Monitoring of Thyroid Therapy in Dogs

By: Patricia A. Schenck, DVM, PhD and Kent R. Refsal, DVM, PhD

Once a diagnosis of hypothyroidism has been made and the dog has started thyroid replacement therapy, thyroid hormone levels should be monitored to ensure that therapy is adequate. Within days, clinical effects of supplementation may be apparent as increased energy and attitude change. However, skin and neurologic signs may take weeks to months to improve after initiation of therapy. We recommend that, in newly diagnosed cases, a serum sample for monitoring be submitted about 6 weeks after the start of therapy. A serum sample should also be submitted after a change in dose or brand of thyroid replacement therapy. In cases of long-term therapy, a sample should be submitted periodically to make sure that the thyroid supplement is adequate and to identify instances where the dose required may be reduced (as with loss of weight).

For monitoring purposes, thyroid hormones and TSH should be measured. Since total T4 (TT4) is given as treatment, TT4 concentration is an important parameter. TT4 concentration gives information about the dose and administration or absorption of the therapeutic product and is interpreted with regard to interval of hours post-pill. Measurement of TSH gives a slightly longer-term picture of the adequacy of therapy. The aim of adequate thyroid supplementation is to keep TSH suppressed to within the reference range. An elevated TSH indicates demand for additional TT4, and thus inadequate supplementation.

Steady-state levels of thyroid hormones will be reached about a week after initiation of therapy and thyroid stimulating hormone (TSH) may not be fully suppressed into the reference range for several days. A monitoring sample can be obtained 10 to 14 days after starting or changing therapy. With long-term therapy, TSH concentration can be expected to reflect the preceding 2 to 4 days of supplementation.

The expected concentration of TT4 depends on the interval post-pill at which the sample was obtained. Thyroid monitoring results can be interpreted with confidence at any time after 3 hours post-pill, as long as that interval is known. When thyroid replacement therapy is given twice daily, TT4 concentrations should be at the top or slightly above the reference range (approximately 3 hours post-pill) and within the lower half of the reference range before administration of the next pill. When thyroid replacement therapy is given only once daily, it may be useful to measure thyroid hormones and TSH in a sample taken more than 12 hours post-pill.

Occasionally, thyroid hormone and TSH results provide discordant messages. When TT4 is low but TSH is well suppressed, it suggests that dose, administration, or absorption was inadequate on the day of the test but that overall the pituitary gland is satisfied that supplementation is adequate. An opposite pattern is sometimes seen, where thyroid hormone concentrations appear adequate but the TSH concentration is elevated. In these cases, thyroid therapy on the day of the test is better than on the preceding days. This could be consistent with poor treatment compliance except on the clinic visit day, or it could reflect an intermittent interfering factor such as gastrointestinal disturbance.

The most common causes of failed thyroid therapy are noncompliance by the owner or avoidance of therapy by the dog, where the dog never receives the prescribed level of medication. High-fiber diets may also limit absorption of supplements. Occasionally some dogs absorb thyroid medication poorly and no underlying problem can be identified.

If thyroid hormone concentrations are adequate and TSH is suppressed, yet clinical signs are not resolved, concurrent disease may be present. If there is concern that the original diagnosis of hypothyroidism was not correct, withdraw thyroid medication and retest in 6 weeks. After 6 weeks with no thyroid supplement, the endogenous thyroid status of the dog can be assessed and is free from interference by thyroid medication.
**In Situ Hybridization for Diagnosis and Research**

By: Matti Kiupel, DVM, PhD, Dipl ACVP and Ingeborg Langohr, DVM, PhD, Dipl ACVP

To establish the cause or pathogenesis of certain diseases, it is often necessary to determine the presence of infectious agents or abnormal gene expression in association with morphologic changes. *In situ* hybridization (ISH) is a powerful molecular tool that allows detection of a particular DNA or mRNA sequence within tissue sections. In essence, any nucleic acid sequence can be specifically detected by the use of a probe that is the “antisense” reverse complementary sequence of the target.

At the DCPAH, we use digoxigenin-labeled oligonucleotide probes, which are single-stranded synthesized DNA strands. Their synthesis is easy to achieve, they are stable, and they hybridize to only specific target nucleic acid. We use probes primarily to detect infectious agents such as viruses, rickettsia and fungi for which immunohistochemistry has low specificity or antibodies are not available that work on formalin-fixed tissues. Whereas most of our probes are species-specific, some are more generic, e.g., papillomaviruses. By screening tissues first with a generic probe, we can search for a wider range of agents and use species-specific probes at a later stage to further speculate the agent of interest. ISH may also be used to localize mRNA and to determine expression levels of specific transcripts such as oncogenes. This provides spatial and temporal information about gene function within specific tissues. All tests can be performed on routine formalin-fixed, paraffin-embedded biopsy or necropsy material as well as research samples. This allows for additional morphologic information on the distribution and expression pattern of the target in complex tissues, as well as for retrospective analyses.

