Frequently-Asked Questions - Endocrinology
Thyroid Function in Dogs

1. Is it OK to submit plasma for a thyroid profile?

EDTA plasma can be used for thyroid tests and provides valid results. Also, serum collected in gel separator tubes provides a valid sample for thyroid profile analyses at Michigan State University.

2. What is lymphocytic thyroiditis (autoimmune thyroiditis)?

Lymphocytic thyroiditis is the underlying cause in many cases of primary hypothyroidism in dogs and the predisposition to its development is believed to be highly heritable. It is an immune mediated disorder characterized histologically by a diffuse infiltration of lymphocytes, plasma cells, and macrophages in the thyroid gland.

Antibodies interact with the follicular cell, colloid, or thyroglobulin antigens and activate the complement cascade and cell-mediated cytotoxicity. The progressive destruction of follicles and secondary fibrosis eventually leads to a failure of thyroid hormone production. More than 60 or 70% of the thyroid tissue needs to be destroyed before we see changes in laboratory measures of thyroid function. This process can take months or years to cause classic hypothyroidism. In some animals it may not progress. Anti-thyroglobulin antibodies are released into the circulation in animals with this condition. A test for these antibodies in serum is included in the Michigan State University Canine Thyroid Diagnostic profile.

3. Can vaccination cause lymphocytic thyroiditis?

This laboratory is not aware of any evidence to support the hypothesis that vaccination could be associated with the induction of immune mediated thyroid disease. In fact, some researchers have looked for and failed to find such an association (Hogenesch et al., 1999 *Advances in Veterinary Medicine* 41: 733-747).

4. How do I interpret MSU's thyroid profile?

In addition to testing for serum levels of total thyroxine (TT4), total tri-iodothyronine (TT3), free thyroxine (FT4), and free tri-iodothyronine (FT3), this profile tests for T4 antibodies (T4AA), T3 antibodies (T3AA), canine thyrotropin (cTSH; thyroid stimulating hormone), and thyroglobulin antibodies (TgAA). The cTSH test provides much needed information in any attempt to diagnose hypothyroidism. Many non-thyroidal factors can cause decreases of TT4, TT3, FT4, and FT3 into the hypothyroid range in a dog with normal thyroid function making it difficult to differentiate sick-but-euthyroid animals from those with hypothyroidism. When thyroid hormone

levels are low due to primary hypothyroidism, most (around 85%) animals will have abnormally high cTSH levels.

The antibodies (T3AA, T4AA, and TgAA) are markers for lymphocytic inflammation within the thyroid gland. T3AA and T4AA are subsets of TgAA which are present in only a proportion of TgAA positive animals. The T3AA andT4AA (THAA) cross-react with T3 or T4 in immunoassays and cause false results in some thyroid hormone assays. It is, therefore, crucial to know whether they are present before interpreting thyroid hormone results.

A step-by-step approach to interpreting the 8-parameter profile would include:

- Check for TgAA. If present, this result indicates the presence of thyroid pathology which is usually lymphocytic thyroiditis, but rarely in older patients, thyroid neoplasia. The absence of TgAA, however, does not exclude the possibility of thyroid dysfunction.
- Check for the presence of T3AA or T4AA. If present, these will interfere with the measurement of both total and free T3 and T4 (except free T4 by equilibrium dialysis) and cause false results to be generated for these tests which should be ignored. In the assays used in the Endocrine Diagnostic Section, the presence of T3AA causes falsely low total T3 (TT3), and falsely increased free T3 (FT3) results. The presence of T4AA causes falsely elevated total T4 (TT4) and free T4 (FT4) results. Fortunately, free T4 measured by the equilibrium dialysis technique (FT4d) which is part of MSU "Premium Profiles" is not affected by T4AA and can be trusted as a valid result even when T4AA are present. The presence of T3AA or T4AA does not interfere with TSH measurement.
- Check T4 concentrations (FT4d if T4AA are present) and TSH. In most dogs with overt primary hypothyroidism, T4 concentrations will be well below the reference ranges and TSH will be high. In euthyroid but sick dogs, T4 will be below and TSH within the reference range. Unfortunately, a proportion (around 15%) of hypothyroid dogs could also have this last pattern of results (low T4, normal TSH).
- Borderline concentrations of just one thyroid hormone measure, or elevated TSH alone does not provide sufficient evidence for a confident diagnosis of hypothyroidism.

5. My patient has normal thyroid hormone concentrations, but has an elevated thyroglobulin autoantibody result. What does this mean?

This most likely reflects the presence of subclinical thyroiditis (but very rarely might indicate thyroid neoplasia in an older dog).

