

## Instructions for Adrenal Function Tests in Dogs and Cats

Serum or EDTA plasma are acceptable samples. Make sure you get sample volume sufficient for analysis – check with your lab. In either case, make sure you spin the blood and separate plasma or serum as soon as possible after collecting the blood (within 30 minutes). EDTA plasma allows immediate spinning, while using clot tube requires waiting for clotting prior to spinning. Sample volume yield is usually greater with EDTA plasma, especially if the patient is polycythemic.

Samples for cortisol assay should be refrigerated or frozen immediately after separation from the RBC, and the samples sent overnight or 2<sup>nd</sup> day, on ice. Cortisol levels are stable in serum or plasma for 5 days at room temperature. Beyond this time, cortisol degradation is significant.

### Adrenal Function Tests for Dogs

#### ACTH Stimulation Test - Canine

Advantages:

1. Takes 1-2 hours (shorter than Low Dose Dex).
2. Assays are cheaper because there are only 2 (three for Low Dose Dex).
3. Fewer false positive due to stress than Low Dose Dex.
  - a. Usually a stress response produces a cortisol of 28-30 or so.
  - b. One study showed 14% of dogs with non-adrenal disease had elevated post-ACTH cortisols.
4. ACTH stim will identify 80-85% of dogs with HAC.
5. Creates a baseline for monitoring Lysodren therapy.
6. The best test for identifying iatrogenic HAC.

Disadvantages:

1. ACTH is much more expensive than dexamethasone.
2. ACTH stim will be normal in 15-20% of dogs with HAC. Many of these dogs have adrenal HAC.
3. Does not distinguish between pituitary and adrenal HAC.

#### *ACTH Stimulation Test Protocols for Dogs*

1. *Low Dose Cortrosyn ACTH Stimulation Test - Canine* - to keep cost down and to stretch out your supply of Cortrosyn, use a specific dose instead of 1 vial/dog.
  - a. Take pre ACTH blood sample, ideally after a 12 hour fast.
  - b. Administer 5 mcg/kg Cortrosyn IV.
  - c. Take post stimulation sample 1 hour after Cortrosyn.

Validation: A few papers have now shown that 5 mcg/kg IV is sufficient with a maximum of 250 mcg/dog (i.e., the whole vial).

Split any left over reconstituted Cortrosyn into aliquots using plastic syringes. For a vial of 250 micrograms Cortrosyn, split it into 5 aliquots of 50 micrograms each or 10 aliquots of 25 micrograms each, or any combination thereof. For about a 10 kg dog or so (probably average size of dog that we do many ACTH stims on), one of the 50 microgram syringes would work. For a 5 kg dog, a syringe of 25 micrograms would work. You can store the aliquots in a frost-free freezer for up to 6 months with no loss of activity as long as it is not thawed and re-frozen. Since no one knows the effect of thawing and refreezing, we don't advise using ACTH that has been thawed and refrozen.

References:

1. Aust Vet J. 1998 Apr;76(4):255-7. Plasma cortisol responses to three corticotrophic preparations in normal dogs. Watson AD, Church DB, Emslie DR, Foster SF. Department of Veterinary Clinical Sciences, University of Sydney, New South Wales.
2. J Small Anim Pract. 2000 Jul;41(7):308-11. Cortisol concentrations following stimulation of healthy and adrenopathic dogs with two doses of tetracosactrin. Frank LA, DeNovo RC, Kraje AC, Oliver JW. Department of Small Animal Clinical Sciences, University of Tennessee, College of Veterinary Medicine, Knoxville, USA.
3. J Am Vet Med Assoc. 1999 May 15;214(10):1497-501. Evaluation of a low-dose synthetic adrenocorticotrophic hormone stimulation test in clinically normal dogs and dogs with naturally developing hyperadrenocorticism. Kerl ME, Peterson ME, Wallace MS, Melian C, Kemppainen RJ. Department of Medicine, Animal Medical Center, New York, NY 10021, USA.
4. J Am Vet Med Assoc. 1998 May 15;212(10):1569-71. Comparison of serum cortisol concentrations in clinically normal dogs after administration of freshly reconstituted versus reconstituted and stored frozen cosyntropin. Frank LA, Oliver JW. Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Tennessee, Knoxville 37901-1071, USA.

