Proper sampling is an important factor to eliminating problems with test results.

Eliminating the confusion in test results

Avoiding certain mistakes will prevent misleading results and help lead to accurate diagnosis

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Laboratory tests are only helpful in veterinary practice if the results can be believed and trusted as being real and, therefore, meaningful. Interpreting results from the CBC and serum chemistry profile done on hemolyzed or lipemic samples often leads to misinformation about the dog's or cat's health state. In addition, insufficient volume of urine used for performing the urinalysis can be misleading.

I assume you have also encountered some of these comments:

"Hemolysis may falsely elevate CPK, AST, LDH, total protein, phosphorus, and uric acid; and falsely decrease GGT, glucose and alkaline phosphatase."

"Moderate icterus may result in decreases in creatinine, cholesterol, AST, and total protein."

"Serum bile acids results may be inaccurate due to lipemia or hemolysis."

"Insufficient amount of urine was submitted to perform the urinalysis."

"The urine protein:creatinine ratio must be evaluated in conjunction with urine sediment findings as hematuria or inflammation can result in abnormal ratios."

Inaccurate blood test results

The most frequent causes of inaccurate laboratory tests results are aged sample, hemolysis, icterus, lipemia, insufficient sample quantity and drug interference from post therapy sampling or contamination. In addition, another less obvious problem is using the wrong type of commercial sample collection tube (Table 1, p. 12).

Hemolysis and its prevention

Grossly or even moderately hemolyzed blood samples should not be acceptable for serum chemistry profile testing. Hemolyzed serum or plasma is pink or red, rather than the normal clear straw or pale yellow color. Serum samples are best collected in serum separator tubes and spun prior to testing with in-hospital equipment or shipment to the reference laboratory. Serum separator tubes avoid problems due to seasonal tem-

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perature extremes.

Most cases of hemolysis can be avoided by observing the following steps:

- For routine blood collections, use a 20- to 22-gauge needle.
- Use only clean, dry, sterile needles, syringes, and tubes and collect blood in room temperature containers.
- When there is difficulty accessing a vein, damage to the red blood cells may result. Correct by collecting a fresh tube when blood flow is established or select another puncture site.
- Be as gentle as possible, drawing the blood evenly. Too much pressure in drawing blood into a syringe or forcefully ejecting blood into a collection tube from a syringe may damage red cells.
- Do not collect a blood sample over a hematoma.
- Allow blood sample to clot completely before centrifuging.
- Do not centrifuge the blood sample for a prolonged period of time.

When using vacuum tubes containing additives (such as anticoagulants, preservatives, or clot activators):

- Tap the vacuum tube gently at a point just below the stopper to release any additive adhering to the tube or stopper.
- Permit the vacuum tube to fill completely to ensure the proper ratio of blood to additive. There will be some dead space at the top of the tube.
- To ensure adequate mixing of blood with the anticoagulant or preservative, use a slow rolling wrist motion to invert the tube gently five or six times. Failure to invert tubes may lead to the formation of microscopic clots. Rapid wrist motion or vigorous shaking may contribute to hemolysis.
- Check to see that all the preservative or anticoagulant is dissolved. If any preservative powder is visible, continue inverting the tube slowly until the powder is dissolved.
- If multiple samples are being drawn, invert each sample as soon as it is drawn. Do not delay. Place the vacuum tube upright in a rack as quickly as possible after collection.
- The serum-separator tube is an additive tube and should be inverted five to six times after collection. Allow the tube to stand 15-30 minutes for complete clotting to occur prior to centrifugation.

