MEAN	2.5 th –97.5 th PERCENTILES
Total neutrophils	10 ⁹ /L	6.22	4.27–9.06	6.6	2.9–13.6
Lymphocytes	10 ⁹ /L	3.08	2.04–4.66	2.6	1.1–5.3
Monocytes	10 ⁹ /L	0.51	0.24–2.04	0.7	0.4–1.6
Eosinophils	10 ⁹ /L	0.35	0.10–1.20	0.9	0.1–3.1
LUC	10 ⁹ /L	0.11	0.03–0.58	—	—
Reticulocytes	%	0.65	0.14–1.48	0.89	0.3–2.4
Reticulocytes	10 ⁹ /L	48.8	10.9–111	58.2	19.4–150
PLT	10 ⁹ /L	338	173–486	316	108–562
MPV	fL	11.5	8.6–14.4	10.6	9.1–12.7

*From Moritz A, Fickenscher Y, Meyer K, Failing K, Weiss DJ: Canine and feline hematology reference values for the Advia 120 hematology system. *Vet Clin Pathol* 33:32, 2004.

FELINE HEMATOLOGY REFERENCE VALUES WITH ADVIA 120 HEMATOLOGY ANALYZER

PARAMETER*	UNIT	DISTRIBUTION	MEAN	2.5 th –97.5 th PERCENTILES
RBC	10 ¹² /L	Log-normal	8.13	5.9–11.2
Hb	g/dL	Gaussian	11.71	8.2–15.3
HCT	L/L	Other	0.38	0.24–0.46
MCV	fL	Gaussian	45.97	37–55
MCHC	g/dL	Other	31.17	26.2–35.9
RDW	%	Other	16.46	13.8–21.1
WBC	10 ⁹ /L	Log-normal	11.99	7.73–18.6
Total neutrophils	10 ⁹ /L	Gaussian	7.83	3.1–12.5
Lymphocytes	10 ⁹ /L	Log-normal	3.13	1.3–7.5
Monocytes	10 ⁹ /L	Log-normal	0.37	0.1–1.1
Eosinophils	10 ⁹ /L	Log-normal	0.36	0.1–2.2
LUC	10 ⁹ /L	Log-normal	0.04	0–0.4
Reticulocytes	%	Log-normal	0.23	0.0–1.2
Reticulocytes	10 ⁹ /L	Log-normal	18.56	4–94
PLT	10 ⁹ /L	Other	281.34	42–630
MPV	fL	Log-normal	15.02	10–26

*From Moritz A, Fickenscher Y, Meyer K, Failing K, Weiss DJ: Canine and feline hematology reference values for the Advia 120 hematology system. *Vet Clin Pathol* 33:32, 2004.

HEMOSTASIS REFERENCE VALUES

TEST	UNITS	CANINE	FELINE
HEMOSTASIS			
Platelets*	×10 ³ /μl	166–575	230–680
PT*	seconds	5.1–7.9	8.4–10.8
aPTT*	seconds	8.6–12.9	13.7–30.2
Fibrinogen	mg/dl	100–245	110–370
FDP	μg/ml	<10	<10

*Coagulation tests were performed with Fibrometer System at Michigan State University's Veterinary Clinical Center laboratory, 1999. Feline values are from Killingsworth C: Screening coagulation tests in the cat. *Vet Clin Pathol* 14:19, 1985.

