15

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Microbiology and Infectious Disease

Infectious agents may be identified directly by cytologic analysis, histopathologic evaluation, culture, viral isolation, antigen detection, or polymerase chain reaction (PCR). 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 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 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.

Historical findings can increase the degree of suspicion for infectious diseases. 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]*, Rocky Mountain spotted fever [RMSF; *Dermacentor* spp.], babesiosis *[R. sanguineus]*) or appropriate travel history (e.g., coccidioidomycosis in the Southwest; RMSF in the Southeast; blastomycosis in the Mississippi, Missouri, and Ohio River valleys) can also suggest an infectious disease. Vaccination history, deworming history, and determination of whether other animals or people in the environment are also 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 mycolonus (canine distemper virus), or testicular swelling plus pain (canine brucellosis).

Finally, clinicopathologic abnormalities can suggest disease caused by infectious agents. Neutrophilic leukocytosis, particularly if found concurrently with a left shift or degenerative neutrophils (see Chapter 4), is consistent with an infectious cause of disease. Gram-negative sepsis is suggested by leukopenia with a degenerative left shift. Monocytosis can be induced by persistent infection with a number of intracellular agents that result in persistent infection. Polyclonal (e.g., multiple infectious causes) or monoclonal gammopathies (e.g., usually induced by neoplasia, rarely associated with canine ehrlichiosis) 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.

Advantages • Inexpensive, readily available, and may allow rapid confirmation and identification of an infectious agent. Assists in establishing normal flora and contaminants versus infection (e.g., interpretation of relative numbers of bacteria and yeasts in the ear canal). Gives visualization of relative numbers of organisms at 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, haemobartonellosis, 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), 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's and Romanowsky's-type

stains (see Chapter 16). The examination is started on low power (10x magnification), with oil immersion (100x) used for inspection of bacterial morphologic features (i.e., rods, cocci) and Gram's stain characteristics (i.e., gram-positive [blue] or gram-negative [pink]). The primary disadvantage of Gram's 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's-type stains. Gram's staining may be variable; organisms in body fluids may stain differently from those grown on a blood agar plate. Gram's stain demonstrates the gram-positive, branching filaments of *Actinomyces* spp. and *Nocardia* spp. (see Color Plate 4C). 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* (Color Plate 4F; see Chapter 9). 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 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's-type stains, and examined for intracytoplasmic aggregations of *Chlamydophila felis* (previously *Chlamydia*).

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), *M. haemominutum* (cats only), *Haemobartonella canis*, *Cytauxzoon felis* (cats only), and *Babesia* spp. sometimes infect 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 10x 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 10x or 40x objective to search for hyphae, spores, conidia, budding yeasts, and fungus-induced damage (e.g., swollen or broken hair shafts). The 40x objective is used to identify arthrospores (dense aggregates of spherical structures that may cover the hair shaft [see Color Plate 3C]). Failure to find arthrospores does not rule out dermatomycosis. Culture is more sensitive for diagnosis of dermatophytosis (see Fungal Culture).

Romanowsky's-type stains (e.g., Wright's) are used in preference to wet-mount preparations and ink when looking for fungi other than dermatophytes (see Chapter 16). Romanowsky's-type stains are also useful in identifying yeasts such as *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Sporothrix schenckii*, *Coccidioides immitis,* or *Cryptococcus neoformans* (see Color Plate 4E) in exudates, CSF, lymph node aspiration cytology, or transtracheal aspiration cytology.

Viral Diseases

Canine distemper virus inclusions in lymphocytes, neutrophils, or erythrocytes (Color Plates 2D and 2E) are diagnostic of infection but are only present transiently, so false-negative results are common. Rarely, FIP-inducing strains of coronavirus result in transient intracytoplasmic inclusions in circulating neutrophils.

CULTURE AND ANTIMICROBIAL SUSCEPTIBILITY

Common Indications • Culture and antimicrobial susceptibility is indicated in most suspected bacterial diseases (Table 15-1), especially when clinical syndromes have been resistant to medications. *Remember:* Skin and mucosal surfaces have a resident microflora (Table 15-2); therefore, care must be taken to avoid contamination.

Advantages • Usually allows the most effective treatment to be administered.

Disadvantages • Requires time for agents to grow; some organisms are fastidious or have special culture requirements; the expense; and 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 following section). 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 transport media* 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 it decreases isolation of organisms (*Bartonella* spp. are

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*BBL Septi-Chec, Becton Dickinson Microbiology Systems, Sparkes, Md.

