Endocrinology

ACTH Stimulation: Clinical Notes and Protocols

SPECIMEN REQUIRED: 0.5 ml of serum or heparinized plasma per sample. Make sure to label samples appropriately as **PRE** and **POST**.
TURNAROUND TIME: 24 hours
I**NDICATIONS FOR TESTING:**

- Diagnosis of canine and feline (rare) hypoadrenocorticism/Addison's disease.
- Diagnosis of canine hyperadrenocorticism (HAC)/Cushing's disease.
- Monitoring therapy for hyperadrenocorticism (mitotane/trilostane).

CANINE PROTOCOL p. 4

FELINE PROTOCOL p. 8

FERRET PROTOCOL p. 9

STORAGE GUIDELINES p. 5 -6

Clinical Notes

Sedation:
Sedation could affect the baseline/pre-cortisol, but should not affect the post-cortisol level. An ACTH stimulation test is designed to maximally stimulate the adrenal glands by administering ACTH. Fear free practice management strategies can still be utilized. Please note sedation in the history to be complete.

Previous Steroid Exposure:
A single dose of dexamethasone at the time of testing (e.g., administered during an Addisonian crisis) is not a concern as it has no cross reactivity with most cortisol assays. It will, however, lead to a decreased cortisol response (by about 35%) from after a few hours to up to 3 days; differentiation between dogs with non- adrenal illness and Addison's should, however, still be possible in most cases especially when given within a short period of time.

Long term administration of glucocorticoids as well as stressful non-adrenal illness can 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 (ocular/aural/dermatologic) or inhaled steroids also need to be considered. Budesonide will also have systemic effects.

ACTH Stimulation: Clinical Notes

Canine Pituitary versus Adrenal Dependent HAC:
The ACTH stimulation test is considered to be less sensitive and slightly more specific than the Low Dose Dexamethasone Test (LDDST) for diagnosis of HAC. It is expected that only 50% of adrenal based disease will show an exaggerated response on an ACTH stimulation test. The ACVIM consensus statement states that the LDDST is the preferred first test for HAC diagnosis since it is slightly more sensitive and may have the ability to differentiate pituitary vs. adrenal dependent disease. The ACTH stimulation test is not a differentiating test. It may be necessary to perform both an ACTH stimulation and a LDDST at independent time points when investigating for Cushing's. Please refer to the ESVE webpage for further information on diagnosis.

Comorbid Disease - Diabetes Mellitus (DM) or Chronic Phenobarbital Therapy
The ACTH stimulation is the preferred test over a LDDST initially if the patient has concurrent DM. Note almost all dogs with DM have an elevated ALP. Concurrent testing for Cushing's in a newly diagnosed diabetic or a sick diabetic is not advised due to the risk of false positive results. Most dogs are not diagnosed with Cushing's and DM at the same time. It is controversial but the ACTH stimulation may be the preferred test over a LDDST if the patient is receiving chronic phenobarbital.

Unexpected Blunted ACTH Stimulation Result Troubleshooting:

1. Consider if it is only very mildly blunted that this could be biologic variation.
2. Double check your product, protocol and dose.
3. Importantly, exclude previous or current exposure of all steroids including topical formulations.
4. Note high dose Ketoconazole or Progestin exposure could cause a blunted response.
5. Consider that it is rare to document an initial blunted test that progresses over time to a flatline and overt clinical hypoadrenocorticism but the possibility exists.
6. Critical illness-related corticosteroid insufficiency (CIRCI) in veterinary medicine is controversial. It would be a differential diagnosis for a persistently hypotensive critically ill ICU patient.
7. One study has noted an association between a blunted ACTH stimulation and inflammatory GI disease however it is not a means of diagnosing inflammatory GI disease.
8. On occasion infiltrative adrenal disease can cause a blunted ACTH stimulation.
9. Depending on how blunted the results are, paired cortisol and eACTH measurements or a UCCR may provide additional information on rare occasion.
10. Consider repeating the ACTH stimulation to document a persistently blunted result.

ACTH Stimulation: Clinical Notes

Monitoring for adrenal recovery while receiving oral steroids.
If monitoring a patient on oral glucocorticoids for adrenal recovery, ensure that oral steroid therapy is discontinued at least 12 -24 hours prior to testing. Exact timing has not been studied however endocrinology references note at least 12 or 24 hours is needed to avoid interference. Hydrocortisone/prednisone/prednisolone can cross-react with the cortisol assay.