In these cases, there is evidence of pathology within the thyroid glands, but not to the extent that it is interfering with thyroid function. Histologically, there are often small foci of lymphocytic inflammation scattered through otherwise healthy appearing thyroid tissue. We estimate that it takes >60% destruction of thyroid tissues by the inflammatory process before we see changes in laboratory measures of thyroid function. It appears that the progression to overt hypothyroidism in these cases is often very slow and that, indeed, some may never have progression of pathology but remain euthyroid for many years, if not for their whole lifetime.

6. What is the purpose of measuring T3 autoantibodies and T4 autoantibodies?

In dogs, antibodies which cross-react with T4 and/or T3 are markers for lymphocytic thyroiditis. These antibodies are generated against T4 and T3 containing epitopes on the thyroglobulin molecule, i.e., they are subsets of TgAA. Positive values indicate thyroid gland pathology, and also tell us about the validity of the thyroid hormone results. T3AA and T4AA are present in around 35% and 14% of hypothyroid dogs respectively. In the Endocrine Diagnostic Section at Michigan State University, T3 antibodies cause a false decrease in TT3 and a false increase in FT3 results. T4 antibodies falsely increase TT4 and may increase FT4 results in the standard thyroid profiles. In a hypothyroid dog with T4AA, T4 may be falsely elevated into the normal range and the true diagnosis masked. To obtain an accurate measure of T4 status in a T4AA positive animal, submit a sample for free T4 by equilibrium dialysis (FT4d) which is not affected by antibody. FT4d is included in Premium Thyroid Profiles. Although these antibodies have a large effect in the laboratory test tube, the clinical impression is that they do not interfere significantly with the availability of thyroid hormone in a thyroxine-treated hypothyroid dog.

7. When should I choose the Premium profile rather than the 'Standard' profile?

The premium profile is an 8-parameter profile that differs from the standard 8-parameter profile only in that it measures free T4 by an equilibrium dialysis method rather than by a two-step radioimmunoassay employed in the Standard Canine Thyroid Diagnostic Profile. This technique is elaborate and time consuming, but allows a more specific measurement of FT4 that is not affected by antibody interference, and is less commonly affected by certain medications or non-thyroidal illness than total T4 or standard measures of free T4. In the dog, choose this test if: 1) T4AA have been documented, 2) non-thyroidal illness is known to be present, or 3) there may have been interfering substances such as steroids or phenobarbital. Premium and Standard versions are also available for our Feline, Other Species, and Canine Thyroid Monitoring Profiles.

8. What is the free T4 by dialysis test used for?

The FT4 by dialysis test is indicated to better identify animals that are euthyroid but whose total- or standard free- T4 is falsely increased due to T4 antibodies or physiologically decreased due to general/systemic illness. A dialysis step first filters out the large antibodies and hormone binding proteins to provide a measurement technique unaffected by antibodies or by changes in T4-protein binding which can occur during non-thyroidal illness. FT4d is useful in cases were total- or standard free- T4 is borderline low and TSH is normal to assist in the differentiation of euthyroid but sick dogs from those 15% of hypothyroid dogs which could have a normal TSH result. FT4 by dialysis may rarely be increased in systemic illness due to decreased protein binding affinity for T4. FT4d is less commonly suppressed by non-thyroidal illness, but does occasionally fall below the reference range in some sick but euthyroid animals.

An important point to recognize is that results from both types of free T4 assays are highly correlated. Most samples that are normal in one assay will be normal in the other. Free T4 will be low with both assay methods in most hypothyroid dogs. There may be a slight improvement in diagnostic sensitivity for feline hyperthyroidism with free T4 by equilibrium dialysis, especially when seen in conjunction with a total T4 assay result. Situations in which measurement of free T4 by dialysis are likely to have an advantage over standard measures include:

Non-thyroidal illness (sick euthyroid). It is well known that thyroid hormone concentrations can decrease as part of a metabolic response to non-thyroidal illness. This decrease occurs in part from changes in thyroid hormone production, characteristics of serum binding, and metabolism of thyroid hormones. In these circumstances, measurement of free T4 in the direct-serum analog assay (standard profile) may underestimate the true circulating concentration of free T4 and give a falsely low result. Free T4 by equilibrium dialysis gives a more accurate result and so will more correctly identify normal free T4 concentrations in sick animals, allowing for better identification of animals that are not hypothyroid. However, even free T4 by dialysis may be low in some euthyroid animals with non-thyroidal illness. Also, a small number of sick but euthyroid cats have been shown to have a false positive result for hyperthyroidism with the assay of free T4 by equilibrium dialysis.