TABLE 15-1. Bacteria Commonly Isolated from Various Sites in Infectious Disorders in Dogs and Cats

Integument
Pyoderma
  Staphylococcus aureus/ intermedius
  Proteus spp.
  Pseudomonas spp.
  Escherichia coli (usually secondary to staphylococci)
Ear
  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 (GI) Tract
Intestine
  Salmonella spp.
  Campylobacter spp.
  Clostridium perfringens
  E. coli
Genitourinary Tract
  E. coli
  Proteus spp.
  Klebsiella spp.
  S. aureus/intermedius
Eye
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.

TABLE 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.
Ear
  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 +   *Streptococcus* spp.
               Staphylococcus spp.
               Bacillus spp.
               Corynebacterium spp.
    Gram −   Enterobacteriaceae (especially *E. coli*, *Enterobacter* spp., *Proteus* spp., and *Klebsiella* spp.)
               Pseudomonas spp.
               Neisseria spp.
               Moraxella spp.

  Anaerobic
    Gram +   *Clostridium* spp.
               Lactobacillus spp.
               Propionibacterium spp.
               Bifidobacterium spp.
    Gram −   *Bacteroides* spp.
               Fusobacterium spp.
               Veillonella spp.
    Other   Spirochetes
               Mycoplasma spp.
               Yeasts

Genitourinary Tract
Distal Urethra and Prepuce
    Gram +   *S. aureus*
               S. epidermidis
               Streptococcus spp.
               Mycoplasma spp.
               Bacillus spp.
               Corynebacterium spp.
    Gram −   *Flavobacterium* spp.
               Haemophilus spp.
               Moraxella spp.
               Pasteurella spp.
               Klebsiella spp.

Data compiled from Greene CE, editor: *Clinical microbiology and infectious diseases of the dog and cat*, Philadelphia, 1998, WB Saunders.

exceptions; they can be cultured from EDTA tubes). 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 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 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

*Trypticase Soy Broth, Becton Dickinson Microbiology Systems, Cockeysville, Md.

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 *E. coli* are important genera. 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 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.

Genitourinary Tract • Urine obtained by cystocentesis is preferred for urine culture. If a patient is severely thrombocytopenic (<50,000/μl), 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 performed if pyuria is identified in absence of calculi, masses, and aerobic bacteria.

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 diskospondylitis should be evaluated for *Brucella canis* infection serologically (see Serologic Tests for Bacterial Infections). Intervertebral joints can be cultured after fluoroscopically guided aspiration or when decompressive spinal surgery is required. Most cases of diskospondylitis develop after hematogenous spread of bacteria from an extravertebral source. Blood and urine are commonly cultured from patients with diskospondylitis; *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.

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 Table 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 indicates oropharyngeal contamination) (see Figure 11-10). 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 (Randolph et al, 1993; Chandler and Lappin, 2002). 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's bacterial transport medium. *Mycoplasma* spp. culture should be specifically requested.

Nasal specimens are best obtained from nasal lavage, 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 Table 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.

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[*]Culturette, American Scientific Products, McGaw Park, Ill.
[†]BBL CultureSwab Plus, Becton Dickinson Microbiology Systems, Sparkes, Md.

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 (Jones, 1998). 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 fastidious *Bacteroides* spp. and *Fusobacterium* spp. 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's 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 caseload and available equipment. With the exception of blood and feces, the majority of culture procedures can be performed in office. Readers are referred elsewhere for details of equipment and operation of an in-office microbiology laboratory (Hirsh and Ruehl, 1986).

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 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 • May be effective even though disk diffusion techniques suggest otherwise (e.g., antibiotics concentrated in urine).

Disadvantages • Expense, inability to perform in office, and need to determine if required concentrations of a certain antibiotic are feasible. Ideally, blood concentrations of drugs should be more than four 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 • Simplicity and suitability for most routine cultures, can be performed in office, and applicability for rapidly growing organisms (e.g., Enterobacteriaceae, *Staphylococcus aureus*).

Disadvantages • Not suitable for slow-growing organisms and anaerobes; inaccuracy in predicting susceptibility of poorly diffusing antibiotics (e.g., polymyxins); 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 prereduced culture media.

Interpretation • Recognizing normal flora (see Table 15-2) 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 Table 15-1. The overlap between resident and pathogenic organisms should be noted.

Staphylococcus intermedius 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. intermedius* in deep pyoderma.

Primary bacterial rhinitis is rare in dogs and cats but can result from infection with *B. bronchiseptica*, *Mycoplasma* spp., and *Chlamydophila felis* (cats). Primary bacterial pneumonia can result from *Bordetella 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 (Ling et al, 1983). 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's and Mycose medium.

Systemic and SC fungi may require 2 weeks' cultivation on Sabouraud's medium for growth to occur.