Timing between suppression tests and an ACTH stimulation test.

If a LDDST test has been performed recently, one should ideally wait one week before performing an ACTH stimulation test. If a HDDST test has been performed one should ideally wait 2 weeks before performing an ACTH stimulation test.

Timing in relation to Trilostane Therapy:
As per Dechra Canada - After the administration of trilostane with food, cortisol levels are most significantly suppressed for 3 to 8 hours. Therefore, in order to obtain results at the peak time of effect, the ACTH stimulation test should be performed at 4-6 hours post-dosing. Timing between suppression tests and an ACTH stimulation test.

Trilostane Goals of Therapy:
In order to receive the best interpretation possible it is valuable to know:

1. Timing of the ACTH stimulation in relation to Trilostane administration. (Trilostane needs to be given with a full meal as per usual.)
2. Dose on a body weight scale along with dose duration.
3. Dosing frequency i.e. once a day versus twice a day.
4. Clinical signs are key to dosing adjustments in addition to the ACTH stimulation results. As per these studies (2020, 2021), we still do not have the perfect test to evaluate clinical control in an otherwise well patient therefore, communication with the owner is important. An ACTH stimulation is an important part of assessing an unwell patient.

In general we note many dogs are doing well when the post cortisol is below 150 nmol/L.

It can be possible to use a pre-pill cortisol for monitoring in particular situations as per available guidelines in the United Kingdom.

ACTH Stimulation: Canine Protocol Cosacthen

Baseline: Collect just prior to Cosacthen administration in a green, red top or SST tube. After collection, separate the serum or plasma as soon as possible and keep it refrigerated.

Cosacthen (tetracosactide)

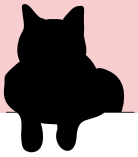
- The official dose is 0.25 mg (1 mL) per dog weighing 4.5–50 kg, administered by IV or IM injection, with the purpose of performing the ACTH stimulation test.
- The Canadian label differs from the UK label. It is possible to use at the veterinarian's discretion an "off-label" dose of 5 µg/kg IV or IM as per the UK label.
- Keep in mind that when using the 5 µg/kg dose, the maximal amount to be administered should be 250 µg, even for dogs weighing more than 50 kg.
- Sampling: Take blood 60 minutes later for the post-ACTH cortisol measurement regardless of the administration route.

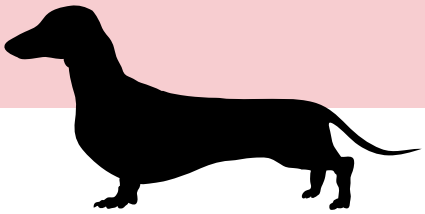
Cosacthen dosing in Small Dogs less than 4.5 kg.

For dogs less than 4.5 kg these are the instructions as per Dechra's technical services.

- The safety study that was done for Cosacthen (tetracosactide) was based on the maximum dose a dog in this weight range would receive, which is 250 µg (1ml) in a 4.5 kg dog (≈ 56 µg/kg). For this reason it cannot be recommended to give a dog weighing less than 4.5 kg the full vial of Cosacthen (250 µg).
- Usage of tetracosactide at 5 ug/kg is effective and this extra label dose is an alternative to the full vial. The issue is that 5 µg/kg in a 4.5kg dog corresponds to 0.09mL (Cosacthen), which is very hard to measure accurately, and the product should NOT be diluted. As 0.1 ml (25 µg) is the minimum recommended volume to be withdrawn into a 1mL syringe, it makes sense for this to be the minimum dose/volume to be administered to dogs weighing 4.5 kg or less.
- An additional **clinical tip** is as follows: "Since it is such a small volume, if you are worried that you may "lose" some product that remains in the needle and/or syringe hub, it should be safe to increase the total volume up to 0.2mL (50 µg). As an example, if you have a 2.5kg dog and you administer 0.2mL (50 µg), this corresponds to 20 µg/kg. We believe this should be safe, because this dose is still below the maximum dose of 56 µg/kg used in the safety study.

ACTH Stimulation: Cosacthen Storage



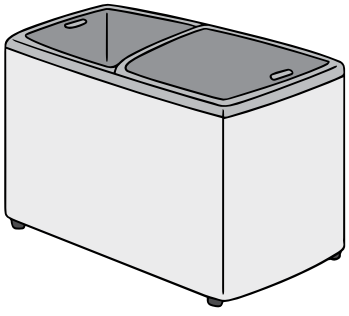


Cosacthen Storage:
As per Dechra Technical Services Information.