2. *High Dose Cortrosyn ACTH Stimulation test Protocol - Canine.*

- a. Take pre ACTH blood sample, ideally after a 12 hour fast.
- b. Administer 1 vial Cortrosyn IV or IM.
- c. Take post stimulation sample 1 hour after Cortrosyn.

NOTE: If giving Cortrosyn IM, make sure to get it into the muscle rather than into the fat. If given intrafat, it will not be absorbed quickly enough to cause stimulation – stim value will be similar to baseline (resembling iatrogenic HAC). For this reason, I prefer to give IV.

3. *ACTH Stimulation Test Using ACTH gel Protocol - Canine.*

- a. Take pre ACTH blood sample, ideally after a 12 hour fast.
- b. Administer 1 unit/lb ACTH gel IM (maximum 50 units).
- c. Take post stimulation sample 2 hours after ACTH gel.

**Low Dose Dexamethasone Test (LDD) - Canine**

Advantages:

1. It takes 8 hrs (longer than ACTH stim).
2. Dexamethasone is much cheaper than ACTH.
3. LDD is more sensitive - will identify 95-98% of dogs with HAC.
4. Can sometimes distinguish between PDH and adrenal HAC.
  - a. Partial or complete suppression at 4 hours, and then rebound to unsuppressed at 8 hours strongly suggests PDH.
  - b. Lack of suppression at all suggests ADH, but can also happen with severe PDH.

Disadvantages:

1. Have to do at least 3-4 cortisol assays, so lab costs are higher.
2. More false positives due to stress than ACTH stim. It's a good idea to do ACTH stim ONLY on that day – no other stressful procedures. In one study, 38% and 56% of dogs with non-adrenal disease at a teaching institution had inadequate suppression at 4 and 8 hours, respectively (Kaplan et al, JAVMA 1995 Aug 15).
3. No baseline is created for monitoring Lysodren or ketoconazole therapy.
4. LDD will be normal in less than 5% of dogs with HAC.
5. Not a good test for identifying iatrogenic HAC – you just get a false negative.

#### *Low Dose Dexamethasone Test (LDD) Protocol – Canine*

1. Get a baseline cortisol first thing in the morning, after a 12 hour fast.
2. Administer 0.01 mg/kg dexamethasone sodium phosphate IV.
3. Take cortisol sample 4 hours after dexamethasone administration.
4. Take cortisol sample 8 hours after dexamethasone administration.

#### *High Dose Dexamethasone Test (HDD) Protocol – Canine*

- Can distinguish between pituitary and adrenal HAC.
  - Suppression of HDD but not LDD indicates Pituitary HAC.
  - Failure to suppress can be either adrenal HAC or severe Pituitary HAC.
1. Get a baseline cortisol first thing in the morning, after a 12 hour fast.
  2. Administer 0.1 mg/kg dexamethasone sodium phosphate IV.
  3. Take cortisol sample 4 hours after dexamethasone administration.
  4. Take cortisol sample 8 hours after dexamethasone administration.

### **Urine Cortisol:Creatinine – Canine**

#### Advantages:

1. Very simple to perform – one urine sample in the morning. Can be collected at home by the owner, to eliminate stress response of coming to the clinic.
2. Very few false negatives.

#### Disadvantages:

1. Screening test only – many false positives. The Kaplan study showed that 76% of dogs with nonadrenal illness had elevated UCC, and there was no significance in UCC when dogs with non-adrenal disease were compared to those with HAC.

### **Resting Cortisol – Canine**

1. basal cortisol > 2 ug/dL that are not receiving corticosteroids, mitotane, or ketoconazole are highly unlikely to have hypoadrenocorticism.
2. basal cortisol <= 2 ug/dL, little to no information regarding adrenal gland function can be obtained and an ACTH stimulation test should be performed.
3. basal cortisol concentrations  $\geq 1.3 \mu\text{g/dL}$  accurately excluded excessive suppression (defined by cortisol concentration after ACTH stimulation < 1.5  $\mu\text{g/dL}$ ) in 254 of 259 (98%) dogs with hyperadrenocorticism being treated with trilostane.
4. basal cortisol concentrations  $\leq 2.9 \mu\text{g/dL}$  correctly excluded inadequate control (defined by cortisol concentration after ACTH stimulation > 9.1  $\mu\text{g/dL}$ ) in 200 of 211 (95%) dogs with Hyperadrenocorticism being treated with trilostane.