SEROLOGIC TESTS FOR BACTERIAL INFECTIONS

Bartonellosis, Feline *(Bartonella henselae)*

Occasional Indications • Most cats with bartonellosis (*Bartonella henselae*) are subclinically infected; however, fever, uveitis, lymphadenopathy, gingivitis, or stomatitis occur in some cats and are indications for antibody testing. Because the organism is transmitted between cats by *Ctenocephalidies felis*, cats with a history of flea infestation are more likely to be infected. The clinical significance of other *Bartonella* spp. that infect cats is unknown at this time.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected by immunofluorescent antibody assay (IFA), enzyme-linked immunosorbent assay (ELISA), and western blot immunoassay. See Appendix I for availability of testing.

Cats infected by fleas are generally bacteremic by week 2; bacteremia can persist for months (Chomel et al, 1996). Antibodies are first detected at approximately week 2, and detectable concentrations persist for months

after resolution of bacteremia. Bacteremia has also been detected in antibody-negative cats. *B. henselae* seroprevalence is often greater than 50% in regions with fleas; in healthy cats in a Birmingham, Alabama shelter, the seroprevalence was 71.4% (Lappin et al, 2000). Because so many healthy cats are seropositive, detection of positive antibody test results does not definitively diagnose clinical bartonellosis in a clinically ill cat; the clinician must still exclude other causes of the clinical syndrome. Because antibody test results do not correlate to blood culture results and because treatment of healthy carriers is not recommended (Lappin et al, 2003), screening healthy cats for *Bartonella* antibodies is not indicated. Detection of local antibody production by the eye has been used to document uveitis as a result of bartonellosis (Lappin et al, 2000).

Definitive diagnosis is based on detection of the organism in blood. Culture for *B. henselae* is generally performed on a 1.5 ml whole blood sample collected aseptically and placed into an EDTA-containing tube. Organism DNA can be amplified from blood or aqueous humor by PCR assay (Jensen et al, 2000; Lappin et al, 2000). Positive blood culture or PCR results are consistent with current infection but do not document clinical illness. Repeated bacteremia has been detected in experimentally inoculated and naturally infected cats (Kordick et al, 1995); therefore, a single negative blood culture or PCR result does not exclude infection. See Appendix I for availability of *Bartonella* spp. blood culture and PCR.

Bartonellosis, Canine *(Bartonella vinsonii)*

Occasional Indications • Dogs from endemic areas or with an appropriate travel history with unexplained myocarditis, granulomatous lymphadenitis, cutaneous vascular disease, hemolytic anemia, polyarthritis, granulomatous meningoencephalitis, or thrombocytopenia should be considered for *B. vinsonii* serologic screening. Based on seroprevalence studies, rural dogs with fleas or ticks are most likely to be exposed.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected by IFA. See Appendix I for availability of testing.

Antibodies can be detected in dogs with and without clinical signs. Seronegative test results make clinical illness caused by *B. vinsonii* less likely. Seropositive test results suggest that

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[*]Dermatophyte test media, Pittman Moore, Mundelein, Ill.
[†]Derm Duet, Bacti Lab., Mountain View, Calif.

the organism could be involved, but the clinician must still exclude other causes of the clinical syndrome.

Definitive diagnosis is based on detection of the organism in blood, but the organism is more difficult to culture than *B. henselae*. Amplification of the organism DNA is usually more successful than culture. (See Appendix I for availability of testing.) Positive blood culture or PCR results are consistent with current infection but do not document clinical illness. A single negative blood culture or PCR does not exclude infection by *B. vinsonii*.

Borreliosis (Lyme Disease)
(Borrelia burgdorferi)

Occasional Indications • Dogs from areas endemic for *Ixodes* ticks or with an appropriate travel history and fever, lameness, glomerulonephritis (Dambach et al, 1997) or nonseptic, suppurative polyarthritis should be suspected of having Lyme disease (borreliosis) and screened for antibodies against *Borrelia burgdorferi*. Serologic testing should be considered in dogs with CNS disease, renal disease, and myocardial disease.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by IFA, ELISA, and Western immunoblot. See Appendix I for availability of testing.

