

ACTH Stimulation Test

Indications

- Diagnosis of canine *and feline (rare)* hypoadrenocorticism [or Addison's disease]
- Diagnosis of canine hyperadrenocorticism (HAC) [or Cushing's disease].
- Monitoring therapy for hyperadrenocorticism [mitotane, trilostane]

Considerations prior to testing

- Sedation is generally not recommended when performing an ACTH stimulation. Making a definitive statement about the effect of sedation is difficult aside from the fact it is not advised. Sedation could affect the baseline/ pre-cortisol, but in theory should not affect the post-cortisol level if collection and ACTH dose correspond to maximal stimulation.
- A single dose of dexamethasone (ex. administered during Addisonian crisis) will not affect results when trying to diagnose hypoadrenocorticism. Hydrocortisone/prednisone/prednisolone can cross react with the cortisol assay.
- Daily administration of glucocorticoids and stress of non-adrenal illness will affect results. The effect of glucocorticoids is dose and duration dependent. Two weeks is generally a minimum withdrawal time for glucocorticoid therapy, but 6 weeks withdrawal or longer will be needed for prolonged or high dose/ immunosuppressive therapy. Topical or inhaled steroids also need to be considered. Chronic topical ophthalmic steroid preparations can cause iatrogenic Cushing's.
- **In the ACVIM consensus statement on the diagnosis of canine Cushing's, the Low Dose Dexamethasone Suppression Test (LDDST) is the preferred first diagnostic test since it is slightly more sensitive and has the ability to differentiate pituitary vs. adrenal dependent disease.** The ACTH stimulation test is not a differentiating test.
- The ACTH stimulation test is considered to be less sensitive and slightly more specific than the LDDST for the diagnosis of Cushing's. About 50% of adrenal based disease will show an exaggerated response on an ACTH stimulation test.
- If monitoring a patient that was over suppressed while receiving mitotane/trilostane ensure that oral steroid therapy is discontinued at least 12 -24 hours prior to testing. Exact timing has not been studied and references note at least 12 or 24 hours to avoid interference.
- **Timing between LDDST/HDDST and ACTH stimulation tests.**
- If a LDDST test has been performed recently, one should consider waiting one week before performing an ACTH stimulation test.
- If a HDDST test has been performed one should consider waiting 2 weeks before performing an ACTH stimulation test

Diagnosis of hypoadrenocorticism/Addison's.

- A resting cortisol of **greater than** 55mmol/L effectively rules out Addison's;
- A resting cortisol of less **than** 55 mmol/L is an indication for an ACTH stimulation test to fully evaluate the adrenal reserve.
- The majority of Addisonian dogs will have an unmeasurable cortisol of less than 27mmol/L at all times with virtually no increase post ACTH stimulation.
- A resting cortisol of less than 27mmol/L as a single test **is NOT** sufficient to diagnose Addison's.

Basic Protocol for ACTH stimulation test in Dogs (See modifications below)

1. Collect a baseline sample into green or red top or SST tube, and separate serum as soon as possible.
2. Inject either 0.25mg (250ug) Cortrosyn per dog, OR a minimum of 5ug/ kg Cortrosyn IM or IV. Intravenous is preferred if the patient is dehydrated or has poor perfusion. (See below for other ACTH products).
3. Collect a post sample at 1 hour if using Cortrosyn regardless of IM or IV route.
4. Separate serum as soon as possible and submit the original tubes and the spun off serum/plasma (0.5 ml) to the lab.
5. Make sure the tubes are labeled correctly as pre or post

Alternative products for ACTH stimulation testing

Synacthen Depot®:

1. Inject Intramuscularly 0.5 mg or 1/2 vial for dogs under 15 kg and 1mg or a full vial for dogs weighing more than 15 kg.
2. Note in normal dogs (weighing 13.5 to 33 kg) Synacthen Depot administered at a dose of .25mg (or 250 ug) IM resulted in an equivalent adrenal cortical stimulation (AJVR 2012). A specific protocol has not been researched in clinically Cushingoid patients.
3. Previous internal laboratory data indicate that a 90 minute post sample is appropriate for Synacthen Depot.
4. No data in cats

Compounded Gels

- Corticotropin gel products are generally dosed at 2.2 IU/kg.
- For e.g. Bexco ACTH gel (40U/ml) collect baseline, 1 hour AND 2 hours post (since there can be variability in when peak stimulation is elicited by these products).
- Compounded/ gel preparations should not be used if Cortrosyn is available.

ACTH Stimulation test combined with Bile Acid panel

- ACTH and bile acids panels can be run simultaneously.
- Please note that patients need to be fasted for a bile acid panel, but cannot be fasted for ACTH stimulation for purposes of monitoring Trilostane therapy.

1. Fast patient for minimum of 12 hours.
2. Collect baseline sample in red top or SST tube, separate serum as soon as possible, label as baseline.
3. Administer Cortrosyn according to previously mentioned protocol.

4. Feed the dog appropriately (regular or higher fat meal if not contraindicated),
5. In one hour collect the post ACTH sample, label.
6. In another hour (2 hours post feeding) collect the post bile acids sample.