CHEMISTRY REFERENCE VALUES

TEST	UNITS	CANINE	FELINE
Arterial blood gas			
pH		7.36–7.44	7.36–7.44
Pco ₂	mm Hg	36–44	28–32
Po ₂	mm Hg	90–100	90–100
Tco ₂	mEq/L	25–27	21–23
HCO ₃	mEq/L	24–26	20–22
Venous blood gas			
pH		7.34–7.46	7.33–7.41
Pco ₂	mm Hg	32–49	34–38
Po ₂	mm Hg	24–48	35–45
Tco ₂	mEq/L	21–31	27–31
HCO ₃	mEq/L	20–29	22–24
A:G ratio (calculated)		0.89–2.68	0.80–1.68
Albumin	g/dl	3.2–4.7	3.0–4.6
ALP (alkaline phosphatase)	IU/L	0–90	4–81
ALT (SGPT)	IU/L	10–94	23–109
Ammonia (resting)	μg/dl	25–92	30–100
Amylase	IU/L	371–1503	531–1660
AST (SGOT)	IU/L	10–62	14–41
Bile acid (fasting)	μmol/L	0.0–15.3	0.0–7.6
Bile acid (2 hour)	μmol/L	0.0–20.3	0.0–10.9
Bilirubin—total	mg/dl	0.1–0.6	0.1–0.7
BSP		0–5%	0–5%
BUN	mg/dl	7–32	18–41
Calcium	mg/dl	9.0–11.9	8.4–11.5
Cholesterol	mg/dl	116–317	64–229
CK	IU/L	51–529	91–326
Creatinine	mg/dl	0.5–1.4	0.7–2.2
Electrolyte profile			
Sodium (Na)	mEq/L	146–156	153–162
Potassium (K)	mEq/L	3.9–5.5	3.6–5.8
Chloride (Cl)	mEq/L	113–123	119–132
Tco ₂	mEq/L	16.9–26.9	12.5–24.5
Anion gap		9–22	10–27
GGT	IU/L	1–6	1–3
Globulin	g/dl	1.5–3.5	2.1–4.0
Glucose	mg/dl	53–117	57–131
LDH	IU/L	42–130	63–193
Lipase	U/L	90–527	
Lipase	Sigma-Tietz units	0.1–1.3	0.1–0.4
Magnesium	mg/dl	1.36–2.09	1.38–2.36
Osmolality—serum			
Calculated	mOsm/Kg	302–325	319–371
Determined	mOsm/Kg	293–321	290–320
Osmolality—urine	mOsm	200–2000	200–2000
Phosphorus	mg/dl	1.9–7.9	2.9–8.3
SDH	IU/L	5.4–33.3	0.4–10
Total protein	g/dl	5.3–7.6	5.5–7.7
Triglyceride	mg/dl	10–500	10–500
Uric acid	mg/dl	0–1	0–1
Serum iron (Abbott)	μg/dl	61–255	34–122
Iron profile*			
Total iron	μg/dl	84–233*	68–215
UIBC	μg/dl	142–393	105–205
TIBC	μg/dl	284–572	†
Saturation	%	20–59	†

Reference values were from Michigan State University's Veterinary Clinical Center laboratory, 1999.

*From Harvey JW, French TW, Meyer DJ: Chronic iron deficiency anemia in dogs. *J Am Anim Hosp Assoc* 18:946, 1982.

†Values not directly determined.

SELECTED FACTORS TO CONVERT COMMONLY USED UNITS TO THE INTERNATIONAL SYSTEM OF UNITS

SUBSTANCE	COMMON UNIT	×	CONVERSION FACTOR	=	INTERNATIONAL UNIT
Albumin (proteins)	g/dl		10		g/L
Ammonia	μg/dl		0.587		μmol/L
Bicarbonate	mEq/L		1		mmol/L
Bile acids	μg/ml		2.45		μmol/L
Bilirubin	mg/dl		17.1		μmol/L
Calcium	mg/dl		0.25		mmol/L
Total CO ₂	mEq/L		1		mmol/L
Pco ₂	mmHg		0.133		kPa*
Cholesterol	mg/dl		0.026		mmol/L
Chloride	mEq/L		1		mmol/L
Creatinine	mg/dl		88.4		μmol/L
Folate	ng/ml		2.27		nmol/L
Glucose	mg/dl		0.0555		mmol/L
Insulin	μIU/ml		0.0417		μg/L
Iron	μg/dl		0.179		μmol/L
Magnesium	mg/dl		0.411		mmol/L
PO ₂	mm Hg		0.133		kPa*
Phosphate	mg/dl		0.323		mmol/L
Potassium	mEq/L		1		mmol/L
Sodium	mEq/L		1		mmol/L
Urea nitrogen	mg/dl		0.357		mmol/L
Xylose	mg/dl		0.067		mmol/L
Enzymes	IU/L		0.017		μkat/L†
Amylase	Somogyi units/dl		1.85		IU/L
ALT (SGPT)	Karmen units/ml		0.48		IU/L
Lipase	Cherry-Crandall units/ml		278		IU/L
Blood cells	Cells/μl		1,000,000		Cells/L‡

*kPa, kilopascal.

†1 kat, 1 katal (i.e., 1 mol/sec), is for reporting enzyme activity, but most laboratories report serum enzyme activity in international units of IU/L or U/L (i.e., 1 μmol/min).

‡Reported as 10⁹ cells/L.Modified from Kaneko JJ: *Clinical biochemistry of domestic animals*, ed 3, New York, 1980, Academic Press, pp 785–791; Lehmann HP, Henry JB: SI units. In Henry JB, editor: *Clinical diagnosis and management by laboratory methods*, ed 17, Philadelphia, 1984, WB Saunders, pp 1428–1450.

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