TRUST, BUT VERIFY
Always check to ensure that the correct type of collection containers are used for laboratory samples.
How to Collect and Prepare Samples for the Laboratory

Laboratory specimen submission may seem like a daunting task, but a little preparation beforehand can lead to timely and accurate results. This article provides an overview of the veterinary nurse’s role in properly collecting, processing, and submitting common laboratory samples for analysis (BOX 1).

LABORATORY REQUIREMENTS

The first step in obtaining any sample is familiarizing yourself with the requirements of the specific laboratory used for testing samples. As part of their quality assurance, most laboratories offer detailed guidelines on specimen types and requirements. This information, along with accession (test request) forms, is usually posted on the laboratory’s website or will be available in informational materials provided by the laboratory. Having general knowledge of the sample requirements ahead of time will ensure that you have appropriate collection supplies on hand and readily available for use as needed. Also, check the turnaround times for the tests required to avoid delays and potential sample degradation (TABLE 1).

PATIENT PREPARATION AND SAMPLE HANDLING

To minimize pre-analytical interference with the test results and to reliably compare the patient’s results with established reference ranges, samples must be collected from a suitably prepared patient.
Excitement and acute stress—often increased by clinic visits, sample collection, and/or illness—can lead to elevated blood and urine glucose levels, as well as neutrophilia, monocytosis, and lymphocytosis. Lipemia is one of several blood sample conditions that can increase hemolysis in a sample and interfere with test results (FIGURE 1). Lipemia can be minimized by fasting the animal for at least 12 hours before blood collection. Unfortunately, fasting is often overlooked in veterinary medicine. A priority for all veterinary professionals should be communicating the need for fasting and suggesting techniques to minimize patient distress, which clients can begin before transporting their animal. Fasting can minimize falsely elevated levels of protein, hemoglobin, blood urea nitrogen (BUN), phosphorus, creatinine, glucose, and sodium, as well as falsely decreased levels of phosphorus and potassium.

Hemolysis is another common blood sample condition that can interfere with results; it often results from improper collection and handling of blood samples before analysis. Hemolysis can be caused by freezing a whole blood sample, lack of or inadequate centrifugation soon after clotting, use of inappropriate needle–syringe combinations, inadequate collection of blood, and failure to handle samples gently when mixing. Hemolysis will interfere with coagulation panels, elevate hemoglobin values, decrease hematocrit values, and interfere with many biochemical assays.

**SAMPLE COLLECTION**

Collection container types and required sample volumes will vary from instrument to instrument, from

---

**TABLE 1 Sample Types, Tests, and Viability**

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>TESTS</th>
<th>VIABILITY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>Complete blood count, manual packed cell volume, cell evaluation, molecular diagnostics, toxicology, serology, virology</td>
<td>• &lt;24 hr, refrigerated; frozen for molecular diagnostics</td>
</tr>
<tr>
<td>Heparinized whole blood</td>
<td>Nonmammal hematology, packed cell volume, toxicology</td>
<td>• &lt;24 hr, refrigerated</td>
</tr>
<tr>
<td>Serum/plasma</td>
<td>Biochemistry panel, serology, endocrine tests, virology titters, molecular diagnostics, toxicology</td>
<td>• &lt;24 hr, refrigerated or room temperature; 7 days to 1 year, frozen, depending on test</td>
</tr>
<tr>
<td>Citrated plasma</td>
<td>Coagulation panels</td>
<td>• &lt;24 hr, refrigerated or frozen</td>
</tr>
<tr>
<td>Other fluids</td>
<td>Cytology, biochemistry panel, culture</td>
<td>• &lt;24 hr, refrigerated</td>
</tr>
<tr>
<td>Feces</td>
<td>Parasite identification, culture, molecular diagnostics, serology, virology</td>
<td>• &lt;24 hr, refrigerated; fixed within 2 hr of collection</td>
</tr>
<tr>
<td>Tissues</td>
<td>Histology, culture, molecular diagnostics, toxicology</td>
<td>• &lt;24 hr, fresh, refrigerated; indefinitely, fixed</td>
</tr>
<tr>
<td>Swabs (cultures)</td>
<td>Bacterial culture, molecular diagnostics, virology</td>
<td>• &lt;24 hr</td>
</tr>
<tr>
<td>Slides</td>
<td>Cytology, cellular morphology</td>
<td>• Indefinitely, stained</td>
</tr>
</tbody>
</table>
laboratory to laboratory, and will differ with each testing procedure. Always check ahead with the laboratory to ensure that samples are collected in the correct type of collection containers and that at least minimum volumes are submitted for analysis. All samples must be labeled properly; they may be rejected by the laboratory if insufficiently labeled. Remember to include the veterinarian or clinic name on each container along with patient and client information. Adding the type of sample to the container is also useful. When serial samples or multiple samples are submitted from the same patient, label the tubes appropriately, indicating date and time of collection, whether collection was before or after medication, and whether collection was before or after the patient had eaten.

