

Tips and Tricks for Endocrine Testing in the Cat and Dog



Dr. Jinelle Webb, MSc, DVSc, Diplomate ACVIM

Endocrine testing

- Endocrine testing is not straightforward
- Most values you interpret have significant daily variation
- Symptoms of endocrine disease typically have numerous possible etiologies, and can be vague and missed by owners
- Testing is often based on stimulation or suppression, which have interpretation required

Canine hyperadrenocorticism

- Do not test a dog without symptoms
- Do not test a dog with only an increase in ALP if not symptomatic
- Do not test a sick dog
- Remember that no test for Canine Cushing's Syndrome is perfect
- Consider results in light of patient



Hyperadrenocorticism

Screening Tests

- Urine cortisol:creatinine ratio
- ACTH stimulation test
- Low-dose dexamethasone test
- Abdominal ultrasound



Sample collection

- Serum, plasma or urine required
- Plasma for endogenous ACTH, and NOT in glass tubes
- Samples can be refrigerated for 7 days
- Samples can be frozen for up to 3 months
- Most important tip is to ensure that the testing is performed correctly at your clinic
- Remember stress can affect the testing

Urine cortisol:creatinine ratio

- Approximately 75% of dogs with non-adrenal illness will have a positive result
- Fairly reliable in ruling out disease
- False negatives are rare but possible
- Most ideal if urine collected prior to arrival at veterinary clinic
- Further testing needed if positive

