ABSTRACT Methods used in clinical laboratory diagnosis in the veterinary laboratory closely parallel the common techniques used in the human laboratory. Immunology procedures include immunohematology, autoimmune testing, and assays for detection of immune deficiencies and infectious diseases. Veterinary immunohematology procedures deal with immune-mediated hemolysis, as well as blood typing, cross matching, and transfusion. Diseases of the immune system in animals include rheumatoid arthritis, systemic lupus erythematosus (SLE), and immunodeficiency disorders. The number of infectious diseases that can be diagnosed in a veterinary laboratory is almost limitless, but perhaps two of the most prevalent and significant are heartworm disease and feline infectious peritonitis (FIP). This is the first article in a three-part continuing education series on veterinary medicine. On completion of the series, the reader will be able to identify similarities between human and veterinary immunologic testing in diagnostic laboratories, describe several common assays used to detect immune and infectious disease in animals, and discuss veterinary reference ranges and issues associated with veterinary specimen analysis.

Immunohematology
Blood Type and Crossmatch
Although crossmatching of donor and recipient blood is common practice before transfusion in domestic animals, blood typing usually is performed only in dogs (Fig 1), in large part because of the lack of naturally occurring antibodies in nonhuman species. At least 11 blood group systems are found in dogs; only one, the dog erythrocyte antigen (DEA)-1 system, is polymorphic. Only DEA-7 has a significant frequency of naturally occurring antibody in nonsensitized animals. Efforts are made during blood typing to assure that donor dogs do not have DEA-1 or DEA-7. DEA-1 is immunogenic and frequently can sensitize a recipient lacking that antigen, and DEA-7 is associated with naturally occurring anti--DEA-7 in approximately 50% of animals negative for that antigen.

The RBC antigen system in cats is simple, with only A and B antigens defined. It appears these antigens have reciprocal antibodies; that is, cats with type A blood have anti-B antibodies, and those with type B blood have anti-A antibodies. Clinically, this requires crossmatching before transfusion to prevent these naturally occurring antibodies.

The RBC antigen system in horses is more complex, demonstrating at least seven systems,
with 1 to 11 antigens in each. However, none of these systems seems to support naturally occurring antibodies. Only crossmatching is performed before transfusion, and the complex antigen systems are used primarily as a genetic marker system for pedigree substantiation.

Coombs’ Test
Hemolytic disease of the newborn (HDN) is virtually nonexistent in utero in domestic animals, but can occur as the result of antibodies passed postpartum after the newborn animal has nursed the immunoglobulin-rich colostrum. That natural antibodies occur so infrequently indicates maternal immunization by previous transfusion, traumatic parturition, or RBC-contaminated vaccine.

Postpartum HDN and other immune-mediated hemolytic anemias in domestic animals require that veterinary diagnostic laboratories offer Coombs’ testing for a variety of domestic animals. The test requires antiserum raised against the species-specific immunoglobulin, which is commonly multivalent against IgG, IgM, and C3.

Autoimmune Testing
A wide variety of autoimmune disorders occur in domestic animals, including skin disease, blood, joint, and systemic organ diseases. The common veterinary laboratory diagnostic tools for autoimmune disease are virtually identical to those used in human diagnostics, but modified for the specific species.
Fig 2. Wright-stained reptilian peripheral blood smear shows toxic change within the heterophils (a granular WBC represented by neutrophils in humans). Note nucleated RBCs (original magnification × 100).

**Rheumatoid Factor**
Rheumatoid arthritis is relatively common in small canine breeds. The commonly used assay detects antibody (rheumatoid factor) that reacts with the animal’s own IgG. The rheumatoid factor is most commonly IgG, but may be IgA or IgM. The assay uses a particle-agglutination method, which utilizes either sheep RBCs or latex particles coated with canine IgG. The particles are reacted with canine serum. Agglutination indicates the presence of antibody against canine IgG (rheumatoid factor).

**Antinuclear Antibody (ANA)**
The ANA assay uses an IFA technique to detect ANAs in animals with systemic lupus erythematosus (SLE). This disease has been diagnosed in canines since 1964, and has now been described in virtually all breeds, with a disproportionate prevalence in poodles and German shepherds. Feline SLE has also been documented.