Blood

Whole Blood
When whole blood is required, a proper ratio of anticoagulant to blood is necessary to prevent overdilution of the sample or clotting if too much blood is added. A general rule of thumb is to fill anticoagulant tubes to within 90% of their capacity; however, it is always prudent to check the tube manufacturer’s guidelines to ensure proper fill volumes. Another guideline for filling any anticoagulant tube is to ensure that the sample is gently inverted at least 8 times while filling the tubes and each time a whole blood sample is taken from the tube for testing. Two commonly used anticoagulants are ethylenediaminetetraacetic acid (EDTA) and heparin. Each laboratory will vary as to whether EDTA or heparin is acceptable for specific tests or whether anticoagulants are interchangeable.

EDTA has minimal effects on cell morphology and thus is commonly used for cell counts and morphologic evaluation of hematology and cytology samples. EDTA comes in lavender-top tubes (LTTs, FIGURE 2) or pink-top tubes. EDTA is available in 2 forms, di-potassium or tris-potassium EDTA, each of which hinders the action of calcium and will also bind to magnesium. If tris-potassium EDTA tubes are inadequately filled, a manually run packed cell volume value may be falsely lowered.

Heparin (FIGURE 2) is another type of anticoagulant that may be used due to EDTA potentially causing cell lysis in certain species of birds, reptiles, and fish, including sea turtles and stingrays. Heparin interferes with thrombin formation by enhancing antithrombin activity and disrupting other coagulation reactions. Because heparin does not preserve cellular morphology as well as EDTA does and promotes platelet clumping, blood slides should be made directly from the needle at the time of collection for any cytologic evaluation required.

Serum
Two main types of tubes are used for serum collection: plain and serum separator tubes (FIGURE 3).

Red-top tubes (RTTs) usually contain no additives; however, some RTTs may contain silica crystals or glass particles to initiate the clotting process. If additives are undesirable for the specific test needed, be sure to use a plain RTT. Some tests, such as endocrine evaluations, will be influenced by activator gel or silicone, and glass may interfere with enzymatic or coagulation tests. Plain serum tubes should stand at room temperature for 30 to 60 minutes to allow

FIGURE 2. Anticoagulated blood tubes. (A) EDTA in a lavender-top tube; (B) heparin in a green-top tube; and (C) citrate in a blue-top tube.
adequate clot formation before centrifugation. Increased serum yield is achieved by rimming the clot. To minimize hemolysis, first allow the clot to fully form before rimming. Then gently insert a wooden applicator stick between the wall of the tube and the clot to detach any fibrin strands from the tube.