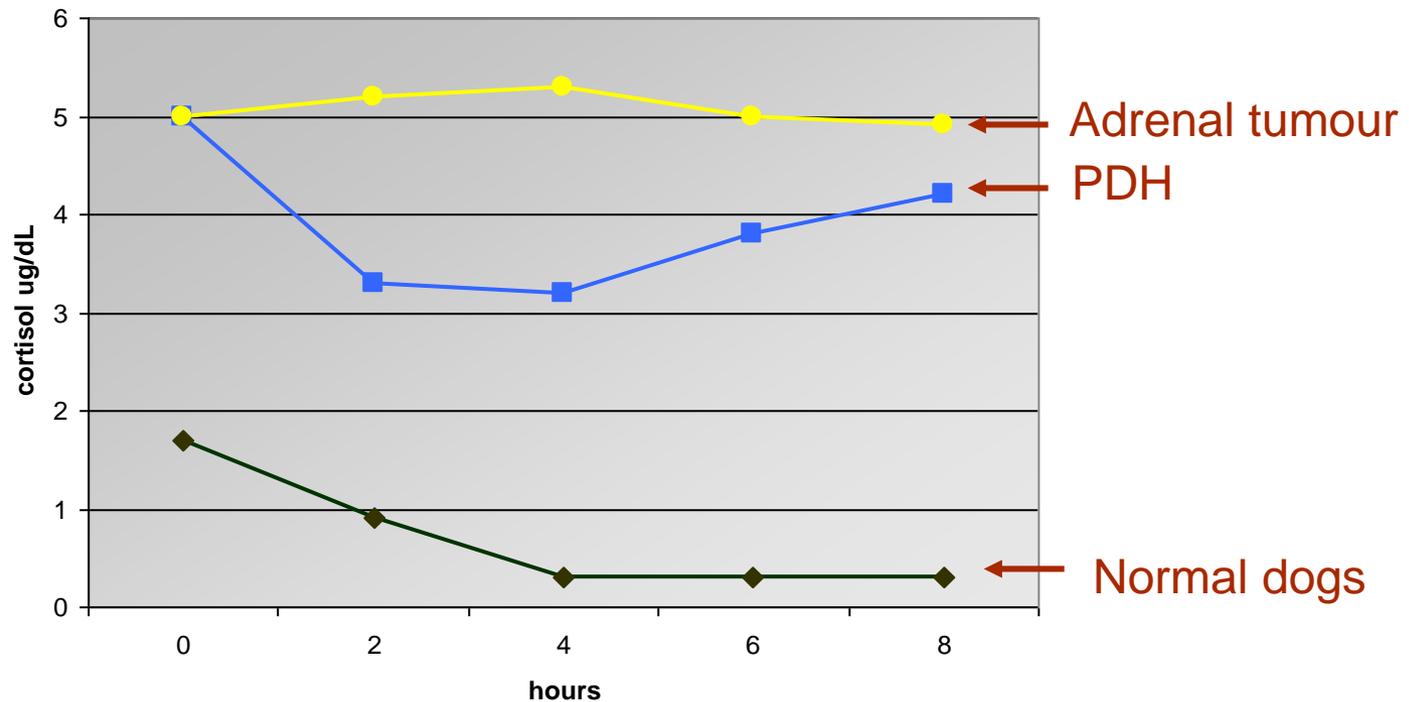
Endogenous ACTH

- Only useful for differentiating pituitary-dependent HAC from adrenal-dependent HAC
- Should be low with adrenal-dependent, and high with pituitary-dependent
- Problem is that with PDH, the level can be low, normal or high
- Some problems with stability
- NOT in glass tubes

Low-dose dexamethasone test

- Administration of 0.01 mg/kg dexamethasone
- Dexamethasone does not cross-react with cortisol assay (*prednisone does*)
- PDH:
 - >99% have increased values at 8 hours
 - 35% have increased 4 hour value
- FAT:
 - >99% have increased values throughout
- Normal dogs: 5% to 56% abnormal result

LDDS test



Mississauga • Oakville

**VETERINARY Emergency
Hospital**

& Referral Group

Low-dose dexamethasone test

- If suppressed fully at 4 and 8 hours, very unlikely to be hyperadrenocorticism
- If not suppressed at all at 4 and 8 hours, likely to be hyperadrenocorticism, although possibility of false positive
- If only suppressed at 4 hours, likely to be hyperadrenocorticism but increased chance of false positive
- Test affected by stress



ACTH stimulation test

- Looking for an exaggerated response
 - Only the post-stimulation value is of use
 - PDH:
 - Clearly abnormal – 30%
 - Borderline – 30%
 - Normal range – 40%
 - FAT:
 - Clearly abnormal – 60%
 - Borderline or normal – 40%
- Normal dogs
 - 15% have abnormal stimulation

ACTH stimulation test

If high suspicion of hyperadrenocorticism:

- Positive result likely has the disease
- Could still have it with a negative result
- Required for monitoring response to medical therapy
- Many previous forms of ACTH for testing are unavailable (ie synacthen)
- Cortrosyn available but expensive
- Options available to reduce cost

Utilizing cortrosyn to reduce \$\$

1. Reconstituted cortrosyn vial contains 100 µg/ml of ACTH, which is 0.1 mg/ml.
2. Aspirate 0.25 and/or 0.5 ml (25 µg or 50 µg) aliquots into plastic syringes.
 - *Please note it is important to use PLASTIC syringes, not glass vials.*
3. Label each syringe with the date reconstituted, amount in that syringe, and name Cortrosyn.
4. Freeze the syringes at -20°C in a non frost-free freezer for up to 6 months. If you elect to refrigerate the syringes, they can be stored for up to 4 weeks.

Utilizing cortrosyn to reduce \$\$

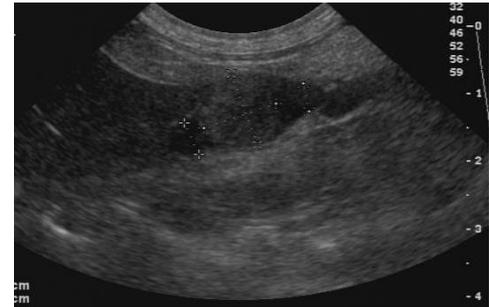
- FOR A **DIAGNOSTIC** ACTH STIMULATION TEST (prior to diagnosis of HAC or hypoadrenocorticism): Administer ***IM or IV*** at a dose of 5 µg/kg (round up if needed). Administer ***IV*** in cats and dehydrated dogs.
- FOR A **MONITORING** ACTH STIMULATION TEST (after a diagnosis, while receiving trilostane or mitotane): Administer ***IV*** at a dose of 1 µg/kg (round up if needed).
- Cortisol levels should be measured prior to injection of Cortrosyn (0 hour), and at 1 hour post administration of Cortrosyn.