IgM and 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. Cross-reactivity with *B. burgdorferi* antigens used in IFA and ELISA occurs with other spirochetes; thus, a positive titer does not document exposure to *B. burgdorferi*. *B. burgdorferi* vaccines induce antibodies that are detected by IFA and ELISA and one of the available point-of-care assays.[*] Western immunoblot can differentiate vaccine-induced antibodies from those from natural infection (Jacobsen, Chang, and Shin, 1996). A point-of-care kit[†] commercially available for detection of *B. burgdorferi* antibodies against the C6 peptide, *E. canis* antibodies, and *Dirofilaria immitis* antigen was licensed recently. Antibodies against the C6 peptide are not induced by vaccination, so positive results

of this kit denote exposure to *B. burgdorferi*. Some nonpathogenic strains of *B. burgdorferi* induce antibody production but not clinical disease (Breitschwerdt, 1995). Some dogs with acute Lyme disease are seronegative on initial testing; documentation of an increasing antibody titer can suggest recent exposure. Antibody titers greater than or equal to 1:1000 have been detected in clinically normal dogs. Healthy dogs develop the same antibody responses as clinically ill dogs, however. Because of these factors, interpretation of serum antibody titers is difficult. Serum antibodies against *B. burgdorferi* only documents exposure to *B. burgdorferi* (or a similar antigen), not clinical disease. Finding a higher titer in CSF than in serum occurs in some dogs with suspected neurologic disease secondary to Lyme disease.

Definitive diagnosis requires demonstration of the organism by culture, histopathologic evaluation of tissue, or PCR. Presumptive diagnoses of clinical Lyme disease in dogs can be based on appropriate clinical, historical, 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, diskospondylitis, or uveitis should be suspected of having brucellosis and screened for antibodies against *B. canis*.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by rapid slide agglutination test (RSAT), tube agglutination test (TAT), agar gel immunodiffusion (AGID), and ELISA (Carmichael and Shin, 1996).

The RSAT and TAT are screening procedures; a 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 autoagglutination in hemolyzed samples. AGID can be performed using cell wall antigens or cytoplasmic antigens. AGID performed with cytoplasmic antigens is the most specific

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[*]LymeCHEK *Borrelia burgdorferi* antibody test, Synbiotics Corp, San Diego, Calif.
[†]Snap3Dx, IDEXX Laboratories, Portland, Me.

[*]D-Tec CB, Synbiotics Corp, San Diego, Calif.

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 4 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 (Carmichael and Shin, 1996). 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 are 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. The most common pathogenic serovars in

dogs include *Leptospira canicola*, *L. icterohaemorrhagiae*, *L. grippotyphosa*, *L. bratislava,* and *L. pomona* (Rentko and Ross, 1992).

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 (Bolin, 1996). 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.

Antibodies are detected by MAT days to weeks after inoculation. Most laboratories assess multiple serovars in the assay. Cross-reactivity exists between some serovars; the serovar with the highest titer is probably the serovar causing infection. Acutely infected dogs are often MAT negative; dogs with suggestive clinical signs of disease but negative MAT results should be retested in 2 to 4 weeks; development of a positive titer confirms recent infection. A fourfold increase in antibody titer over 2 to 4 weeks confirms recent infection. Vaccination can induce positive MAT titers.

Definitive diagnosis requires demonstration of the organism by urine dark-field microscopy, phase contrast microscopy, or culture. Examination of urine for leptospires 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. In acute disease, leptospiremia occurs for a few days to 2 weeks after infection, during which time the organism may sometimes be cultured from blood. Urine can be cultured, but repeated culture may be needed because of intermittent shedding. The combination of increasing antibody titers with appropriate clinical pathologic abnormalities and clinical findings suggests clinical leptospirosis. PCR can be used to demonstrate the organism in urine, blood, or tissues, but is not widely available at this time, is not standardized between laboratories, and is expensive.

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 TAT. Cross-reactivity with *Brucella abortus* and certain strains of *Proteus vulgaris* has been documented with human serum. (See Appendix I for availability of testing.) Time between acquisition of infection and a positive titer is not known. A single titer of 1:80 or higher or a fourfold increase in titer between acute and convalescent sera (3 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.

SEROLOGIC 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 must be interpreted in conjunction with cytology, radiology, histopathology, and culture.

Analysis, Artifacts, and Interpretation • AGID, counterimmunoelectrophoresis (CIEP), and ELISA are used to detect circulating antibodies in serum (Sharp, 1998). Serum antibodies can represent exposure or infection. Results in many dogs with nasal aspergillosis are falsely negative. Owing to persistence of titers in some treated dogs (12 months), monitoring titers to assess therapeutic response is not recommended.

Radiographic demonstration of nasal turbinate destruction suggests aspergillosis or nasal neoplasia. Cytologic analysis (see Color Plate 3D) and culture of canine nasal exudate alone are not diagnostic because fungal elements may be nondetectable in affected dogs although being found in non-infected 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* if the organism is not demonstrated by cytology, histopathology, or culture. 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 (Legendre, 1998). Because subclinical canine infections are unusual, positive serologic results are considered significant. False-negative results occur in animals with peracute infection or with advanced cases overwhelming the immune system. Many cats with blastomycosis are seronegative. Antibody titers do not always revert to negative after successful treatment.