In general, ISH is regarded as a time-consuming, labor-intensive and technically complex method. We recently acquired an automated system that completes the entire protocol in 1 day under controlled conditions. This system also allows for consistency in our results. The cost for a single ISH test is $45/sample (see current Fee Schedule on the DCPAH CD or website). We can likewise develop and validate an assay that meets your specific needs if you provide us with the sequence of a specific infectious agent or gene of interest and with proper control tissues.

**Blood Spot Analysis for Heavy Metals and Pesticides**

By: Andreas Lehner, MS, PhD; Wilson K. Rumbeiha, BVM, PhD, DABT, DABVT; Alan Shlosberg, BVSc; Margaret Johnson, MT (ASCP)

Traditional approaches to the detection of heavy metals and organic pollutants involve testing of fairly large volumes of whole blood from the affected animal. This makes testing of very small animals (birds, frogs, fish, turtles, snakes, neonates, etc.) or wildlife populations difficult. The DCPAH Toxicology Lab has developed and validated a new approach to heavy metal and organic pesticide testing which requires only 50 μL of blood, with the goal of increased options for clients. The new technique may be of interest to those performing field research, examining large populations, or working on animals with small blood volumes.

Blood samples are spotted directly on filter paper (50 μL, to fill the inscribed circles), dried for 4 hr, and sealed in a plastic bag with desiccant for transport to the lab (Figure 1). In the laboratory, the dried blood spots are excised from the card and used for analysis. The test is sensitive for detection of heavy metals and persistent organic pollutants with limits of detection of 10 and 5 ppb, respectively.

Currently, tests on the dried blood spots have been validated for heavy metals (arsenic, cadmium, lead, mercury, selenium, and thallium), which affect the nervous, renal, and hepatic systems, and persistent organic pollutants (DDT, lindane, and PCB-153), which cause hyperexcitability, seizures, and reproductive failure.

In addition to the small sample volume required, advantages of the dried blood spot method include easy storage and shipment at room temperature. Samples may be shipped internationally after disinfection with a brief USDA-approved heating regimen. Tests can be done on individual animals, but this method is particularly suited for population studies. Dried blood spot tests will be priced competitively and discounts will apply to large sample submissions. Kits for dried blood spot collection will be available from DCPAH and will include complete materials for 25 samples and sample collection/handling instructions. A formal announcement of the launch of these tests will be made on our website. Please call the Toxicology Section (517.353.1683) for further information.
Garbage In, Garbage Out: Tips for Avoiding Pre-Analytic Sample Problems

By: DCPAH Clinical Pathologists

The accuracy of results is only as reliable as the quality of the sample tested. Pre-analytic factors can negatively impact a sample, making it impossible for the Clinical Pathology Section to provide accurate test results that reflect any abnormalities in a patient. Some of these factors are physiologic in the patient and cannot be controlled. However, many of the sample problems that we see are avoidable and a direct result of improper sample collection, handling, or transport.

Delayed Separation of Serum from the Clot

One of the most frequent problems is submission of serum for chemistry testing that has not been removed from a clot tube or is in a serum separator tube (SST). Do not rely on the gel in an SST to keep serum separate from the clot, because the gel may not seal completely or may break down in transit.

This is illustrated by a sample from an adult horse (Figure 1) that arrived at the Clinical Pathology laboratory in an SST 2 days after collection. The serum had moderate hemolysis. Abnormal results on a serum chemistry profile included:

- hyperkalemia (8.2 mmol/L; reference interval 2.6-4.8)
- hyperphosphatemia (8.3 mg/dL; reference interval 5-4.7)
- hypoglycemia (<5 mg/dL; reference interval 78-124)

These abnormalities can be explained by problems with the sample. There is no way to accurately predict what the results would have been if the sample had been handled properly. Consumption of glucose by cells in the clot resulted in false hypoglycemia. While hemolysis may occur in vivo, it often occurs during sample collection, centrifugation, or shipping. The degree to which hemolysis interferes with some chemistry testing depends on the analyzer and methods used, and may occur for several reasons:

1) The red color may interfere with spectrophotometric assays. 2) Erythrocytes may release substances that positively or negatively interfere with enzyme reactions. 3) Erythrocytes contain high concentrations of some substances that will falsely elevate serum concentrations when released from the cells.