Monitoring HAC with cortisol

- Study (ACVIM 2017) looking at using only cortisol levels to monitor trilostane therapy
- Looked at pre-pill and 3 hour post pill cortisol levels for trilostane (PDH and FAT)
- Encouraging preliminary results that the pre-pill level, along with clinical signs, can be used to monitor trilostane dose
- More study needed before this approach can be adopted.

Abdominal ultrasound

- Ultrasonographer must be comfortable in imaging the adrenal glands
- NOT as sole screening test
- PDH:
 - Expect bilaterally enlarged, symmetric glands
- FAT:
 - One adrenal tumour (rare cases have 2)
 - Alternate gland small or not visible
- However, benign adrenal nodules are common



Hyperadrenocorticism - FAQs

What is the best test for HAC?

- Difficult question, but likely the LDDS is slightly better than the ACTH stimulation test.

I have a geriatric dog with PU/PD, an elevated ALP and an elevated UCCR. Can I start treatment for HAC?

- No. Many older pets will have an elevated ALP and UCCR. This pet MAY have HAC, but further testing is needed.

Hyperadrenocorticism - FAQs

Why is there not a panel that includes endogenous ACTH with provocative testing?

- Endogenous ACTH is really only used to differentiate PDH from an adrenal mass. It has issues with stability, interpretation and cost.

I have a pet with uncontrolled diabetes mellitus where I suspect HAC. Help!

- Ideally try to control the DM as best as possible, and then you may need to utilize several tests for HAC (LDDS and U/S, for example).

Hypoadrenocorticism

- Random cortisol can be used as a screening test
- If normal, then no further testing indicated
- If <27 ng/dL, then a **diagnostic** ACTH stimulation test should be performed to attain a definitive diagnosis
- Need to ensure good client communication in use of a random cortisol; aware that a second, more expensive test may be needed

Hypoadrenocorticism - FAQs

If my ACTH stimulation test come back markedly elevated, does the dog have HAC?

- Not likely. There is likely another illness present.

I want to test a dog that is receiving long term steroids. Can I perform an ACTH stimulation test? What if the steroid is only topical?

- No. The use of steroid will have suppressed adrenal function and this will be reflected on your testing. This can happen in some cases of topical steroids too.

Hypoadrenocorticism - FAQs

If I can't use the ACTH stimulation test for a dog receiving steroids, can I rely on my ultrasound findings?

- No. Long term steroid use will have reduced the size of the adrenal glands.

So how do I determine if the pet has hypoadrenocorticism?

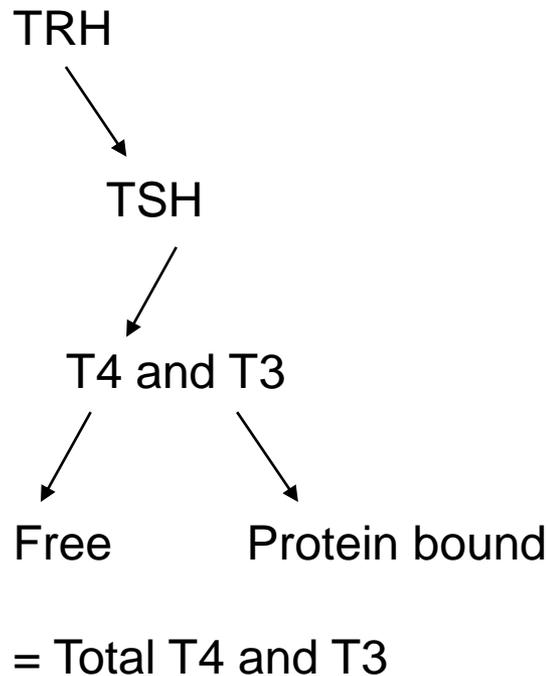
- The only way is to wean the pet off the steroids, wait for a period of time (weeks), and re-test. There is some risk to doing this.