When using vacuum tubes without anticoagulants:

- Permit the vacuum tube to fill completely.
- Let the filled vacuum tube stand for a minimum of 30 minutes and not longer than 60 minutes prior to centrifugation, preferably recommend no more than two hours. This allows time for the clot to form. If the blood sample is allowed to stand for longer than 60 minutes, chemical activity and degeneration of the cells within the vacuum tube will take place, and test results may be altered.
- Centrifuge the blood sample at the

dvm Table 1: Commercial Sample Containers

- **Red-stopper tube:** Contains no anticoagulant or preservative. Use: Serum or clotted whole blood. Serum must be separated from cells within 45-60 minutes of venipuncture. Send serum in a plastic transport tube.
- **Mottled red/gray or cherry red-stopper (serum-separator) tube:** Contains clot activator and gel for separating serum from cells, but not anticoagulant. Do not use serum-separator tubes to submit samples for therapeutic drug monitoring. Always check the test description to determine whether a serum-separator tube is acceptable. Use: Serum. May be used for assays requiring serum unless otherwise stated. Separate serum from cells within 45 minutes of venipuncture. If sample is centrifuged before clotting is complete, a fibrin clot will form on top of the cell. This finding is frequent in hemolyzed samples.
- **Lavender-stopper tube:** Contains liquid K_3 EDTA. Use: EDTA whole blood or plasma. Send plasma in a plastic transport tube labeled "Plasma, EDTA." Send whole blood in a lavender-stopper tube.
- **Gray-stopper tube:** Contains sodium fluoride (a preservative) and potassium oxalate (an anticoagulant). Use: Sodium fluoride whole blood or plasma. Send plasma in a plastic transport tube labeled "Plasma, Sodium Fluoride." Send whole blood in a gray-stopper tube.
- **Blue-stopper tube:** Contains sodium citrate. Be sure to use only tubes with a 3.2 percent sodium citrate concentration. These are easily identified by the yellow diagonal stripes on the label. Use: Sodium citrate plasma. Send plasma in a plastic transport tube labeled "Plasma, Sodium Citrate." Send whole blood in a blue-stopper tube.
- **Green-stopper tube:** Contains sodium heparin or lithium heparin. Use: Heparinized whole blood or plasma. Send plasma in a plastic transport tube labeled "Plasma, Sodium Heparin" or "Plasma, Lithium Heparin." Send whole blood in a green-stopper tube.
- **Yellow-stopper tube:** Contains 1 ml acid citrate dextrose (ACD) solution. Use: ACD whole blood. Send whole blood in a yellow-stopper tube.
- **Royal blue-stopper tube:** Contains sodium EDTA for trace metal studies. Use: EDTA whole blood or plasma. Send whole blood in a royal blue-stopper tube. Send plasma in a plastic transport tube labeled "Plasma, EDTA".

end of the waiting period in strict accordance with the manufacturer's instructions for speed and duration of centrifugation (usually 10-15 minutes).

Lipemia and its prevention

Normal serum or plasma is a clear and light yellow to straw in color. Turbid serum or plasma appears cloudy or milky. Serum or plasma may be cloudy due to bacterial contamination or chronic or transient high lipid levels in the animal's blood. Animals that consume foods within the 24-hour period immediately preceding collection of a blood sample may have temporarily elevated lipid levels, which may be manifested by cloudy or lipemic serum. Lipemic serum or plasma may not be a true indicator of the animal's physiologic state. Regardless of diet and length of fast, some animals may produce cloudy samples – such as adult Schnauzers. To avoid dietary-induced high lipid levels prior to testing, animals should be excluded foods for 12-14 hours prior to sample collection.

> Always draw whole blood in an amount 2.5 times the required volume of serum required for a particular test.

Sample quantity not sufficient

One of the most common and expensive errors in sample collection is the submission of an insufficient volume of sample for testing. The laboratory sends out a report marked QNS (quantity not sufficient), and the animal has to be returned for a repeat collection at additional expense and inconvenience to the owner and to the veterinarian. To ensure an adequate sample volume:

- Always draw whole blood in an amount 2.5 times the required volume of serum required for a particular test. For example, if 4 ml serum is required, draw at least 10 ml whole blood.
- In general, for testing 20 analytes in the serum chemistry profile, 3 to 4 ml of whole blood is needed to obtain heparinized plasma, while 4 to 5 ml of clotted blood is needed to express serum. Two to 3 ml of EDTA blood and citrated blood is suffi-

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cient to perform hematology and coagulation tests, respectively. One ml of whole blood is sufficient to perform three to four analytes in the serum chemistry profile.