In the common ANA assay, substrate cells (usually human laryngeal tumor cells [HEp-2 cells]) are affixed to a microscopic slide and flooded with the serum from the affected animal. If ANA is present, it will affix to a variety of structures in the nucleus of the substrate cells. After rinsing, an antiglobulin conjugated with fluorescein is reacted with the substrate cells. The presence and pattern of ANA attachment can then be viewed under a fluorescent microscope.

**LE Cell Preparation**
The LE cell preparation is another tool used to diagnose SLE. As in humans, this test in veterinary diagnostics has poor sensitivity and specificity, but can be performed inexpensively and easily and does not require species-specific reagents. The method requires that some of the cells within a blood or body fluid specimen be traumatized to expose nuclear material. If ANA is present, it will attach to the exposed nucleus, causing swelling and loss of chromatin structure. This sensitized nuclear material is then phagocytized by the intact macrophages or neutrophils in the specimen, creating the diagnostic LE cells.

**Direct Fluorescent Antibody**
DFA techniques can be used to diagnose a variety of autoimmune diseases in animals. Affected tissue is harvested at biopsy. The tissue is sectioned and mounted on slides, and flooded with species-specific fluorescein-conjugated anti-immunoglobulin. Tissues with autoimmune antibody attachment can then be viewed under the fluorescent microscope.

**Indirect Fluorescent Antibody**
IFA techniques can be used to demonstrate the presence of autoimmune antibody in animal plasma. The ANA test is a variant of this technique. Purchased or prepared substrate cells (commonly skin, renal, or hepatic cells) from a specific affected tissue are flooded with plasma from the affected animal. After washing, the cells are flooded with species-specific fluorescein-conjugated anti-immunoglobulin. The presence of autoantibody directed against specific tissue components, such as cutaneous basement membrane or renal glomerular basement membrane, can be viewed under the fluorescent microscope.

**Coombs’ Test**
Direct and indirect Coombs’ testing is used to assist with the diagnosis of immune-mediated hemolytic anemia in animals. The application of this test in veterinary diagnostics requires the use of species-specific antiglobulin.

**Immune Deficiency and Quantitative Immunoglobulins**

**Failure of Passive Transfer in Neonates**
Failure of passive transfer of maternal antibody in neonates is the most common immune deficiency of the animal kingdom. The effects of this condition depend on the relative contribution of placental transfer of maternal antibody to the neonate vs colostral transfer. Canine and feline placenta types allow limited transplacental
immunoglobulin transfer. Equine and bovine placentas do not allow immunoglobulin transfer from mother to neonate, and patent neonatal immunity depends totally on successful postpartum suckling in these animals. Intestinal absorption of colostral immunoglobulin is most efficient in the first 6 hours postpartum, and is virtually nonexistent after 24 hours. Lack of colostrum appears to have little effect in puppies, and perhaps slight effect in kittens. Its greatest effect is in cattle and horses, and it is extremely difficult to rear these young animals successfully without colostrum.

A veterinary laboratory can diagnose failure of passive transfer with a variety of quantitative or semiquantitative immunoglobulin assays. Time is critical. Although the animal should be at least 18 hours old, to allow maximum transfer of colostral immunoglobulin, deficient animals must be treated within 72 hours. Semiquantitative stat or point-of-care methods to measure neonatal immunoglobulin levels such as particle agglutination and precipitation in inorganic salt solution are usually adequate, and are preferred.

**Congenital and Acquired Immunodeficiency Diseases**

Defects in almost every aspect of the immune system have been described in animals, including severe combined immunodeficiency disease, cellular dysfunctions, complement dysfunctions, Chédiak-Higashi syndrome, Pelger-Huët nuclear anomaly, hypogammaglobulinemia, defective barrier systems, and infection-induced immune system suppression (Fig 2). The immunology laboratory can assist with the diagnosis of immune deficiencies in the humoral system with quantitative and qualitative immunoglobulin assays. Immunoglobulin quantitation is usually accomplished with radial immunodiffusion, rocket immunoelectrophoresis, ELISA, or radioimmunoassay. The primary challenge with these techniques is development of species-specific reagent systems.

**Infectious Serology**

Each infectious disease presents its own set of diagnostic challenges for the veterinary laboratory. Two, however, are particularly worthy of further discussion because of their prevalence, diagnostic significance, and developing technology.