- Note that Cosacthen requires refrigeration between 2-8°C and once the vial has been breached any remaining product should be discarded (since it contains no preservative) or immediately placed in aliquots and frozen, to prevent contamination.
- Dechra has also investigated (internal information) the freezing and aliquoting of Cosacthen (considered an extra label practice).
- It was confirmed that Cosacthen remains stable and viable when stored in a plastic syringe at a constant -20 °C for up to 6 months. To maintain stability of the product while frozen, it **should NOT** be kept in a frost-free freezer (i.e. one that goes through freeze-thaw cycles to avoid ice/frost buildup) as repeated cycles of freezing and thawing of Cosacthen will impact its stability. Cosacthen is only stable for 7 days in a frost-free freezer. There is no information available on storing Cosacthen within a separate styrofoam box within a frost-free freezer.
- A chest freezer is a good option. Given the 250 µg/mL concentration of Cosacthen you could create 5 aliquots of 0.2 mL (50 µg) or, provided you have small enough syringes, 10 aliquots of 0.1 mL (25 µg).

GENERAL STORAGE GUIDELINES:

- Prior to placing the syringe cap on the aliquot, please remember to draw in a small bubble of air to ensure there is sufficient room for the product to expand once it freezes.
- DO NOT dilute the product.
- Hold syringes in the palm of your hand to thaw and warm prior to use.
- Do not refreeze aliquots once thawed.



ACTH Stimulation - Canine Protocol
Cosyntropin Administration & Storage

Baseline: Collect just prior to Cosyntropin administration in a green, red top or SST tube. After collection, separate the serum or plasma as soon as possible and keep it refrigerated.

Cortrosyn (cosyntropin)

- The reconstituted drug product should be inspected visually for particulate matter and discolouration prior to injection.
- Collect your baseline sample and inject either 0.25mg (250µg) Cortrosyn per dog, OR a minimum of 5µg/kg Cortrosyn IM or IV.
- IV is preferred if the patient is dehydrated or has poor perfusion.
- Collect a post ACTH sample 60 minutes later regardless of a IM or IV route.
- Separate as soon as possible and submit the spun off serum/plasma (0.5 ml).

Storage:
It has been noted that reconstituted Cortrosyn is stable in the fridge for 4 weeks when stored in plastic containers. There is no specific published reference on this and the product insert notes the product should be discarded after use. Clinical pharmacologists advise against storage in the fridge unless it has been mixed in an ISO 5 environment. Otherwise as this is a sterile product, they suggest storage for only 8-12 hours in the fridge. Similar to Cosacthen, aliquoting and freezing is the preferred storage method to avoid contamination. Once reconstituted, Cortrosyn is bioactive for at least 6 months when stored in a -20 °C non frost-free freezer (again plastic storage e.g. syringe). Clinical pharmacologists will note that the risk of bacterial contamination must be weighed against savings when using aliquots that have been stored for up to 6 months. Specific guidelines would advise only 45 days while other studies have used aliquots within 3-6 months. If you only have a frost-free freezer, then one could consider storing the aliquots within a styrofoam box with sufficient additional freezer packs inside to **prevent thawing**. See here on monitoring your freezer controls.

- **Prior to placing the syringe cap on the aliquot, please remember to draw in a small bubble of air to ensure there is sufficient room for the product to expand once it freezes.**
- **Hold syringes in the palm of your hand to thaw and warm prior to use.**
- **Do not refreeze aliquots once thawed.**

ACTH Stimulation - Dated Protocols

The following products are not advised if Cosacthen or Cosyntropin are available.

S**ynacthen Depot®**

Synacthen Depot® is labeled for IM use only and must not be administered IV.

Dose: 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.

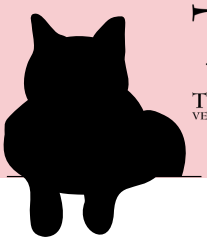
Note in normal dogs (weighing 13.5 to 33 kg) Synacthen Depot® administered at a dose of 0.25mg (or 250 µg) IM resulted in an equivalent adrenal cortical stimulation (AJVR 2012). A specific protocol has not been researched in clinically Cushingoid patients. Previous internal laboratory data note a 90 minute post is appropriate for Synacthen Depot®

Storage:
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 only).