- For most serum and plasma tests, check to be certain that the transport tube is half full. Certain tests (such as prothrombin time) require a 90 percent to 100 percent full tube in order to achieve the proper blood-to-anticoagulant ratio; otherwise, the sample may be found to be QNS.

Inaccurate urine tests results

From a chemical and cytologic viewpoint, marked changes can occur in urine on standing and shipping. For the best possible results, urine should be examined immediately after collection. If shipping urine for analysis, it is important to:

- Collect at least 5 ml of fresh urine in a sterile leak-proof container.
- State method of collection, such as catheter, mid-stream, cystocentesis, and other means.
- Preserve urine for sediment cytologic examination by using l drop of formalin (40 percent formaldehyde) to 30 ml of urine, and state that this preservative has been used. An unpreserved sample should be included for chemical tests.
- If bacterial culture is required, submit a urine-soaked swab too.

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Thyroid testing

Hypothyroidism is diagnosed in dogs based on historical and physical examination findings, blood and urine test results (CBC, serum chemistry profile, urinalysis and specific endocrine function test results (usually serum T4 and free T4 concentrations). Personally, I believe hypothyroidism is over-diagnosed in veterinary practice and no dog should be confirmed as having hypothyroidism until the dog is eating and functioning reasonably well. Concurrent illnesses such as diabetes mellitus, chronic renal failure, hepatic insufficiency and infections can cause euthyroid-sick syndrome, resulting in decreases in serum T4 concentrations. Drugs, such as anesthetics, phenobarbital, primidone, diazepam, trimethoprim-sulfas, phenylbutazone, salicylates and glucocorticoids, may decrease serum T4 concentrations. Free T4 concentrations are used to differentiate between "euthyroid sick" syndrome and true hypothyroidism. Theoretically, serum free T4 concentration is not subject to spontaneous or drug-induced changes that occur with serum T4 concentration. Glucocorticoids will decrease both serum T4 and free T4 concentrations in dogs.

Phenobarbital treatment is common in dogs and may alter thyroid testing. Phenobarbital treatment is associated with decreased total serum T4 and decreased free T4 concentrations. This may be due to induced metabolism of T4, increased biliary excretion of T4 due to increased bile flow or increased peripheral deiodination of T4 to T3. TSH concentrations may be either normal or increased. Therefore, low free T4 concentration in a dog treated with phenobarbital can lead to a misdiagnosis of hypothyroidism. Low free T4 and total T4 concentrations may normalize by four and six weeks after discontinuation of phenobarbital at which time thyroid testing, along with serum cholesterol levels and clinical evaluation, can be repeated.

Adrenal testing

Hyperadrenocorticism is diagnosed in dogs based on historical and physical examination findings, blood and urine test results (CBC, serum chemistry profile, urinalysis and urine cortisol-to-creatinine ratio), and specific endocrine function test results (ACTH stimulation test, low-dose dexamethasone

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suppression test, and possibly high-dose dexamethasone suppression test). The ACTH stimulation test is less sensitive for the diagnosis of adrenocortical tumors and pituitary-dependent hyperadrenocorticism. I prefer the urine cortisol-to-creatinine ratio as an effective screening test and the low-dose dexamethasone suppression test to confirm hyperadrenocorticism. The low-dose dexamethasone suppression test and urine cortisol-to-creatinine ratio are also not able to differentiate between adrenocortical tumors and pituitary-dependent hyperadrenocorticism. The high-dose dexamethasone suppression test is useful in distinguishing between adrenocortical tumor and pituitary-dependent hyperadrenocorticism.

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Phenobarbital also speeds the clearance of dexamethasone but should not affect low-dose dexamethasone suppression testing in most dogs. Phenobarbital does not affect the ACTH stimulation test or endogenous ACTH concentrations. Dogs that are treated for hyperadrenocorticism with mitotane (Lysodren) that are also being treated with phenobarbital may require higher loading and maintenance dosages of mitotane. This is most likely due to phenobarbital-induced increases in the elimination of mitotane.