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 lymphadenopathy, osteomyelitis, uveitis, pericarditis, and nodular or ulcerative skin lesions can be screened for antibodies against *Coccidioides immitis* if the organism is not demonstrated by cytology, histopathology, or culture. Feline disease is rare but has been associated with nodular or ulcerative skin lesions, pulmonary interstitial disease, osteomyelitis, uveitis, and CNS disease.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by complement fixation (CF), AGID, ELISA, latex agglutination, and tube precipitin (TP) tests. TP detects IgM antibodies; CF and AGID detect IgG antibodies (Greene, 1998). 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 *C. neoformans* antigen if the organism is not demonstrated by cytology, histopathology, or culture.

Analysis, Artifacts, and Interpretation • Measurement of antibodies against *C. neoformans* is not clinically useful. Cryptococcal antigen is detected in serum, aqueous humor, or CSF using latex agglutination (LA).[*]

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 (Malik et al, 1996a). Positive titers occur in some animals after apparently successful clinical responses suggesting persistent low-grade infection or false-positive results (Flatland, Greene, and Lappin, 1996; Jacobs et al, 1997). 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 (Color Plate 4E) 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 (Malik et al, 1996b). CSF may contain the yeast, but concentration techniques (i.e., cytocentrifugation) should be used. Routine cytology stains (e.g., Wright's) 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 (Kano et al, 2001).

Histoplasmosis *(Histoplasma capsulatum)*

Rare Indications • Animals with weight loss, pulmonary interstitial disease, uveal disease, diarrhea, or lymphadenomegaly can be serologically screened for antibodies against *H. capsulatum* if the organism is not

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[*]Cryptococcus latex agglutination test, Cima Scientific, Dallas, Tex.

demonstrated by cytology, histopathology, or culture (Wolf, 1998).

Analysis, Artifacts, and Interpretation • Primarily, AGID is used to detect circulating antibodies in serum. Presence of serum antibodies confirm 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 Color Plate 3E), biopsy, or culture. 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. Serologic diagnosis is unreliable, generally not recommended, and used only to establish a presumptive diagnosis when the organism cannot be demonstrated by cytology, histopathology, or culture but other abnormalities suggest the disease.

SEROLOGIC TESTS FOR PROTOZOAL INFECTIONS

Babesiosis (*Babesia canis canis, B. canis rossi, B. canis vogeli,* and *B. gibsoni* in Dogs; *B. cati, B. felis, B. herpailuri,* and *B. pantherae* in Cats)

Rare Indications • Babesia serology is indicated 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, the species infecting cats are not found in the United States.

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by IFA. (See Appendix I for availability of testing.) In most laboratories, titers greater than 1:40 are considered positive (Breitschwerdt, 1995). 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. Some dogs with *B. gibsoni* are seronegative on IFA using *B. canis* antigen; IFAs are available using *B. gibsoni* antigens. Dogs suspected of having babesiosis yet seronegative for *B. canis* should be screened for antibodies against *B. gibsoni*. Antibodies 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 this titer magnitude (Birkenheuer et al, 1999). 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 remain high for at least 6 months. Untreated, seropositive dogs should be 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's and Giemsa's). 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). PCR is now available commercially and can be used to document organism presence, but positive results do not always correlate to clinical illness (Ano, Makimura, and Harasawa, 2001).

Neosporosis *(Neospora caninum)*

Rare Indications • Serology for *N. caninum* (the cause of neosporosis) can be performed in dogs with clinical evidence of polyradiculomyositis, including progressive ascending rigid paralysis, dysphagia, muscle atrophy, and (rarely) myocardial dysfunction or pneumonia (Lindsay and Dubey, 2000).

Analysis, Artifacts, and Interpretation • Circulating antibodies are detected in serum by IFA. (See Appendix I for availability of testing.) 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, *T. 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 *N. caninum* only documents infection, not clinical disease.

Definitive diagnosis is based on demonstration of the organism in tissues. The organism can be differentiated from *T. gondii* structurally and by immunohistochemistry (Lindsay and Dubey, 1999). *Neospora caninum* DNA can be amplified from tissue by PCR assay, and PCR can be used to distinguish the organism from *T. gondii*. *Neospora caninum* oocysts are found in the feces of some dogs (McAllister et al, 1998).

Toxoplasmosis *(Toxoplasma gondii)*

Occasional Indications
Healthy cats: Toxoplasma gondii–specific antibodies form in serum, aqueous humor, and CSF of healthy and diseased cats. 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 (Lappin, 1996). See Appendix I for availability of testing.