Feline hyperaldosteronism

When should you perform aldosterone levels?

- Cats with azotemia, hypokalemia and/or hypertension may actually have an elevation in aldosterone rather than renal failure.
- This is from either an adrenal adenoma or carcinoma, which produces excess aldosterone.
- Can perform an aldosterone level, but can be grey zone with other diseases.
- Consider abdominal ultrasound or MRI.

Thyroid disease



Mississauga • Oakville

**VETERINARY Emergency
Hospital**

& Referral Group

Sample collection

- Serum recommended; EDTA plasma can be used for some components
- Samples can be refrigerated for 5 days, and most components up to a week
- Samples can be frozen for up to a month
- If requesting the fT_4 by equilibrium dialysis, ship on ice to avoid a false increase in this level

Free T₄ measurement options

Free T₄ – assay options:

- Equilibrium dialysis
 - Filters out large antibodies and hormone-binding proteins
 - Use in patients with suspected or documented thyroglobulin autoantibodies, non thyroidal illness, or on medication known to affect levels
- RIA/Chemiluminescence
 - Faster turnaround time, lower cost

Free T₄ Summary

- Feline hyperthyroidism
 - Slight improvement in diagnostic sensitivity
- Canine hypothyroidism
 - Only improved diagnostic capability in small number of specific cases

Bottom Line:

- In almost all cases, fT₄ results will be the same with both methods
- Do not use only fT₄ to diagnose

Feline hyperthyroidism

- Present in 10% of geriatric cats
- TT_4 is diagnostic for feline hyperthyroidism, and has a sensitivity of over 91%
- However, TT_4 can be normal in a cat with hyperthyroidism
 - Daily variation
 - Interaction of drugs or systemic illness
- What should you do if the TT_4 is normal in a cat with a high index of suspicion?

Feline hyperthyroidism

- Recheck the TT_4 a few days later
- Measure the fT_4 , which is elevated in >98% of cats with hyperthyroidism
- Why not start with the fT_4 ?
 - Up to 12% of cats with non-thyroidal illness will have an elevated fT_4
- Why not measure TSH? Should be low.
 - Feline TSH assay not widely available
 - TSH cannot differentiate at low end of range (normal range extends to zero).

Feline hyperthyroidism - FAQs

My patient has normal thyroid hormone levels, but I think that she is hyperthyroid.

- If the level is at the high end of normal, especially for TT_4 , hyperthyroidism is still possible.

My patient has low thyroid hormone levels. Does he have hypothyroidism?

- This is very unlikely, especially if the clinical suspicion was hyperthyroidism. Consider other systemic illness.

Feline hyperthyroidism

- How about using scintigraphy for diagnosis?
 - Very sensitive test
 - Cost and availability limits use
 - Requirement for sedation
 - Has been used prior to therapy for carcinoma, as will assess for metastatic disease.

Feline hyperthyroidism

Monitoring

- Controversial on whether timing is important
- Likely a good idea to be consistent when sample is taken (ie not 1 hour post pill, then next time 10 hours post pill)
- Typically only monitor TT_4 levels
- Recent study indicated that fT_4 did NOT identify more cases of iatrogenic hypothyroidism than using just the TT_4

Canine hypothyroidism

- Different forms of hypothyroidism, however most cases are due to inability of the thyroid gland to synthesis thyroid hormone.
- Diagnosis is a challenge, there are many false positive cases
- Complicating factors for TT_4 level
 - Time of day, age, breed, room temperature, presence of estrus, obesity, malnutrition, systemic disease, drug interactions

Canine hypothyroidism

- Age
 - Higher levels in puppies
 - Lower levels in geriatrics, likely due to systemic disease or drug therapy (ie NSAIDs)
- Breed
 - Sighthounds, Basenjis have lower levels
- TT_4 is often included in geriatric profiles, however really it should only be performed in a dog with clinical signs of hypothyroidism

Canine hypothyroidism

- Start with TT_4 , as a level mid range or high normal rules out hypothyroidism
- If the TT_4 is low, then measure fT_4 and cTSH
- Most cases of hypothyroidism have a low fT_4 , although not all cases
- Most cases of hypothyroidism have a high cTSH, due to lack of negative feedback
- However, ~30% of cases have a high normal cTSH for unknown reasons

Canine hypothyroidism

- TSH and TRH stimulation tests are not recommended (side effects and cost)
- Thyroglobulin autoantibodies can be measured, but are present in some normal dogs
- Some cases are a challenge. In a case with high index of suspicion and equivocal results, can retest or consider a trial of supplementation.