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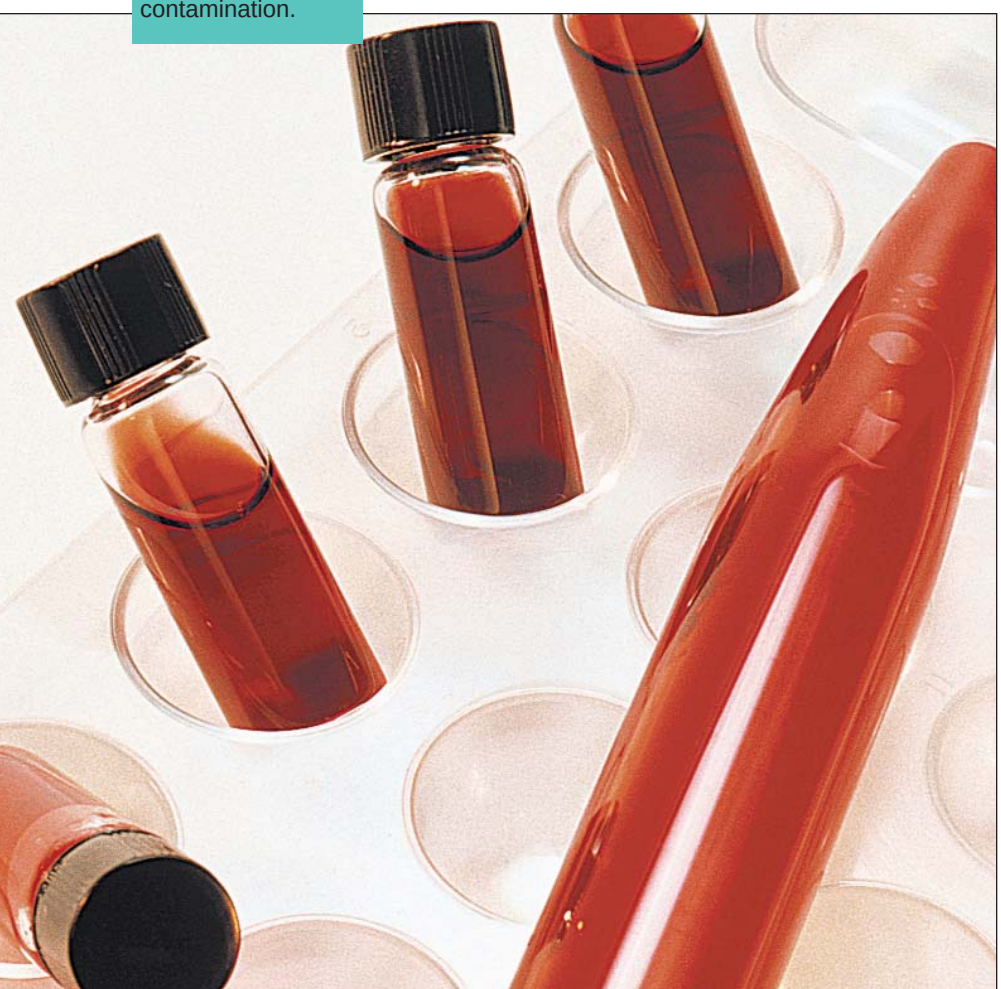

Summary

Laboratory tests are only helpful in veterinary practice if the results can be believed and trusted as being real and, therefore, meaningful. The most frequent causes of inaccurate laboratory tests results are aged sample, hemolysis, icterus, lipemia, insufficient sample quantity and drug interference from post therapy sampling or contamination. Another less obvious problem is using the wrong type of commercial sample collection tube. Interpretation of thyroid and adrenal test results can also contribute to wrong interpretation of tests results. **DVM**

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Suggested reading

- Ettinger SJ, Feldman EC: Textbook of Veterinary Internal Medicine: Diseases of the Dog and Cat, 5th edition. 2000.
- Willard MD, Tvedten H, Turnwald GH: Small Animal Clinical Diagnosis by Laboratory Methods, 3rd edition. 1999