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% (Lappin, 1996). IgM titers persist in some clinically ill cats for greater than 16 weeks; these cats are frequently coinfected 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 (Lappin, 1996). Healthy and clinically ill dogs occasionally develop detectable IgM titers. Kinetics of post-infection 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 authors, however, have 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. A LA* and an indirect

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*Toxotest-MT, Tanabe USA, Inc, San Diego, Calif.

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 for availability of testing.

Fecal Examination • Fecal oocysts can be demonstrated using flotation techniques with various solutions with specific gravities 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, 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 (Lappin, 1996). The organism also can be detected transiently in aqueous humor and blood of healthy, experimentally inoculated cats (Lappin, 1996); however, making the positive predictive value of the PCR for clinical disease less than 100%.

Trypanosomiasis (Chagas' Disease) *(Trypanosoma cruzi)*

Rare Indications • Serologic testing for antibodies against *T. 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 (Barr, 1998). See Appendix I for availability of testing.

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

[†]TPM-Test, Wampole Laboratories, Cranbury, N.J.

can be demonstrated in tissues. PCR can be used to amplify organism DNA.

SEROLOGIC TESTS FOR RICKETTSIAL INFECTIONS

Canine Ehrlichiosis *(Ehrlichia canis)*

Common Indications • Serologic testing for ehrlichiosis 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 are detected in serum by IFA; they do not cross-react with *Rickettsia rickettsii* or *E. platys* antigens. *Anaplasma phagocytophila* (previously *Ehrlichia equi*), *E. chaffeensis, E. risticii*, and *E. ewingii* (Anderson et al, 1992) can also cause disease in dogs. Cross-reactivity of antibodies against these agents with *E. canis* antigen varies, and infected dogs can be serologically negative. See Appendix I for availability of testing for antibodies against other Ehrlichial species.

Antibodies against *E. canis* can be detected as early as 7 days and are almost always present by 28 days after inoculation (Neer et al, 2002). 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 (Bartsch and Greene, 1996). Clinically ill, seropositive dogs should be treated a minimum of 28 days and until clinical and laboratory abnormalities have resolved (Neer et al, 2002). Whether to treat healthy, seropositive dogs is controversial; the issues involved in this decision were recently reviewed (Neer et al, 2002).

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. Morulae are rarely found on routine blood smear or bone marrow aspiration cytology unless the dog has been immunosuppressed or the neutrophilic strain *(E. ewingii)* is present. *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 (McBride et al, 1996) and has potential benefit for use in monitoring treatment. (See Appendix I for availability of testing.) The *Consensus Statement on Ehrlichial Disease of Small Animals* from the Infectious Disease Study Group of the ACVIM (Neer et al, 2002) 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 Ehrlichiosis (*Ehrlichia* spp.)

Rare Indications • Serologic testing for ehrlichiosis is indicated for cats with thrombocytopenia, anemia, leukopenia, hyperglobulinemia, proteinuria, polyarthritis, fever, or lymphadenomegaly if no other obvious cause exists (Bouloy et al, 1994; Peavy et al, 1997; Stubbs et al, 2000).

Analysis, Artifacts, and Interpretation • IFA has detected circulating IgG antibodies against *E. canis*, *E. risticii*, and *Anaplasma phagocytophila* (previously *E. equi*) in serum of cats. PCR has detected DNA of *E. canis* (Breitschwerdt et al, 2003) and *A. phagocytophila* (Lappin et al, 2003) in the blood of clinically ill cats. Some cats with presumed *E. canis* infection have been seronegative. Antibodies against *E. canis, E. risticii*, and *A. phagocytophila* can be detected in serum from healthy cats

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[*]Snap3Dx, IDEXX Laboratories, Portland, Me.

and therefore cannot be used alone to make a definitive diagnosis of ehrlichiosis. A tentative diagnosis of feline ehrlichiosis is based on the combination of clinical signs, positive serologic test results, exclusion of other known causes, and response to tetracyclines. Definitive diagnosis is based on demonstration of morulae in leukocytes or amplification of DNA from the blood by PCR. See Appendix I for availability of PCR testing.

Infectious Cyclic Thrombocytopenia
(Ehrlichia platys)

Occasional Indications • Serologic testing for *E. platys* infection is indicated for dogs from endemic areas or with appropriate travel history and thrombocytopenia or endogenous uveitis.