**Serum separator tubes** have either a gel matrix at the bottom or on the sides of the tube that will separate the clot from the serum after centrifugation. The tops of these tubes are a marbled red/gray, red/black, or solid yellow. Even when serum separator tubes are used, the serum is ideally removed and placed into another plain RTT for submission. If centrifugation speeds and times are inadequate, the gel may not form a complete barrier with the clot, which can affect biochemical assays or cause hemolysis. Tubes may be centrifuged again if the barrier does not completely separate serum from the clot. When serum or plasma is left in contact with cells, glucose levels may be decreased, phosphorus levels may be increased, and the sample may hemolyze during prolonged storage.

**Plasma**

Plasma is the supernatant separated from the cellular layer of an unclotted sample. At the time of collection, do not mix anticoagulants, which may adversely interfere with each other and with the test procedure. For example, do not use a heparinized syringe to collect a sample to be placed in an EDTA tube.

**Lithium heparinized plasma** can be used instead of serum for biochemistry panels on some instruments due to minimal interference with electrolyte assays.

Heparinized plasma can be immediately processed because there is no need to wait for the sample to clot. For species other than mammals, laboratories that accept heparinized samples for hematology may use 1 tube for both hematologic and biochemical assays.

**Citrate tubes** have a blue top (FIGURE 2) and come with either 3.2% or 3.8% solution. When citrate binds to calcium in the sample, the blocked calcium prevents clotting. For accurate results, citrate tubes must be adequately filled to ensure a citrate:blood dilution of 1:9. Coagulation studies require citrated plasma to be removed from cells within 1 to 6 hours, depending on the specific test. The secondary tube must clearly be labeled as citrated plasma to avoid confusion with serum or other types of plasma.

When sample volume is limited, EDTA plasma may be used for plasma protein, glucose, and BUN measurement as well as some serologic tests.

**Other Fluids**

For cytologic evaluation of other fluids (e.g., urine, cerebral spinal fluid, effusions, fine-needle aspirates, washes), submit fluid samples in LTTs; for biochemical evaluation or bacterial culture, submit the samples in sterile RTTs. To be diagnostically viable, samples must be free of lubricants or ultrasonography gel. Slides to be submitted to the laboratory along with the sample should be prepared at the time of sample collection. Washes and reproductive and respiratory secretions should be placed in sterile RTTs for submission.

---

**FIGURE 3.** Serum tubes (left) and serum separator tubes (right). Notice that plain red-top tubes have “no additive” listed on the label.

**FIGURE 4.** Examples of commercial secondary containers with biohazard label. A pouch on the back holds the paperwork separate from the sample, and absorbent material is placed in the pouch with the sample. Biohazard labels *must not* be added to the outer container.
Commercial urine specimen containers often leak while in transit. To avoid leakage, transfer urine to a 15- or 50-mL sediment tube with a screw cap for biochemical and sediment analysis. Ideally, a line smear (a stop smear) prepared from the sediment is submitted along with the urine since cellular degradation in urine occurs so quickly. To enhance cellular evaluation, prepare line smears and allow them to air dry before shipment. Preservatives are available; however, not all laboratories will accept preservative-treated samples.

Feces
Fresh fecal samples are required for parasite ova identification, microbial culture, and molecular diagnostics. Required volumes can be submitted in an airtight container, refrigerated, and shipped with ice packs. For select tests, such as Giardia and Cryptosporidia fluorescent antibody tests, the sample can be diluted with 10% formalin before submission. Special media are available for Tritrichomonas foetus samples, which require the inoculation of a pouch before submission to the laboratory.

Tissues
Fresh tissue is used for a variety of analyses, including histopathology, microbial culture, molecular diagnostics, toxicology, and virology. To avoid contamination, each tissue must be labeled properly and packaged separately. For the samples to remain diagnostic, they must be transported quickly to the laboratory. If tissue must be fixed in formalin, use a tissue:formalin ratio of 1:10. Samples can be placed in a larger volume of formalin for 24 hours to fix and then transferred to a smaller container for transport; smaller samples are preferable. Ensure that container openings are wide enough to accommodate swelling and hardening of tissues during fixation.