In this horse, hyperkalemia and hyperphosphatemia can be explained by leakage from erythrocytes. Hyperkalemia is not expected when hemolyzed samples from cats or dogs, except for some Akitas and Shiba Inus.

Tips to prevent hemolysis include:

- avoid excess negative pressure during blood collection
- for tubes with clot activators, gently mix (do not shake) blood following collection
- avoid heating or freezing whole blood samples
- employ proper centrifugation
- separate serum from the clot promptly after collection

Aged Samples

Another common pre-analytic problem is submission of aged blood or other body fluids for analysis. Cells deteriorate in vitro. Deterioration may make it impossible to identify cell features or even cell types on cytologic and hematologic preparations.

Figure 2 is an image of a blood smear that was prepared from 7-day-old blood that arrived in the laboratory. Deterioration of the leukocytes made it impossible to provide a WBC differential.

To maximize information from blood or body fluids:

- submit 2 unstained, air-dried slides prepared from a gently-mixed sample (see the fall 2008 newsletter at http://www.animal-health.msu.edu/News/2008_Fall.pdf for tips on making slides of body fluids)
- ship the smears in a plastic holder to protect from breakage
- insulate the slides from the ice pack and fluid
- submit the EDTA tube with an ice pack, making sure that the fluid is insulated from direct contact with the ice pack to avoid freezing
- ship overnight to minimize transport time

Toxicology has improved its glomerular filtration rate (GFR) test with the goal of faster turnaround and increased options for clients. GFR is determined from clearance, i.e., the volume of plasma that has been cleared of a particular substance over time. Iohexol is a suitable triiodinated substance for GFR calculations, which are valuable in determining relative kidney function in canine/feline patients or drug clearance in animals undergoing chemotherapy.

Our traditional GFR method required time-consuming HPLC quantitation of iohexol isomers. DCPAH has validated a new approach that sensitively measures entire plasma iodine composition by ICP/MS. The new analytical approach has shortened turnaround, and samples are now run twice a week (Tuesdays/Thursdays) rather than just once as previously. The HPLC method is still available for ongoing research projects but will be retired once demand drops off. There are no changes in applicable fees for GFR (#70029) or iohexol (#70007).

Faster Iohexol/GFR by ICP/MS

By: Andreas Lehner, MS, PhD; Wilson K. Rumbeiha, BVM, PhD, DABT, DABVT; Margaret Johnson, MT (ASCP)

Visit the DCPAH website (www.animal-health.msu.edu) for other tips to avoid pre-analytic errors. You may also contact the Clinical Pathology Section directly at 517.355.1774 if you have questions about sample collection or handling.

FIGURE 1: Hemolyzed serum from a 2-day-old sample that was collected into a serum separator tube. Note that the tube was not properly centrifuged and the serum was not removed from the clot.

FIGURE 2: Smear (modified Wright’s stain) from 7-day-old dog blood. Note the poorly preserved leukocytes (B, C, D) when compared to fresh dog blood (A).
DCPAH Shipping Solutions – Mailer Price Increase

The following mailers will increase in price on April 1, 2010:

<table>
<thead>
<tr>
<th>Description</th>
<th>Price per Box</th>
<th>Total Price</th>
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<tr>
<td>Insulated Mailer/Includes FedEx Overnight Return</td>
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<td>$15.00</td>
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<td>$33.00</td>
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<tr>
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<td>12 boxes w/US Postal Service Return Postage</td>
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<td>$7.00</td>
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<tr>
<td>Biopsy Small w/FedEx Overnight Return</td>
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<tr>
<td>Biopsy Large w/FedEx Overnight Return</td>
<td>$15.00</td>
<td>$15.00</td>
</tr>
<tr>
<td>Insulated Mailer/No Shipping</td>
<td>$25.00</td>
<td>$25.00</td>
</tr>
</tbody>
</table>

Please note that the price difference between FedEx and USPS mailers is very small yet the benefit is HUGE – guaranteed, traceable, door-to-door service for only a few dollars more! Please contact us at 517.353.1683 and order yours today!

When we upgraded to our new laboratory operating system in 2005, we were able to offer you many different options for receiving your test results. This flexibility has proven popular with our clients, since each clinic has different reporting needs. However, as postage costs continue to increase, we must make some changes to these options in order to keep our overhead costs down, thereby continuing to keep our test prices reasonable. Effective April 1, 2010, all clients who are receiving reports by both mail and fax will receive results by fax only. If you are a mail/fax client, you may wish to select either e-mail or WebView as your report back-up choice.

Simply contact the lab at 517.353.1683 to make this change. All clients currently receiving results by mail only will be contacted via letter and encouraged to convert to fax, e-mail, or WebView options.

Looking for Results?
Changes In DCPAH Report Distribution!

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