Effects of other medications. Circulating concentrations of thyroid hormones can be reduced secondary to administration of certain medications, for reasons similar to those listed for non-thyroidal illness. Clinicians are aware of these effects with drugs such as glucocorticoids, anti-epileptic medications, and phenylbutazone. Again, the assay of free T4 by equilibrium dialysis will better identify animals that are euthyroid. Administration of sulfonamides in high enough doses has a direct effect on the thyroid glands to block production of thyroid hormones and therefore creates a true (albeit reversible) hypothyroidism. Thus, sulfonamides will cause low thyroid hormones results and elevation of thyroid stimulating hormone regardless of the measurement technique for free T4.

T4-autoantibodies. A small number of dogs with lymphocytic thyroiditis will produce circulating thyroid autoantibodies that bind to T4. These endogenous autoantibodies cause interference in routine direct-serum immunoassays and cause a false increase of the measured concentration of T4 and free T4 when measured by direct assays. Assay of free T4 by equilibrium dialysis will provide an accurate measurement, because the interfering autoantibody in the patients' serum does not diffuse across the dialysis membrane and is not transferred into the assay tube. If a dog has a known T4 autoantibody, it is then best to use free T4 by dialysis to monitor T4 supplementation. Total and free T4 results will be accurate with either profile in dogs that have a positive T3 autoantibody result only.

9. Can thyroid stimulating hormone (TSH) be used to diagnose hypothyroidism and hyperthyroidism?

Thyroid stimulating hormone (TSH) is released by the pituitary in response to low serum T4 levels, due to loss of negative feedback. An increase in TSH concentration therefore indicates a failure of adequate thyroid hormone production (hypothyroidism). Abnormally high concentrations of T4, as seen in hyperthyroidism (a very rare condition in the dog) will inhibit TSH secretion and cause low serum concentrations of TSH. Unfortunately, current assays for canine TSH do not have sufficient sensitivity to differentiate normal from low concentrations. It is for this reason that the low end of the canine reference range extends to zero and the test cannot be used reliably to identify hyperthyroid states.

10. My patient has low thyroid hormone concentrations, but thyroid stimulating hormone is not elevated. What does this mean?

Depending on the clinical presentation, one of two main possibilities is likely.

1. The more common explanation is that T4 values often decline in animals with non-thyroidal illness and in animals receiving certain drug therapies (some glucocorticoids or anticonvulsants). Non-thyroidal illness may suppress TSH release from the pituitary via glucocorticoid-mediated inhibition or lower T4 concentrations by altering serum protein binding affinities.
2. The second possibility (if clinical evidence for hypothyroidism is strong and there is no illness or interfering medication identified) is that the animal truly has hypothyroidism but it is one of approximately 15% of cases in which TSH is not found to be elevated. If clinical signs are not suggestive of hypothyroidism, this picture could also be consistent with advancing age or normal breed differences (esp. sighthounds - see below). It is not clear why as many as 15% of hypothyroid dogs can have a normal TSH concentration, but suggested explanations include: rare cases of secondary hypothyroidism (where the pituitary gland is unable to secrete adequate TSH), the presence of TSH-suppressing concurrent significant non-thyroidal illness, and structural differences in the TSH molecule that interfere with its detection.

With this pattern of test results, a trial with T4 supplementation is suggested only if there is a strong clinical presentation consistent with hypothyroidism and if no non-thyroidal illness can be detected. An objective case review should be conducted after 6-8 weeks of therapy for evidence of clinical improvement. Thyroid supplementation can be discontinued if no improvement has occurred in that time, and the diagnosis reconsidered. A therapeutic monitoring sample taken during treatment should help confirm whether adequate amounts of thyroid medication were being absorbed. Even when there is clinical improvement, strictly speaking, therapy should be discontinued to see if the original clinical signs return in the absence of medication. Admittedly, this latter protocol is rarely followed in clinical practice. While the administration of thyroid hormone to animals which do not have hypothyroidism is generally considered to have minimal risk, large scale studies in human medicine have shown detrimental effects of such treatment in patients that have decreases in serum thyroid hormone concentrations due to non-thyroidal illness. (Brent GA and Hershman JM. Thyroxine therapy in patients with severe non-thyroidal illness and low serum thyroxine concentrations. J Clin Endocrinology and Metabolism. 1986, 63:1)

11. My patient has normal thyroid hormone concentrations, but thyroid stimulating hormone is elevated. What does this mean?

We are aware of a few circumstances which will cause this pattern of test results. However, there may be other possibilities about which we have yet to learn. Spurious elevations in TSH are occasionally seen both as a laboratory artifact and as a non-repeatable physiological anomaly. These two possibilities are identified by submitting a repeat sample for analysis.