Analysis, Artifacts, and Interpretation • Circulating IgG antibodies against *E. platys* are detected in serum by IFA. Antibodies against *E. platys* do not react with *E. canis* antigens. (See Appendix I for availability of testing.) The cutoff for a positive IgG antibody titer varies by laboratory. Experimentally infected dogs become antibody positive 13 to 19 days after infection (French and Harvey, 1983). Low antibody titers in suspected clinical cases should be rechecked after 21 days. Because many dogs are subclinically infected, positive antibody titers do not prove clinical disease. Definitive diagnosis requires demonstration of the organism within platelets (difficult, owing to cyclic parasitemia).

Rocky Mountain Spotted Fever
(Rickettsia rickettsii)

Occasional Indications • Serologic testing for 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 • The clinician can measure antibodies against *R. rickettsii* in canine serum by IFA, ELISA, and LA (Greene et al, 1993). 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 (Greene et al, 1993). After experimental inoculation, IgM antibodies can be detected by IFA by day 9, peak by day 20, and are negative by day 80 (Breitschwerdt, 1995). 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, historical, 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 four-fold 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 (Gasser, Birkenheuer, and Breitschwerdt, 2001). 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 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.

SEROLOGIC TESTS AND IDENTIFICATION TECHNIQUES FOR 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, 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 Color Plates 2D and 2E), direct fluorescent antibody staining of cytologic or histopathologic specimens, histopathologic evaluation, or PCR documentation of distemper viral DNA in peripheral blood, CSF, or conjunctival scrapings. (See Appendix I for

availability of PCR testing.) 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 parvovirus and canine parvovirus or coronavirus 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 is more useful. In-office ELISA for canine parvovirus in feces seem 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 may also detect feline parvovirus. Virus isolation or electron microscopy is required to identify other canine or feline viruses in feces.

Feline Infectious Peritonitis (FIP)

Rare Indications • FIP is an appropriate differential diagnosis in cats with fever; uveitis; retinal hemorrhage; nonseptic abdominal or

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[*]TiterCHEK CDV/CPV, Synbiotics Corp, San Diego, Calif.

[*]TiterCHEK CDV/CPV, Synbiotics Corp, San Diego, Calif.
[†]ASSURE/Parvo canine parvovirus antigen test, Synbiotics Corp, San Diego, Calif.
[‡]Witness CPV canine parvovirus antigen test, Synbiotics Corp, San Diego, Calif.
[§]SNAP Parvo antigen test, IDEXX Corp, Portland, Me.

pleural exudates or modified transudates; anemia; hyperglobulinemia; and renal, hepatic, or neurologic abnormalities. Results of currently available tests cannot be used to definitely 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. 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. 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 reverse transcriptase polymerase chain reaction (RT-PCR) of feces. However, positive test results do not indicate FIP because antibody-positive, healthy cats can pass coronaviruses (Addie and Jarrett, 1992). 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. PCR can also detect coronavirus particles in effusions, tissues, and blood (Barr, 1996). Detecting coronavirus by PCR in effusions and tissues predicts FIP, but detection in blood does not. 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 (Shelly, Scarlett-Kranz, and Blue, 1998).

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. Western blot immunoassay is performed in some commercial laboratories. An in-office ELISA is available for FIV antibodies and FeLV antigen combined.[*] 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 (Barr, 1996). Positive ELISA results should be confirmed via Western blot immunoassay or IFA. Finding 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 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. The ability of virus isolation or PCR to accurately differentiate naturally exposed and vaccinated cats is currently unknown.

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.

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. Testing of serum gives the best results; tears and saliva should not be tested. Several point-of-care ELISA tests are available.[*][†][‡][§][‖] 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 recommended.

FeLV infection has six stages (Zenger and Wolf, 1992). Stages 1 through 3 are dissemination stages; bone marrow infection occurs in stage 4. Infected neutrophils and platelets are released from the bone marrow in stage 5, and virus appears in systemic epithelial tissues (including salivary glands and tear glands) during stage 6. ELISA can detect p27 antigen in serum during stages 2 through 6; p27 in cells is not detected by the IFA until stages 5 and 6. Thus serum ELISA is the first assay to become positive after infection, positive results occurring 2 to 30 weeks (generally 2 to 8 weeks) after infection.

Seropositivity can be detected by serum ELISA before a cat develops persistent (i.e., stages 4 to 6) infection; thus, some cats yield negative results after development of neutralizing antibodies. In healthy cats, seropositive results by serum ELISA should be confirmed by IFA or retesting by ELISA in 4 to 6 weeks. Some ELISA-positive cats reverting to negative status have become latently infected. The majority of latently infected cats are ELISA-negative on all testing, but virus can be isolated from bone marrow. Virus can also be localized to other tissues. False-positive ELISA results can be the result of poor laboratory technique.