Compounded Gels

- Corticotropin gel products are generally dosed at 2.2 IU/kg.
- E.g., Bexco ACTH gel (40 IU/ml) collect baseline, 1 hour AND 2 hours post (since there can be a variable peak response elicited by these products).

ACTH Stimulation - Feline Protocols



Note the ACTH stimulation is not the advised test of choice for diagnosing feline HAC due to poor test sensitivity (more than 50% of cats will have a normal stimulation). A UCCR is considered a reasonable screening test in cats and performs best if the urine sample is collected at home 48-72 hours after the last veterinary visit. The LDDST is the preferred test for HAC in cats.

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 sex hormones can also be evaluated if desired (e.g., progesterone). A LDDST can be considered to investigate for concurrent glucocorticoid excess.

Baseline: Collect just prior to Cosyntropin administration in a green, red top or SST tube. After collection separate the serum or plasma as soon as possible.

Cortrosyn (Cosyntropin)
Timing differs between IV versus IM administration. **IV is preferred as it is more reliable.**

IV Route: Give a total Cortrosyn dose of 125 µg/cat IV or the lower dose of 5 µg/kg followed by a 30 minute and 60 minute post sample. Some endocrinologists advocate the 125 µg/cat dose over the 5µg/kg dose however based on a 2011 AVMA paper in small number of normal cats the lower dose should be acceptable and a single 60 minute post cortisol would also be acceptable when screening for Addison's.
IM Route: If administering Cortrosyn IM, the peak cortisol is variable therefore a 30 minute and 60 minute and 120 minute collection is advised.

Cosacthen (tetracosactide)
Cosacthen is only approved for use in dogs. Dechra does not have any safety or efficacy data regarding the off-label use of Cosacthen in cats. Dechra advocates use of all of its approved veterinary medicinal products only according to the details included on the SPC of each product. Any deviation from these instructions is entirely based on the benefit:risk assessment performed by the treating veterinary surgeon based on the individual patient's needs and written informed owner consent should be obtained.

Dechra's Recommended Protocol for ACTH stimulation test (based on review of literature and internal discussions with Peter Graham): Consider this additional paper for reference.

Dose: 125 µg/cat (0.5 ml of Cosacthen) or 5 µg/kg
Route of administration of Cosacthen: IV or IM
Sample Timing: Collect basline prior to injection of Cosacthen and 60 minutes post-injection.

ACTH Stimulation - Ferret Protocols

An ACTH stimulation would be indicated to diagnose hypoadrenocorticism. Spontaneous hypoadrenocortism in ferrets is considered extremely rare. If the primary concern is adrenal associated endocrinopathy see additional notes below.

Cortrosyn (cosyntropin)

Baseline - Collect a baseline cortisol sample just prior to Cosyntropin administration in a green, red top or SST tube. After collection, separate the serum or plasma as soon as possible and keep refrigerated.
Dose: 5µg/kg IV or IM
Sample Time: Post cortisol is collected at 60 minutes after cosyntropin administration.

Reference. Rosenthal KL, Peterson ME, Quesenberry KE, Lothrop CD Jr. Evaluation of plasma cortisol and corticosterone responses to synthetic adrenocorticotropic hormone administration in ferrets. Am J Vet Res. 1993 Jan;54(1):29-31. PMID: 8427469.

An **ACTH stimulation is not indicated for adrenal tumor endocrine disease, i.e.** Adrenal-associated endocrinopathy (AAE). AAE associated clinical manifestations are primarily due to elevated sex hormones. The most common clinical sign of AAE in ferrets is progressive, symmetrical alopecia with or without pruritis. AAE is diagnosed based on incidence and characteristic clinical presentation. Confirmatory diagnosis is made based on a serum hormone assay panel that consists of estradiol, androstenedione, and 17α-HPG which is available as a send out test to the University of Tennessee.*

Protocol per Tennessee: Adrenal Panel for Ferrets.
a. Collect baseline serum sample in red top tube (0.5 ml). Do NOT use serum separator tube.
b. Allow sample to clot then centrifuge and separate serum.
c. Send sample cold on an ice pack by overnight delivery.
d. The following hormones will be assayed: Estradiol, Androstenedione and 17α-HPG. The assay is run each week.

***Reference:**
Bakthavatchalu V, Muthupalani S, Marini RP, Fox JG. Endocrinopathy and Aging in Ferrets. Vet Pathol. 2016 Mar;53(2):349-65. doi: 10.1177/0300985815623621. PMID: 26936751; PMCID: PMC5397995. (Open Access)