Cultures
Samples for microbial culture, molecular diagnostics, and virology must be collected by using the appropriate type of swab and placed in transport media that will enhance the viability of suspected pathogens. For example, molecular diagnostic tests require synthetic fibers and plastic shafts. For some virus testing, calcium alginate, cotton fibers, and wooden shafts are not acceptable.

Slides
Laboratory personnel prefer that slides be submitted unstained. Ideal samples include air-dried blood samples, rolled swab slides, and cytology slides with a thin area for evaluation. Avoid contamination with formalin vapors from formalin-fixed tissue samples by packing slides in a separate container in such a way as to prevent breakage while in transit. Formalin will partially fix cells and create an artifact that can render the slide nondiagnostic.

PREPARING SAMPLES FOR TRANSPORT
Specimen transport guidelines are established by the United States Department of Transportation and the International Air Transport Association. Anyone who handles and prepares biological, potentially hazardous materials must have documented training. Samples are categorized as A or B biological substances, according to their potential for causing serious or fatal disease in humans. Responsibility for designating samples correctly falls to the person preparing the sample for transport. If samples are improperly packaged, the shipper may be subjected to fines, jail time, or extra handling fees.
Category B, Biological Substances, UN 3373, generally includes diagnostic samples from animals.

Category A, Infectious Substances, UN 2900, includes samples to be tested for more serious animal diseases, which may cause disability or fatality in humans exposed to the contents.

To protect handlers of the package while in transit and personnel at the destination facility, a triple-layer packaging scheme is required.\textsuperscript{10,11}

**Primary container:** A leak-proof, primary container is used to house the sample, not to exceed 1 liter (liquids) or 4 kilograms (solids). Primary containers may be made of plastic, glass, or metal, and include blood tubes, plastic bags for tissues, culture swabs, and jars.\textsuperscript{3,10,11} Use indelible markers to label each container.\textsuperscript{3} Place absorbent cushioning around the primary container to sufficiently absorb any liquid that may leak.\textsuperscript{3,10} Wrap glass containers individually in cushioning material to prevent breakage.\textsuperscript{3,10,12}

**Secondary container:** This layer is most commonly a scalable plastic bag (FIGURE 4), but it can also be insulated polystyrene foam (e.g., Styrofoam) inserts. Secondary containers must be leak-proof.\textsuperscript{10,11} Multiple samples and ice packs, if required, may be contained within the secondary container, along with properly completed paperwork sealed in a separate bag.\textsuperscript{3,4} Ice packs should be placed in sealed plastic bags with absorbent material to prevent condensation from wetting package contents.\textsuperscript{3}

**Tertiary container:** The third (outer) layer is usually a rigid cardboard shipping box, often with a polystyrene foam insert that can withstand transport.\textsuperscript{3,10-12} Labels must include the shipper’s name, phone number, address, and the biological category (e.g., “Biological Substance Category B” and “UN3373”) (FIGURE 5). Make sure that the correct address is placed on the outer container; some laboratories have separate branches that deal only with certain types of specimens or a separate department and location for the diagnostic laboratory.

If dangerous components, such as dry ice, are added to the package, additional regulatory labeling and change of designation from Category B to Category A may be required. Dry ice must be properly ventilated to prevent bursting of the shipping container.\textsuperscript{3,12}

**CONCLUSION**

Commitment to lifelong learning is part of the Veterinary Technician Oath.\textsuperscript{13} With continual advances in laboratory diagnostic testing, veterinary nurses play a vital role in ensuring the viability of samples en route to the laboratory. By researching and staying current with test requirements, veterinary nurses are instrumental in communicating preliminary preparation with clients and discussing with the veterinary team ways to reduce erroneous test results. This flexibility and continued learning are what make veterinary nurses invaluable assets for properly collecting, preparing, and submitting samples to the laboratory. TVN

**References**