Sample Submission: Errors and Pitfalls.

Holly S. Bender, *University of Blacksburg*
Carolyn S. McCoy, *University of Blacksburg*
Sample Submission: Errors and Pitfalls

Holly S. Bender DVM, PhD
Carolyn S. McCoy MT (ASCP)

Personnel at large commercial or institutional labs are generally graduates of sophisticated training programs, and are trained to handle samples appropriately. In our experience, most of the technologists in these establishments have an intense interest in seeing that your test results are as accurate as possible. Fortunately, sample handling is so routine to these people, that correct processing becomes second nature. These laboratories generally set guidelines so that data from your samples will truly reflect your patient’s condition.

Although most people understand the importance of careful sample handling by their laboratories, the most crucial components of sample preparation begin long before the sample reaches the lab. The quality of the laboratory results begins with your sampling technique. Unfortunately, practitioners and paraprofessionals often become distracted by dying patients, distraught clients or a hectic day. As sample handling seems like the least of their problems at the time, it is often neglected and major artifacts may be introduced before the sample reaches the lab. Keeping in mind a few general principles may save you countless erroneous results that may lead to misdiagnosis.

With the current information explosion in biomedical sciences, time is more and more valuable in the veterinary curriculum. Due to this time squeeze, proper procedures in sample acquisition and handling are sometimes neglected in our training programs. We spend much more time teaching the important skill of interpreting lab data, but unfortunately, even the best veterinary diagnostician cannot make sense of fallacious data.

We set guidelines in our laboratory for sample submission so that we can catch as many of the errors and pitfalls as possible before the sample is analyzed. When we discover a problem that will adversely affect the results of a test, we reject the sample and request that a new one be drawn. We do this because we firmly believe that erroneous data is worse than no data at all. This was initially met with disappointment and anger from some of our clinicians and students, but time has shown that the extra time that it takes to redraw the sample has been well worth the effort.

Proper sample handling begins with meticulous venipuncture technique. It is best to use the needle with the largest bore possible to avoid trauma to the sample. All too often shock, dehydration or a fractious patient makes obtaining a sample more art than science. Repeated probing under the skin may ultimately yield a blood sample, but it is often contaminated with subcutaneous fluids rich in tissue thromboplastin. This may lead to major problems with samples that must be collected in anticoagulant. The extrinsic clotting pathway and platelet aggregation are initiated before the sample reaches the anticoagulant. This ruins hematology samples, as unpredictable numbers of cells and platelets become enmeshed in the clot, thus invalidating erythrocyte, leukocyte and platelet counts. Even if the clots are fished out from the tube, your counts will be erroneous. This ultimately invalidates the entire CBC because all of the useful parameters are based upon accurate counts.

Even if the entire clotting pathway is not activated, difficult venipuncture often leads to the clumping of platelets. Regardless of which method you use, accurate platelet counts are not possible on samples that have platelet clumps. Fortunately, we can often detect many of these samples by scanning a stained slide. We do not attempt platelet counts in samples with clumps in order to avoid alarming clinicians with spurious thrombocytopenias.

As a general rule, a greater number of artifacts will be introduced with the number of times a sample is transferred. If your patient has large enough veins for you to draw a sample directly into a vacutainer, it is best to do so. As this is often impractical in small or dehydrated patients, samples must be first drawn into a syringe and transferred to a tube with extreme care. To transfer blood from a syringe to a vacutainer tube, remove the rubber stopper from the tube (this applies to all tubes except for citrated (blue topped) tubes used for coagulation studies). It is important to remove the needle from the syringe before transferring the sample, because passing the blood back through a needle often lyses erythrocytes. Allow the blood to gently flow down the side of the tube, as samples are often traumatized and lysed if the blood is rapidly squirted into the vacutainer. Remember, these samples contain fragile living cells that are not accustomed or built for harsh treatment. A correct diagnosis may depend on retention of the characteristic morphology that these cells display in circulation.

Hemolysis not only ruins cell counts in samples submitted for CBC’s, it also may cause erroneous chemistry values. Unfortunately, the effect of hemoglobin on serum chemistries is not always constant between methodologies. It also may be difficult to predict the effect of hemoglobin of the different species on the various analytes.

If you are filling multiple tubes from one syringe, be sure to fill tubes containing anticoagulants first, as clotting may have already begun. Then continue to fill remaining tubes. It is important not to overfill tubes as the stoppers will not seal properly in overfilled tubes. Then, recap the tubes and gently mix all tubes containing anticoagulant.

Light blue tubes containing sodium citrate for coagulation studies are handled differently. These should be filled by leaving the needle on the syringe and inserting it directly into the citrated tube. Allow the tube to free fill. This is because the vacuum in the tube is calibrated for a specific volume of blood in relation to the concentration.
of anticoagulant. If the tube is overfilled or underfilled, coagulation studies will be adversely affected. These specimens are rejected at our lab.

If you use multi-sample vacutainer needles, please note the following sequence of draw: blood culture, red, navy, green, light blue, EDTA, sodium floride. This sequence avoids contamination of samples with anticoagulants and inhibitors. It is best to discharge approximately one ml of blood between green and light blue tubes so that no heparin is introduced into the citrated tube. In human settings, a red topped tube is purposely drawn before the citrated tube to clear any traces of tissue thromboplastin from the sample. We would be wise to follow this lead.