Physiologic explanations for this pattern of test results include recovery from significant non-thyroidal illness when that illness had been associated with low serum thyroid hormone concentrations, and recovery from a reversible sulfa-drug-induced hypothyroidism. In these

instances, elevated serum TSH concentrations are observed as the pituitary encourages the suppressed thyroid glands to return to function. A similar finding might follow withdrawal of prolonged thyroid medication from a dog which had normal thyroid function. For this reason, in cases where a dog has been receiving thyroid supplement and the original diagnosis of hypothyroidism is questioned, thyroid supplementation should be stopped for approximately 6 weeks prior to diagnostic testing.

The principal pathologic explanation for this combination of test results is the presence of subclinical hypothyroidism (partial thyroid failure). When the functional reserve of the thyroid glands is decreasing (>60% destruction), the first physiologic response is to increase TSH production and encourage the small amount of remaining thyroid tissue to work much harder in an attempt to maintain normal serum thyroid hormone concentrations. Often, this compensatory mechanism is successful and low-normal thyroid hormone concentrations are maintained by the increased TSH production for many months, despite the "partial" thyroid failure. Because, normal thyroid hormone levels are being maintained it is not yet clear whether we should expect to see clinical signs of dysfunction in thyroid hormone dependent tissues. Those clinical signs which are present in these animals may reflect another non-thyroidal, illness and consequently a therapeutic trial might not result in marked clinical improvement.

The best approach in these cases is to retest at a later date. If subclinical hypothyroidism is present, the elevated TSH is likely to be persistent and T4 concentrations may fall further with time as the decrease in thyroid functional mass continues. If the animal is recovering from a serious illness or the effects of medication, TSH can be expected to return to reference range and T4 concentrations will remain normal.

12. The only abnormality in a thyroid profile is a low total T3 concentration. Could this be consistent with hypothyroidism?

First check T3AA to see if the TT3 result can be trusted to be valid. If T3AA is positive, then the interfering effect of this antibody is the most likely explanation for the low TT3 result. See above for the meaning of positive antibodies and the further interpretation of the 8-parameter profile.

If T3AA is negative, consider the following: when comparisons are made between the performance characteristics of T4 and T3 in their ability to differentiate normal from hypothyroid dogs, the T4 assays generally perform better. In the absence of T4 cross-reacting antibodies, T4 results more directly reflect thyroid hormone production by the thyroid glands than T3, whose concentration is heavily based on modulation of peripheral de-iodinases responsible for T4 to T3 conversion. Such a finding may reflect a "low T3 state of medical illness" suggesting a significant non-thyroidal illness.

The opposite situation, of low T4 with normal T3 concentrations in the absence of antibodies, also occurs. In this case, the fact that T3 concentrations are being maintained makes it unlikely that the animal would have clinical signs of hypothyroidism. We often attribute a combination of low T4, normal T3 and normal TSH concentrations to a "low-T4-state-of medical-illness". Whether there is any pattern to which dogs or which diseases are associated with Low-T4, or Low-T3 states of medical illness (or both) has not been determined.

13. What drugs affect thyroid hormone concentrations?

Phenobarbital and glucocorticoids cause a "sick euthyroid" pattern of low thyroid hormone levels without an increase in TSH.

The mechanism by which phenobarbital exerts its effect are not clear but may include a central component. In the long-term administration of phenobarbital there may even be slight decreases in FT4d and increases in TSH.

Glucocorticoids are known to suppress TT4 and FT4, but their effect on FT4d is generally less dramatic suggesting that their principal effect is through alterations in protein binding.

Sulfa-containing medications affect the thyroid directly to decrease T4 and T3 production, and cause a consequent increase in TSH. This is a true but reversible hypothyroidism. The pituitary thyroid axis recovers in less than 3 weeks following withdrawal of the medication.

Reports of a minimal effect of carprofen (Rimadyl) on TT4 exist. FT4d is less affected. However these studies have not included control animals and the existence of a biologically significant effect remains controversial.

Once an animal is receiving thyroid hormone medication, thyroid testing can only tell if that medication appears to be well absorbed. No comment can be made about the patient’s endogenous thyroid functional status. Because of the suppressive effects of thyroid medication on the endogenous thyroid in normal dogs, a withdrawal period of 6-weeks is recommended before a diagnostic sample can be taken with which the dogs' endogenous thyroid status can be assessed free from interference by the thyroid medication.

14. Is the thyroid profile affected by estrus or pregnancy?

Higher total thyroid hormone concentrations have been documented in normal bitches during periods of progesterone influence (metestrus and pregnancy). However, whether these numeric differences are of sufficient magnitude to cause misdiagnoses is yet to be determined but perhaps unlikely. The effect of reproductive stage on TSH has not yet been reported.