A positive IFA test result has 99% correlation with virus isolation. False-negative 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. The viremia may be transient or sustained. Unless the IFA test is performed during a transient infection, the animal will likely (>95%) remain positive for life.

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 transient stage 2 to 3 infection. The American Association of Feline Practitioners has summarized testing recommendations for FeLV or FIV infections (http://www.aafponline.org/about/guidelines_retrovirus_testing_2001.PDF).

Some cats with latent infection localized to bone marrow have positive bone marrow IFA results. The most reliable means of identifying latent FeLV infections is virus isolation or PCR performed on bone marrow cells, but neither technique is widely available. A latently infected cat may become viremic (i.e., IFA- and ELISA-positive) after extreme stress or administration of glucocorticoids.

DIAGNOSIS OF DIROFILARIASIS (DIROFILARIA IMMITIS)

Cytology (Knott's Test or Filter Test)

Common Indications • Cytologic evaluation for microfilaria 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).

Advantages • Very specific (microfilaria morphology differentiates *D. immitis* microfilaria

[*]Snap FIV antibody/FeLV antigen Combo, IDEXX Corp, Portland, Me.
[†]SNAP FeLV antigen test, IDEXX Corp, Portland, Me.
[‡]ASSURE/FeLV feline leukemia virus antigen test, Synbiotics Corp, San Diego, Calif.
[§]ViraCHEK/FeLV feline leukemia virus antigen test, Synbiotics Corp, San Diego, Calif.
[‖]Witness FeLV feline leukemia virus antigen test, Synbiotics Corp, San Diego, Calif.

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.

Disadvantages • Up to 40% of dogs have spontaneous occult dirofilariasis (Zimmerman, 1992) and must be diagnosed by serologic testing and radiographic examination. All cytology tests have poor sensitivity in cats.

Analysis, Artifacts, and Interpretation • A positive test result diagnoses heartworm disease, except in juveniles less than 4 to 5 months of age that could have received the microfilaria by transplacental transfer. Up to 40% of infected dogs are amicrofilaremic. Infected dogs receiving ivermectin or milbemycin as heartworm preventive are commonly amicrofilaremic. Clinical or laboratory signs of heartworm disease despite one or more negative microfilaria tests indicate serologic testing for circulating heartworm antigen, chest radiographs, echocardiography, or a combination of these.

Heartworm Adult Antigen Titer

Common Indications • Amicrofilaremic dogs or cats with clinical signs, laboratory abnormalities, or thoracic radiographic changes consistent with dirofilariasis. Dogs on ivermectin, selamectin, or milbemycin preventive can have sterile female adult worms and be amicrofilaremic. The test can also be used to assess efficacy of adulticide treatment.

Advantages • Greater sensitivity when compared with microfilaria detection techniques.

Disadvantage • More expensive than microfilaria detection techniques.

Analysis, Artifacts, and Interpretation • ELISA can detect circulating heartworm antigen in serum; several kits are commercially available.[*][†][‡][§][‖][¶] Intestinal parasites, *D. reconditum*,

hemolysis, and concurrent use of diethylcarbamazine, ivermectin, or milbemycin do not alter results of antigen assays. 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 animals 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 (Synder et al, 2000).

Definitive diagnosis requires detecting circulating microfilariae, characteristic radiographic signs (i.e., right-sided cardiac enlargement, increased diameter with or without tortuosity of pulmonary arteries, pulmonary interstitial infiltrate), or circulating heartworm antigen. Amicrofilaremic dogs with clinical signs of disease should be evaluated for serum antigen and with thoracic radiographs. In a previously infected and treated dog, it may be impossible to differentiate "new" from "old" radiographic lesions. In such cases, serum antigen testing is indicated.

Heartworm Antibody Titer (Feline)

Rare Indications • 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

[*]Witness HW heartworm antigen test, Synbiotics Corp, San Diego, Calif.

[†]DiroCHEK HW heartworm antigen test, Synbiotics Corp, San Diego, Calif.

[‡]SNAP heartworm antigen test, IDEXX Corp, Portland, Me.

[§]PetCheck heartworm PF antigen test, IDEXX Corp, Portland, Me.

[‖]Solo Step CH test cassettes heartworm antigen test, Heska Corp, Fort Collins, Colo.

[¶]Solo Step CH test strips heartworm antigen test, Heska Corp, Fort Collins, Colo.

[**]Witness FHW feline heartworm antibody test, Synbiotics Corp, San Diego, Calif.

[††]ASSURE/FH feline heartworm antibody test, Synbiotics Corp, San Diego, Calif.

[‡‡]Solo Step FH test cassettes heartworm antibody test, Heska Corp, Fort Collins, Colo.

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 (Synder et al, 2000).

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