Low T4 values are observed fairly frequently in practice. How should I interpret a low T4 result?

Relying on a low T4 value alone can lead to a misdiagnosis of hypothyroidism, especially in the absence of clinical signs or laboratory abnormalities suggestive of this disorder. T4 levels are often affected by a number of factors including age, breed (e.g., sighthounds have much lower levels than other breeds), level of activity, nonthyroidal illness (NTI), and medications such as phenobarbital, sulfonamides, and corticosteroids. And lastly, even in clinically normal dogs, T4 levels can fluctuate in and out of the normal reference range. In summary, if hypothyroidism is suspected based on a low T4 value alone, I recommend that clinicians run other tests to help confirm the diagnosis.

What other tests are used to help confirm the diagnosis?

It is important to emphasize that thyroid diagnostic tests do not always lead to a definitive diagnosis, no matter how many tests are performed. The exceptions would be a Tc99 thyroid scan or a thyroid biopsy. These tests, however, are expensive and not widely available (thyroid scan), and therefore, are not very practical. In lieu of the latter two testing procedures, I recommend running the following three blood tests to help confirm the diagnosis: free T4 by equilibrium dialysis (FT4ED), thyroglobulin autoantibody (TgAA) level, and an endogenous canine TSH (cTSH). If the pet owner can only perform one of these tests because of budget concerns, I would usually recommend the FT4ED. However, if the ultimate goal is to identify autoimmune thyroiditis, which is the heritable form of hypothyroidism, the TgAA would be the test of choice. What does a positive TgAA mean? What are the most common inconsistencies or misconceptions regarding the different confirmatory thyroid tests and test results?

What does a positive TgAA mean?

Elevated thyroglobulin antibodies are present in the serum of dogs with autoimmune thyroiditis which is a heritable trait and the most common cause of hypothyroidism. Dogs testing positive for TgAA should not be bred. However, the presence of antibodies does not establish the animal’s current thyroid status. Recent evidence suggests that at least 20% of dogs, where the only abnormality is a positive TgAA, will progress within a year to develop additional clinical or
laboratory abnormalities consistent with hypothyroidism. False positive TgAA results can occur for 30-45 days following rabies vaccination. False negative results occur in 8% of dogs verified to have T3 or T4 autoantibodies.

cTSH: The diagnostic sensitivity of the cTSH assay is poor compared to the TSH assay in people (70% versus 95%). In other words, up to 30% of dogs with hypothyroidism will have normal or low cTSH results. An elevated cTSH level is therefore consistent with hypothyroidism but a low or normal level doesn’t rule out the diagnosis. This subset of hypothyroid dogs with normal or low TSH levels are thought to produce a different bioform of TSH that the assay doesn’t recognize.

FT4ED: Compared to T4 concentrations, the FT4ED is less likely to be lowered by NTI and drugs. However, advanced cases of Cushing’s syndrome and other severe NTI and high serum levels of phenobarbital have been associated with very low FT4ED concentrations.

What is the best way to diagnose hypothyroidism in a dog with Cushing’s syndrome, diabetes mellitus, or being treated with phenobarbital, sulfonamides or glucocorticoids? Is the cTSH test useful in these situations?
The cTSH level has questionable diagnostic utility in the above situations. For example, cTSH is occasionally elevated in dogs on phenobarbital, and commonly elevated in animals treated with sulfonamides, and unchanged or low in patients treated with corticosteroids or have Cushing’s syndrome. In general, any dog with NTI or is being administered corticosteroids or sulfonamides and has a low T4 and hypothyroidism is suspected, the NTI should be medically managed first or the drugs discontinued if possible. Next the dog should be re-evaluated at a later date for hypothyroidism using the recommended confirmatory tests. In some cases, a thyroid hormone trial for 1-2 months may be indicated. With dogs on phenobarbital, the clinical picture dictates whether a thyroid hormone trial should be considered.

What about dosing and monitoring of T4 replacement therapy?
Dose: Thyroid hormone dosing is a little confusing because there are different recommendations in the US and Europe. In the US, thyroxine is typically dosed at 0.1 mg per 12 pounds of optimum body weight given BID (the dosage in sighthounds is lower; 0.1 mg per 15 pounds of optimum body weight). Twice daily dosing is based on the plasma half-life of thyroxine which is 12-16 hours. Many European practitioners use the same dose of thyroxine given SID. The clinical response to SID dosing is often acceptable, presumably because the biological action of thyroid hormones far exceeds that of the plasma half-life.

