Matrix Issues Associated With Analysis of Veterinary Specimens

Analysis of veterinary samples can cause problems for laboratories whose primary focus is human sample testing. Some of these issues, which can affect several laboratory departments, are due to differences in sample matrix and are often species dependent.

**Hematology**

Analysis of animal blood for CBC may be straightforward or difficult and labor intensive, depending on the species and the hematology analyzer used. Many animal blood samples contain high protein levels, especially globulins, which can lead to protein buildup, particularly in small-diameter tubes, apertures, and shear valves. Frequent rinsing, cleaning, and maintenance procedures may be necessary. Animal blood also tends to have different or shorter coagulation mechanisms, which can result in different reference ranges for the various species. If human ranges are used to set automated coagulation instruments, shorter animal coagulation times will be missed and falsely elevated results reported. Abnormal findings may range from platelet clumping (especially in feline samples) to clot formation. Examination of blood specimens prior to analysis is essential to detect clots, which can cause analyzer malfunction.

Platelet clumping is common in veterinary specimens, and leads to falsely decreased platelet counts. Platelet clumping may be due to prolonged time between sampling and analysis or to drawing technique. Because of the small diameter of some animal veins, blood samples cannot be directly drawn into vacuum tubes, but are drawn into syringes and then placed in EDTA anticoagulated tubes. Therefore, the coagulation cascade, including platelet aggregation, has already begun.

Anticoagulants used for submission of hematology specimens also affect the viability of the samples. Mammalian blood is generally best drawn into EDTA anticoagulated tubes. Many avian and most reptile samples submitted in EDTA become hemolyzed and gelatinous and cannot be analyzed, and therefore must be drawn into heparin anticoagulated tubes.

**Urinalysis**

Animal urine specimens vary greatly in constituents, analytes, and viscosity. Urine samples from many species, especially horses (Fig 1), contain a large amount of mucus in urine samples from horses can make microscopic urinalysis difficult. Serum samples may contain yellow pigment due to diet; this situation can confuse inexperienced technologists.
Fig 2. Collection of a clean urine sample from birds, such as the blue and gold Macaw pictured here, is very difficult due to the urinary system joining the gastrointestinal system to form the cloaca.

large amount of mucus, which can make analysis, especially microscopic analysis, difficult. Urine specimens from dogs and cats often contain marked amounts of blood and fibrin. Extensive coloration of the urine may produce false color changes on dipstick chemistry pads, which can cause problems in automated urine test strip reading, yielding false-positive or false-negative results. Crystals, especially struvite calculus (triple phosphate), are common in dog and cat urine, and can be significant, accounting for one fourth to one third of the urine sample volume. Collection of a clean urine sample in avians and exotic species is difficult because the gastrointestinal and urinary systems join to form a common external opening, the cloaca (Fig 2). The urine samples are therefore often heavily contaminated with fecal material, and many also contain a large concentration of urates, which may greatly increase viscosity.

Urine collection methods may introduce artifacts, resulting in false-positive glucose readings, for example. Bleach and other disinfectants often used by well-meaning owners to clean a collection vessel in the home, or collection of urine from the examination table (often the only sample available) will produce results strongly positive for glucosuria (3–4+). Glucosuria without concurrent hyperglycemia should always be rechecked, and the method of urine collection and transport should be determined.

Medications or vitamins given to animals can cause false-positive or false-negative urine dipstick reactions. For example, vitamin C (ascorbic acid) causes false-negative reactions for glucose with the urine dipstick method, but causes false-positive reactions for glucose with reagent tablets. Various antibiotics precipitate in urine, resulting in crystaluria.

Cytology
In veterinary medicine, the vast majority of cytology specimens are nongynecologic aspirates (ie, body fluids) collected with a fine needle. The aspirates are collected on slides, and are air-dried as opposed to being sprayed with cytofixative. Most veterinary cytologists stain these specimens with an eosin–methylene blue stain (eg, Giemsa, Leishman, Wright stain). The body fluids can contain blood clots, fibrin strands, infectious agents, and a wide variety of debris, and are submitted in EDTA anticoagulated and red-top tubes. Many are markedly viscous, lipemic (chylous), or hemolyzed, and cell counts (manual or automated) and other chemical analyses cannot be performed. Elevated cell counts (>10,000/μL) are common, and cytospin slides must be prepared to ensure proper cell density determinations.