After the blood is collected, make certain that the specimen is clearly labeled with the patient and owner name and the date. It is also important to document the time of collection, either on the laboratory submission form or on the blood collection tube. Check with your laboratory, most likely they have established a labeling protocol to ensure that you receive results for the correct patient.

At this point, please remember that your work is not done! You are handling a vital sample, filled with living and breathing cells, easily denatured enzymes and chemicals that readily deteriorate. Do not leave the sample lying on your desk, or worse, in the dashboard of your vehicle. If you are transporting the specimen to an outside lab, it is wise to separate the serum or plasma from the cells by centrifugation within 4 hours of collection.

The following guidelines were established in our lab to avoid common problems. In our laboratory, the following guidelines apply to chemistry samples, using our instrumentation:

<table>
<thead>
<tr>
<th>Test</th>
<th>Handling Guidelines</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine aminotransferase-</td>
<td>do not freeze</td>
</tr>
<tr>
<td>Alkaline phosphatase-</td>
<td>stable at refrigerator temp only 6 hrs</td>
</tr>
<tr>
<td>Ammonia-</td>
<td>unstable, collect on ice or separate and freeze immediately</td>
</tr>
<tr>
<td>Amylase-</td>
<td>stable at refrigerator temp for 24 hrs</td>
</tr>
<tr>
<td>Bilirubin, (total, conj, unconj)-</td>
<td>protect from light, in light not more than 2 hrs</td>
</tr>
<tr>
<td>Calcium-</td>
<td>pH dependant, store stoppered at refrigerator temp less than 2 hrs</td>
</tr>
<tr>
<td>CO₂-</td>
<td>see comments for calcium</td>
</tr>
<tr>
<td>CSF Protein-</td>
<td>do not use EDTA tube, falsely increases value</td>
</tr>
<tr>
<td>CK-</td>
<td>stable at refrigerator temp only 4 hrs</td>
</tr>
<tr>
<td>Glucose-</td>
<td>remove serum from cells, glucose concentration will decrease at a rate of 7% in one hr</td>
</tr>
<tr>
<td>LDH-</td>
<td>Do not refrigerate as it decreases stability</td>
</tr>
<tr>
<td>Lipase-</td>
<td>stable at refrigerator temp for 24 hrs</td>
</tr>
</tbody>
</table>

Samples submitted for hematology also have specific guidelines. Do not submit clotted samples for the reasons detailed above. It is important to remember that EDTA is the anticoagulant of choice for hematology studies. This is because heparin, citrate or other anticoagulants alter the morphology and make accurate differential counts impossible. Citrate also introduces an unacceptable dilutional effect, and heparin inhibits the lysing agent used in our laboratory.

EDTA tubes must be filled with a minimum volume of blood before the anticoagulant is diluted enough so that cell counts will not be significantly reduced. Our minimum fill requirements are as follows:

- 10ml EDTA tube- 2.5ml
- 5ml EDTA tube- 1.25ml
- 3ml EDTA tube- 1.5ml

We have found by experience that platelet clumps are much more prevalent in samples drawn in 3 ml EDTA tubes than in larger tubes. Therefore, our small animal samples submitted for platelet counts are drawn in 5ml tubes. Large animal samples tend to clot in 5ml EDTA tubes, therefore large animal CBC's are collected in 10ml tubes.

EDTA is a great anticoagulant if the sample is fresh. However, many problems may be introduced by allowing the cells to remain in the tube for several hours. We have found that slides made from samples older than 6-8 hours are often unreadable because the leukocytes swell, become vacuolated, and even extrude their nuclei. If analysis of your sample will be delayed for several hours, it is best to make slide smears of the blood and allow them to air dry. This process fixes the sample for an indefinite period of time, and there is no need for fixation or staining before the sample reaches the lab. This also applies to slide smears prepared for cytologic examination.

Other parameters of the CBC are also affected by storage of the sample at room temperature. Platelets will clump and deteriorate within a couple of hours. Refrigeration can slightly prolong this change. Erythrocytes tend
to swell after 6 hours, raising the PCV and the MVC and lowering the MCHC. Refrigeration can delay these changes beyond 24 hours.

Coagulation studies using citrated tubes, require a precise volume of blood as detailed above. Our technologists check this volume against prefilled controls to prevent spurious results. Citrated plasma is acceptable for coagulation testing only if it is less than 4 hours old and/or frozen.

References


The following are licensed veterinary diagnostic kits for cats and dogs from USDA lists as of March 1980. These will be discussed as to ease of use and specificity and sensitivity.

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Test System</th>
<th>Detection</th>
<th>Sample Required</th>
<th>Firm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brucellosis</td>
<td>NSAT/2ME-NSAT</td>
<td>Ab* to B. canis Ag*</td>
<td>Serum</td>
<td>Pitman-Moore</td>
</tr>
<tr>
<td>Disease</td>
<td>Ind Immunoflu</td>
<td>Ab-extracted virus</td>
<td>Coated to polymer</td>
<td>Shaw / Marion</td>
</tr>
<tr>
<td>Heartworm</td>
<td>Latex agg</td>
<td>Circulating Ag of</td>
<td>Serum</td>
<td>Immunogenat</td>
</tr>
<tr>
<td>Test KIt</td>
<td>Dirofilariosis</td>
<td>Immitis to Ab</td>
<td>coated beads</td>
<td>Idex, Inc</td>
</tr>
</tbody>
</table>

PROC. 9th ACVIM FORUM

NEW ORLEANS, LA, MAY 1991