Monitoring: The ultimate goal of thyroid replacement therapy is complete clinical abnormalities. However, there are now well defined laboratory goals that can be expected to correspond with good clinical resolution. Therapeutic monitoring (post-pill testing) can be performed as early as 2 weeks following the start of therapy or after making a subsequent dosage alteration, although steady state
tissue response may take up to 4-6 weeks. It is recommended to test T4 levels (Test T496) at 4-6 hours after the morning administration of thyroid medication, ideally given on an empty stomach because thyroxine binds to calcium and soy in foods. T4 levels in the upper half of the normal reference range or mildly elevated would be considered optimal. However, in sighthounds optimal levels are just below the midpoint of the normal reference range.

Any final recommendations related to testing for hypothyroidism?

If I was to leave your readers with any take-away message, it would be that hypothyroidism is often diagnosed without adequate laboratory evidence

ANTECH Q&A on Free T4

The measurement of free fraction of thyroid hormone (free thyroxine) can be essential in the accurate determination of thyroid status in patients with inconclusive TT4 levels or in cases where confirmation of thyroid status is desired prior to therapy. The fT4-ED assay offered by ANTECH (test code: T460) has been shown to have superior diagnostic accuracy in canine and feline patients over the analog or direct fT4 assays because the presence of non-thyroidal disease in many clinical situations significantly reduces the diagnostic accuracy of both the TT4 and analog fT4 assays. The following Q&A explains why the fT4-ED test is the preferred test in these situations.

Question: Some veterinary reference labs have replaced the existing Free T4 by Equilibrium Dialysis (fT4-ED) assay with an analog-method chemiluminescent fT4 immunoassay (fT4-CH). Which is the preferred fT4 assay?

Answer: ANTECH's fT4-ED test remains the gold standard test, with superior accuracy compared to the fT4-CH test. While the fT4-ED test offered by ANTECH has demonstrated sensitivities of 98% for detecting hypothyroidism in dogs[1] and 98.5% for detecting hyperthyroidism in cats[2] in peer-reviewed research, the new fT4-CH assay substituted by IDEXX Reference Labs has shown a sensitivity of only 80% for identifying hypothyroidism in dogs and 87% for identifying hyperthyroidism in cats[3].

Question: Why is the fT4 by Equilibrium Dialysis more accurate?

Answer: The key step in enabling accurate measurement of the non-protein bound, biologically active ("free") T4 fraction, is to separate it from protein-bound T4 by dialysing serum across a semi-permeable membrane. All analog fT4 methods, including the fT4-CH assay, lack the fT4-ED assay's critical dialysis step and are interfered with by thyroid binding proteins and (much less commonly) by anti-T4 autoantibodies. This prevents them from accurately assessing thyroid function in dogs with NTI and dogs with T4 autoantibodies.

Question: Can you highlight the differences between TT4, analog fT4 and fT4-ED test results in common clinical scenarios?

Answer: The table below, provided by Peter Graham, Cambridge Specialist Laboratories and formerly Director of the MSU Canine Thyroid Health Study, highlights the most common clinical scenarios and how the fT4-ED test typically provides better discrimination, resulting in higher diagnostic accuracy.
# Common Thyroid Testing Scenarios for Dogs

<table>
<thead>
<tr>
<th>Common DOG Scenarios:</th>
<th>TT4 result</th>
<th>Analog method FT4 result</th>
<th>FT4-ED result</th>
</tr>
</thead>
</table>
| Low TT4 dog suspected of hypothyroidism with normal TSH | Low | Low (no add'l information) | • Low if hypothyroid  
• Normal if euthyroid with NTI |
| Normal TT4 in dog suspected of hypothyroidism | Normal | Normal (no add'l information) | • Low if hypothyroid with T4AA  
• Normal if euthyroid |
| Euthyroid dog with NTI | Low-normal or Low | Low-normal or Low | Normal |
| Hypothyroid dog with T4AA | Normal or High | Normal or High | Low |
| Hypothyroid dog | Low (High or Normal if T4AA present) | Low (High or Normal if T4AA present) | Low |
| Healthy euthyroid dog | Normal | Normal | Normal |

# Common Thyroid Testing Scenarios for Cats

<table>
<thead>
<tr>
<th>Common CAT Scenarios:</th>
<th>TT4 result</th>
<th>Analog method FT4 result</th>
<th>FT4-ED result</th>
</tr>
</thead>
</table>
| Normal TT4 in cat suspected of hyperthyroidism | Normal | Normal (no add'l information) | • High if hyperthyroid  
• Normal if euthyroid |
| Hyperthyroid cat with NTI | Normal | Normal | High |

*Courtesy of Peter Graham, BVMS PhD CertVR DipECVCP MRCVS


## Useful Resources

More information and current peer-reviewed research on canine and feline thyroid testing:


Please share your feedback with us regarding topics you’d like us to address in future newsletters! Just click on the following email link to submit your idea: newsletter@antechmail.com