Chemistry
The majority of differences in chemical analysis between veterinary and human samples are related to reference or expected ranges for the various species, rather than matrix issues. With currently used analyzers, carryover between samples is generally not an issue. The increased protein concentrations that may affect hematology analyzers may similarly affect chemistry instruments.

The degree of interference that hemolysis contributes to potassium measurement is species dependent. Intraerythrocytic potassium concentration depends on the species or breed of animal. Specimens from most dogs (except Akitas) and cats contain minimal intraerythrocytic potassium concentrations; therefore, delayed separation or hemolysis does not affect potassium results. Horse specimens contain moderate concentrations of intraerythrocytic potassium, and delayed separation or hemolysis will result in significantly elevated potassium values. Avian and reptile (Fig 3) specimens contain variable concentrations of intraerythrocytic potassium, depending on the species.
Another common finding in veterinary samples is lipemia. Many veterinary patients are not fasted prior to sampling, resulting in a high percentage of lipemic specimens. Lipemia in veterinary samples causes the same problems as lipemia in human samples: falsely positive or negative results, depending on the reagents and chemical analyzer used. Icterus and yellow coloration of serum due to diet are other concerns in analysis of veterinary samples. Serum samples from herbivores (e.g., cows, sheep, horses) contain variable amounts of yellow pigmentation due to diet, and specimens from dogs and cats may have significant coloration due to commercially prepared foods with vegetable sources of protein. The yellow coloration does not interfere with chemistry analysis, but can cause confusion for inexperienced technologists.

**Serology**

The number of serology tests performed in veterinary medicine is extensive. Several of the more common tests are for feline coronavirus, feline leukemia virus, feline immunodeficiency virus, heartworm antigen, *Ehrlichia*, Lyme disease, antinuclear antibodies, and equine infectious anemia (Coggin test). The methods for these tests include indirect fluorescent antibody, enzyme-linked immunosorbent assay (ELISA), agar gel immunodiffusion (AGID), and latex agglutination. Hemolysis can interfere with the ELISA method by causing nonspecific binding of conjugate to the wall of the wells, resulting in false-positive reactions. Hemolysis can also interfere with AGID, because coloration of the agar gel obscures the precipitin line, making it difficult to read. Lipemia may interfere with some serology tests, because of steric hindrance (in some ELISA-based tests), or if severe, by decreasing the amount of aqueous sample or interfering with electrolyte measurement. Increased protein concentrations, especially globulins, common in sick animals, may cause interference with serology testing because of nonspecific binding, similar to excessive hemolysis. Antibody-antigen complexing, fibrin clots, and excessive protein concentrations may affect serologic analysis of body fluids.

**Conclusion**

Veterinary specimens can be analyzed accurately when a number of issues are addressed. Matrix issues can affect most departments of the laboratory, and include intracellular (e.g., potassium) and extracellular (e.g., lipemia, coagulation) components. Automated instruments may be affected by veterinary specimens that contain excessive protein buildup, clots, fibrin strands, and collection artifacts. The technologist who is aware of these issues and is properly prepared to handle them will be able to provide veterinarians with accurate and timely results.

**Suggested Readings**

Kaneko JJ, ed. *Clinical Biochemistry of Domestic Animals*. 4th ed. New York, NY: Academic Press; 1989.
Meyer W, Coles FH, Rich LJ. *Veterinary Laboratory Medicine: Interpretation and Diagnosis*. Philadelphia, Pa: Saunders; 1992.
Willard MD, Tvedten H, Turnwald GH. *Small Animal Clinical Diagnosis by Laboratory Methods*. 2nd ed. Philadelphia, Pa: Saunders; 1994.