Note that if using Synacthen Depot® the post sample for the ACTH stimulation will be at 90 minutes after the preprandial/baseline cortisol sample. The post bile acids should still be two hours from the preprandial sample.

ACTH stimulation protocol for Cats

Note the ACTH stimulation is not the test of choice for diagnosing feline Hyperadrenocorticism due to the lower sensitivity (more than 50% of cats will have a normal stimulation).

A UCCR is also a good screening test in cats for Cushing's. Note urine for a UCCR should be collected at home and it should be 48-72 hours since the last veterinary visit.

An ACTH stimulation is the only test to diagnose hypoadrenocorticism in cats. Adrenal reserve should ideally be evaluated in cats with hyperaldosteronism prior to surgical adrenalectomy. Other hormones can also be evaluated if desired (progesterone).

1. Baseline: collect a baseline sample in a green or red top or SST tube and separate the serum or plasma as soon as possible. Note timing differs between IV versus IM administration. Intravenous is preferred as it is more reliable
2. Give a total Cortrosyn dose of .25 mg (125 ug)/cat IV or the lower dose of 5 ug/kg followed by a 30min and 1-hour post sample.
3. Separate as soon as possible and submit the original tubes and the spun off serum/plasma (0.5 ml) to the lab.
4. Make sure the tubes are labeled correctly as pre or post

If administering Cortrosyn IM, the peak cortisol is variable. Thus a 30 minute and 60 minute and 120 minute collection would be advised.

Synacthen Depot® has not been well evaluated in cats.

Storage Tips

- Reconstituted Cortrosyn is stable in the fridge for at least 4 weeks when stored in plastic containers. It is stable in a -20°C non frost-free freezer for 6 months (again plastic storage for e.g. syringe).
- Depot Tetracosactide can be stored in plastic syringes at -20°C for up to six months without interfering with the biological stability of the hormone (ACVIM 2017 Abstract)

Monitoring Trilostane therapy – always correspond with Patient's Clinical Status.

- Dogs must NOT be fasted prior to the ACTH stimulation test; trilostane must be administered with a meal prior to testing.

- Current Dechra recommendations support beginning the ACTH test at 4-6 hours post pill. Some patients may have a maximum effect at 3 hours post pill.
- Keeping the same timing of the test may be important: for an individual, always start the ACTH stimulation test at the same time post-pill interval for better monitoring.
- Algorithms for monitoring Trilostane can be found here - Dechra Monitoring Algorithm (<https://www.dechra-us.com/therapy-areas/companion-animal/endocrinology/canine-hyperadrenocorticism/monitoring-cushings>)
- Dechra within the United Kingdom now advocates the use of pre-pill cortisol levels as a means of monitoring Trilostane therapy. This is also being used by endocrinologists/specialists within North America. Interpretation of this test needs to be done in strict combination with clinical signs and it is not “fool proof”. Further information/algorithms can be found at this link Pre-Pill Cortisol Monitoring Dechra
- Pre-pill monitoring research continues

Atypical or Occult Hyperadrenocorticism.

Consider whether first both an ACTH stimulation and LDDST have been performed. Consider an 8 hour cut-off for the LDDST could be considered positive if over 30-35 nmol/L. Consider that the disease is slowly progressive and repeat testing in a period of months may be appropriate.

Consider Food Dependent Cushing's -see below.

In certain circumstances an ACTH stimulation test with a pre and post cortisol and non-cortisol steroid hormone panel can be considered if there remains a high index of suspicion of HAC. The full ACTH hormone panel is sent out to the University of Tennessee and includes -pre and post cortisol, androstenedione, estradiol, progesterone, 17OH progesterone and aldosterone. If cortisol is elevated then this would be consistent with typical HAC. Non-cortisol steroid hormone elevations are not specific for atypical HAC. Non-adrenal illness or stress can also elevate the non-cortisol steroid hormones. There is evidence for and against these hormones playing a causative role in the disease.

Food Dependent Hypercortisolism in Dogs - Rare. Diagnosis is based in part on pre and 3 hour post prandial Urine Cortisol Creatinine Ratios. This is a consideration if initial screening is negative for Cushing's despite a strong clinical concern. Dogs are often younger than typical HAC cases.

Additional Resources

The ACVIM consensus statement on diagnosis of hyperadrenocorticism is an excellent resource for diagnosis of Cushing's disease and is available at:

<http://onlinelibrary.wiley.com/doi/10.1111/jvim.12192/epdf>

Extending your supply of Cortrosyn:

Dr. Mark Peterson offers insights into veterinary endocrinology, including how to lower the cost of ACTH stimulation testing for clients.

<https://endocrinevet.blogspot.ca/2011/03/how-to-extend-your-supply-of-cortrosyn.html>

CVT 15 Web Chapter 18. Interpretation of Endocrine Diagnostic Test Results or Adrenal and Thyroid Disease. Robert J. Kemppainen, Auburn, Alabama. Ellen N. Behrend, Auburn Alabama.

Pre-trilostane and three-hour post-trilostane cortisol to monitor trilostane therapy in dogs - Macfarlane article open press - <https://veterinaryrecord.bmj.com/content/179/23/597>