Canine hypothyroidism - FAQs

My patient has low thyroid hormone levels, but the TSH is normal.

- The most likely cause is non thyroidal illness, however ~15% of dogs that truly have hypothyroidism have a normal TSH level.

Should I measure fT4 by equilibrium dialysis rather than by 2-step or chemiluminescence?

- fT4ED is more time consuming and costs more. There are only a small number of cases where it is indicated.

Canine hypothyroidism - FAQs

My patient has normal thyroid hormone levels, but the TSH is high.

- The most likely cause is spurious, which can be ruled out with a repeat test. Other causes:
 - Recently recovered from non thyroidal illness or treatment with certain drugs
 - Recently withdrawn levothyroxine
 - Subclinical/early hypothyroidism
- Recommend retesting at a later date

Canine hypothyroidism - FAQs

My patient has a normal thyroid panel but had an elevated % thyroglobulin autoantibody

- The most likely cause is subclinical thyroiditis (or rare cases of thyroid neoplasia). Not all of these cases will develop clinical hypothyroidism.

I still think this patient has hypothyroidism, should I try a trial of medication?

- Only if clinical suspicion remains very high. Although this is not considered harmful, human studies have shown detrimental effects.

Canine hypothyroidism

Monitoring

- Controversial on how to monitor
- Taught to measure TT₄ level 4-6 hours after giving the levothyroxine
- Bioavailability through oral absorption varies between 13-87%, which can be daily variation
- Value obtained one day could be quite different than another day
- Diet can affect absorption (high fibre diet can reduce absorption)

Mississauga • Oakville

**VETERINARY Emergency
Hospital**

& Referral Group

Canine hypothyroidism

Monitoring

1. Only test if there is clinical evidence of on-going hypothyroidism
2. Consider measuring the TSH, but you **HAVE** to first evaluate pre-treatment TSH value. If the TSH was not high to begin with, you cannot use this test to monitor.
 - a. If TSH normal while receiving levothyroxine, then higher dose not indicated
 - b. If TSH high, then a higher dose is indicated

Canine hypothyroidism

Monitoring

3. Consider measuring fT4

- a) Does not vary throughout day, so can measure at any time
- b) Cost is significantly higher than TT_4

Bottom line – only test if the dog still has clinical signs of hypothyroidism, and consider which test is best. You may need more than the TT_4 .

Canine hypothyroidism - FAQs

How soon after starting therapy can I test?

- TT_4 and fT_4 should be in a steady state within a week, recommendation is to wait 10-14 days
- TSH may take a few weeks to normalize
- Clinical signs can take weeks or more

My TT_4 is low but my TSH is normal

- There is daily variation in absorption, so as long as the TSH was high at time of diagnosis, this indicates adequate therapy

Canine hypothyroidism - FAQs

My TT₄ is normal but my TSH is high

- If the TSH is high, then there is inadequate therapy. This could be due to poor compliance, intermittent poor absorption, underlying gastrointestinal disease, etc.

Not all clinical signs have resolved

- If the thyroid panel shows adequate supplementation and enough time has passed, consider concurrent disease or an incorrect diagnosis.

Canine hypothyroidism - FAQs

If a definitive diagnosis was not obtained at initiation of therapy, how do I now confirm the diagnosis?

- The only way to determine if a pet is truly hypothyroid in this situation is to stop the medication, and retest at least 6 weeks later. Please note that you do NOT need to wean the medication.

Questions?



Mississauga • Oakville

VETERINARY Emergency
Hospital

& Referral Group