15. Total T4 and/or free T4 is (are) low, but everything else is normal. What does this mean?

We generally attribute this kind of finding to the "low-T4 state of medical illness" suggesting the presence of a significant non-thyroidal illness. This lowered thyroid hormone concentration we believe is due to the effects of the illness on hormone production and/or serum protein binding and perhaps excretion. The opposite situation of normal T4’s but low T3’s may also be observed.

16. Do some breeds of dogs have thyroid hormone reference ranges that differ from the general dog population?

Sighthounds (Greyhounds, Salukis, Afghan hounds, Whippets, Borzois, Irish Wolfhounds, Pharoah hounds, Ibizan hounds, etc.) have lower TT4 and FT4 reference ranges although these are generally not published. In some case studies, the TT4 reference range extends to zero. As such, a dog with hypothyroidism cannot be differentiated from a normal dog using T4 tests alone. Fortunately, general canine population ranges for TSH appear to apply well to these

breeds. Thus a sighthound with low TT4 and FT4 concentrations, but with a normal TSH concentration, is unlikely to be hypothyroid and does not require thyroid supplementation.

17. Should I use steroids to treat lymphocytic thyroiditis?

Treating with corticosteroids might be desirable to decrease the immune/inflammatory components of this disease, but our laboratory does not advocate their use. There are no published studies evaluating this approach in dogs. There are more disadvantages to steroid use than advantages, because of their many side effects. A decrease in inflammation can be achieved without corticosteroids by using T4 therapy. Treatment with exogenous T4 causes atrophy of the endogenous thyroid through negative feedback. Over time, the resulting decrease in thyroid mass results in a concomitant decrease in the inflammatory process.

18. Thyroglobulin autoantibody, T3 autoantibody, and T4 autoantibody are reported as a percentage. What does this mean?

Thyroglobulin autoantibody, T3 autoantibody, and T4 autoantibody are reported as a percentage of the value of the positive control for the assay. For TgAA, samples that are > 35% of the positive control value are considered to be positive for the presence of lymphocytic thyroiditis.

19. A dog is receiving thyroid supplement but hypothyroidism was never initially diagnosed. How can I determine if this dog is really hypothyroid?

Once an animal is receiving thyroid hormone medication, thyroid testing can only tell if that medication appears to be well absorbed. No comment can be made about the patient's endogenous thyroid functional status. Because of the suppressive effects of thyroid medication on endogenous thyroid hormone production in normal dogs, a withdrawal period of 6 weeks with no thyroid supplement is recommended before a diagnostic sample can be taken. After 6 weeks of no thyroid supplement, the dogs' endogenous thyroid status can be assessed, and is free from interference by thyroid medication.

20. An extra test was performed on my sample for nonspecific binding (NSB). What does this mean?

In most assays, there is specific binding, and nonspecific binding which is usually low. This nonspecific binding can vary from individual to individual, and may be high enough to cause an apparently positive TgAA value. In these cases, an extra test to evaluate the contribution of nonspecific binding is performed. In many instances, the specific TgAA is still positive indicating lymphocytic thyroiditis. However, in some instances, the specific TgAA becomes negative when nonspecific binding is taken into account.

21. My patient has a thyroid mass, yet thyroid hormone concentrations were normal to low. Why aren't they elevated?

Most thyroid masses in the canine are nonfunctional and do not secrete excess concentrations of thyroid hormones. The majority of thyroid tumors are thyroid carcinomas, and only a small percentage result in hyperthyroidism. Occasionally, a thyroid mass may cause an increase in TgAA.

22. I have a patient that is aggressive. Is this dog likely to be hypothyroid?

There have been anecdotal reports of hypothyroidism resulting in aggression. However, this association has not been proven in any retrospective or prospective studies where large numbers of dogs have been evaluated. In one retrospective study performed here at Michigan State University, there was actually a negative correlation between a history of aggression and hypothyroidism. In other words, dogs with a history of aggression were actually less likely to be hypothyroid.

23. Is the TSH assay valid in cats? Why would I measure TSH in cats?

The TSH assay is valid in cats. It is not routinely measured, as most cats are being tested for hyperthyroidism in which TSH should be very low. However, the normal reference range extends to zero for TSH in the cat, so measurement of TSH is not useful in the diagnosis of hyperthyroidism. Measurement of TSH in the cat is most useful if hypothyroidism is a possibility, or if there is a possibility of oversuppression of thyroid function with methimazole therapy. In those cases, the TSH concentration will be elevated.