**Heartworm Disease**

Heartworm disease is caused by infection with *Dirofilaria immitis*, which is endemic in all parts of the United States and is particularly common in Atlantic and Gulf Coast saltwater marshes, where mosquitoes are prevalent. The life cycle of this parasite begins with an infected mosquito that passes larvae to a healthy mammal during a bite. The larvae migrate to muscle or fat, and develop into young adult worms within 90 days. The adult migrates to the right ventricle and pulmonary arteries, where it matures within 4 months. In a patent infection, females become gravid by 5 months, and shed microfilaria 6 to 7 months after the initial infection. Canines are the definitive host for *D. immitis*, but felines and other small mammals can become infected. Noncanine hosts generally mount a vigorous immune response to infection, and the development of a patent infection with adult worms is uncommon and often self-limiting.

In the past, detection of heartworm infection depended on detection of either the microfilaria by direct examination of stained blood smears (Knott test, Fig 3) or the shedding antigen from adult worms in the serum of infected animals (ELISA method). The specificity of the heartworm antigen method is good, and a positive result is an excellent predictor of a patent infection with productive female worms. Although the test is marketed for use in canines, clinical experience has demonstrated the antigen test to be useful in felines and other small mammals. However, several factors create a problem with the sensitivity of this assay. The heartworm antigen method can...
only detect antigen shed from adult female worms. Therefore this method will produce a negative result if the infection is early in development or includes only male worms. Also, if the worm burden is low, the antigen level may not rise above the lower sensitivity limit of this ELISA method. These limiting conditions are commonly present early in canine infection and in infections in non-canine species.

Heartworm antibody testing (IFA method) also is available. The antibody assay has a much higher sensitivity than the antigen assay, demonstrating somatic antibodies (against larval antigens) within 1 to 2 weeks of exposure and cuticular antibody (against adult and filarial antigens) within 2 to 3 months. The assay commonly yields positive results during the prepatent period, in infection with male-only worms, and in infections with low worm burden. The antibody assay is often the assay of choice in noncanine species, because worm burdens are often low or unisex. However, this assay also yields positive results after any exposure to heartworm, even if the exposure fails to develop into an infection. After an exposure or infection has cleared, the antibody assay generally remains positive for 9 to 12 months. This test is also limited by the availability of species-specific fluorescein-conjugated antiglobulin.

**Feline Infectious Peritonitis (FIP)**

FIP is caused by infection with a feline coronavirus that causes thoracic and abdominal effusions, characteristic multifocal fibrinonecrotic plaques, and wasting and death. The virus is passed by the fecal-oral route, and will spread rapidly through a breeding colony or domestic household. Like the common rhinovirus in humans, many coronaviruses can infect felines, but only a few cause clinical FIP. The laboratory’s ability to assist with the diagnosis of FIP was severely limited by the nonspecific nature of the immunofluorescent antibody assay used to detect anti-coronavirus antibody. The assay demonstrates a positive titer in a cat that is or was infected with any coronavirus. The majority of adult cats demonstrate some level of anti-coronavirus antibody. The clinician is faced with determining what level is significant, whether the titer is rising or falling, and whether the clinical symptoms are consistent with FIP or with some other benign coronavirus infection.

PCR is of help in making this difficult diagnosis. PCR techniques focus on detection of a specific region on the virus genome that is present only in the coronavirus subset known to cause FIP. This specific region is known as the 7B gene, and PCR techniques are capable of determining whether this gene is present in the infecting coronavirus. However, while it is true that all FIP-causing coronaviruses demonstrate the 7B region, not all 7B-positive coronaviruses cause FIP. The key to FIP infection seems to be the ability of the 7B region to produce and secrete a specific protein, the 7B-region specific protein. If the coronavirus is secreting this specific protein, it is capable of causing FIP.

A new ELISA method has recently been developed that can detect the presence of this 7B-region specific protein. The method has been described as the feline infectious peritonitis—specific ELISA (FIPSE), and appears to have good predictive value in the diagnosis of the disease. This test is currently available in a limited market while clinical correlation studies are carried out.

**Conclusion**

Immunology and serology procedures in a veterinary diagnostic laboratory closely parallel those in human laboratories. However, because of the number of nonhuman animal species and the number of congenital and acquired diseases found in each species, the number of diagnostic laboratory procedures that can be performed in a veterinary laboratory is almost infinite. I have summarized some of the most common immunology procedures performed in a veterinary laboratory in the most common companion animal species.

**Reference**

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