5. During trilostane treatment, basal cortisol concentrations between 1.3 and either 2.9 µg/dL or ≤ 50% of the pretreatment baseline cortisol concentration correctly predicted acceptable control of adrenal gland function in 147 of 168 (88%) dogs with Hyperadrenocorticism.

## **Abdominal Ultrasound – Canine and Feline**

Advantages:

1. Well tolerated by most patients.
2. Can visualize adrenal glands to evaluate for pathology. Two plump adrenals would suggest PDH. One large adrenal and the other atrophied would suggest adrenal HAC. Adrenal tumors can be bilateral.
3. Identifying an adrenal tumor that invades the caudal vena cava suggests malignancy, and indicates poor prognosis, probably precluding Lysodren therapy for many owners.
4. Hyperechoic, enlarged liver can support diagnosis of HAC. Liver cytology would show steroid hepatopathy. Sometimes gall bladder sludging is present.
5. Gives a great deal of additional information about the condition of the patient. Might identify concurrent severe diseases which might preclude expensive long term Lysodren therapy.
6. Can evaluate for calcium oxalate calculi – HAC patients are predisposed.

Disadvantages:

1. Can be difficult to evaluate adrenal glands in small animals with a great deal of gas in the gut, and in patients with increased abdominal fat.
2. Very subjective interpretation, and results depend much on the skill and experience of the sonographer.

## **Endogenous ACTH – Canine and Feline**

I use this as a last resort when I am convinced that the dog may have Cushing's, and the ACTH stim and LDD are just not convincing enough. It can be helpful, but sample handling is tedious (see Appendix 15). You have to spin down the blood right away, put it in a plastic tube and ship it overnight on ice to Michigan (form - Appendix 15).

## **Adrenal Function Tests for Cats - Protocols**

Procedures only discussed here. Advantages and Disadvantages of each listed above for dogs apply to cats also.

### *Low Dose Dexamethasone Test Protocol - Feline*

1. Get baseline cortisol after a 12 hour fast.
2. Administer 0.1 mg/kg dexamethasone sodium phosphate IV (higher dose).
3. Take cortisol sample 2 hours after dexamethasone administration.
4. Take cortisol sample 4 hours after dexamethasone administration.
5. Take cortisol sample 6 hours after dexamethasone administration.
6. Take cortisol sample 8 hours after dexamethasone administration.
7. Cats may escape suppression earlier than dogs, so the extra samples are needed. Lack of suppression at all does not suggest adrenal disease as it does in dogs.

### *High Dose Dexamethasone Test Protocol – Feline*

1. Get baseline cortisol after a 12 hour fast.
2. Administer 1.0 mg/kg dexamethasone sodium phosphate IV (higher dose).
3. Take cortisol sample 2 hours after dexamethasone administration.
4. Take cortisol sample 4 hours after dexamethasone administration.
5. Take cortisol sample 6 hours after dexamethasone administration.
6. Take cortisol sample 8 hours after dexamethasone administration.
7. Cats may escape suppression earlier than dogs, so the extra samples are needed.
8. Helpful in identifying adrenal tumors.

*ACTH stimulation Test Protocol - Feline*

1. Get baseline cortisol after a 12 hour fast.
2. Give 1/2 vial (125ug) or 5 ug/kg cosyntropin IM or IV.
3. Take cortisol sample 30 minutes after ACTH administration.
4. Take cortisol sample 1 hour after ACTH administration.
5. Cats metabolize ACTH and cortisol quickly, so the extra samples are needed.

References:

5. JAVMA 2011 238(2):176-82. Cortisol and aldosterone response to various doses of cosyntropin in healthy cats. Amy E Declue; Linda G Martin; Ellen N Behrend; Leah A Cohn; David I Dismukes; Hollie P Lee. Comparative Internal Medicine Laboratory, Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO 65211, USA. [decluea@Missouri.edu](mailto:decluea@Missouri.edu).