



## **Adrenal Function Testing Protocols**

The following protocols are listed along with brief summaries of the utility, methods, and interpretations of the tests. Interpretations are based on canine patients as they are most tested for adrenal dysfunction, while feline patients rarely have Cushing's and can have slightly different interpretations for the adrenal function tests. If a cat is being tested it may be worthwhile contacting a pathologist if guidance is desired. Note that no single test is 100% sensitive or specific for hyperadrenocorticism (Cushing's), that the diagnostic accuracy of a given test is dependent upon the type of patient being tested [i.e. the accuracy of a test is much higher in a patient that has actual physical exam, laboratory and clinical findings that are suggestive of a particular disease than in a healthy patient or patient with few non-specific findings or in a patient that has an obscured clinical picture due to presence of another disease process (pancreatitis, liver disease, etc.)] and depending on the situation, multiple tests or repeating of tests may be indicated. In addition, once hyperadrenocorticism is diagnosed and localized, additional periodic testing for the purposes of monitoring will be indicated. More elaborate information regarding sensitivity, specificity and diagnostic accuracy of a given test as well as more information regarding interpretation of tests is widely available in the literature, though a very good text to consider is Feldman and Nelson's Canine and Feline Endocrinology, 4th edition. If you have a question regarding a particular case, please feel free to contact the duty clinical pathologist.

### **Low-dose dexamethasone suppression test (LDDST):**

This test is used to differentiate between normal adrenal function and hyperadrenocorticism. The test is typically performed on dogs that have clinical signs, physical exam findings and/or laboratory findings that already allow suspicion of hyperadrenocorticism. This test is infrequently used in the few suspected cases of feline hyperadrenocorticism, and the dosage is listed in brackets below. Dexamethasone injection should suppress cortisol output by normal adrenal glands indirectly by a negative feedback mechanism where dexamethasone suppresses ACTH release by the pituitary gland. Dexamethasone will not be measured as cortisol by the test method and one-time doses should not interfere with test results.

**NOTE:** Method has not been validated for plasma samples so must collect serum in a plain red top tube (do not use SST tubes).

1. Collect at least 1ml of blood in a red top tube. This is the baseline sample and should be labeled "pre".
2. Inject 0.01mg/kg dexamethasone IV for dogs (0.1mg/kg for cats)
3. Collect 1ml of blood in a red top tube 3 hours (some texts list 4 hours, which is fine) and 8 hours post injection -- label as "3 hr. post" and "8hr post", respectively.



**Interpretation:** Suppression below 40 nmol/L is considered "normal". Conversely, according to Feldman and Nelson's Canine and Feline Endocrinology, 4<sup>th</sup> ed., dogs with hyperadrenocorticism have plasma/serum cortisol levels that are consistently greater than 40 nmol/L at the 8-hour mark. In some cases, this test can help distinguish between pituitary-dependent hyperadrenocorticism (PDH) and a functional adrenal tumor (FAT). In some cases of PDH, an escape pattern can be seen where suppression of cortisol output is noted at the 3-hour mark, though by the 8-hour mark, cortisol values rise above 40 nmol/L once again. Suppression is defined by a >50% decrease of cortisol output from the baseline or a cortisol value of <40 nmol/L at the 3-hour mark. Lack of suppression can be found with either PDH or FAT.

**High-dose dexamethasone suppression test (HDDST):** This test is used to help differentiate between pituitary-dependent hyperadrenocorticism (PDH) and functional adrenal tumor (FAT) in a canine patient that already has an established diagnosis of hyperadrenocorticism and has not yet received any therapy.

**Method:**

**NOTE:** Method has not been validated for plasma samples so must collect serum in a plain red top tube (do not use SST tubes).

1. Collect at least 1ml of blood in a red top tube. This is the baseline sample and should be labeled "pre".
2. Inject 0.1mg/kg dexamethasone IV in dogs (1mg/kg in cats).
3. Collect 1ml of blood in a red top tube at 4 and 8 hours post injection -- label as "4 hr. post" and "8 hr. post" respectively.

**Interpretation:** In a patient with PDH, cortisol levels should suppress to <50% of the baseline value at either the 4hr or 8hr marks, or both. 4hr and/or 8hr cortisol levels <40nmol/L also support PDH. FAT patients will not have suppressed cortisol values at either mark. It should be noted approximately 25% of PDH patients also will not suppress at either mark, therefore lack of suppression does not definitively differentiate between PDH and FAT.

**ACTH stimulation test:** This test is used to differentiate between normal adrenal function and hyperadrenocorticism (Cushing's) in dogs or to differentiate between normal adrenal function and hypoadrenocorticism (Addison's) in dogs and cats. The test can also be used to monitor response to therapy for a previously diagnosed hyperadrenocorticism patient. This is the only test which can confirm iatrogenic Cushing's - if a patient is receiving steroids (for example, prednisone) and testing is desired to rule in/out iatrogenic Cushing's it is best to have



the patient off of steroid medication for 3-4 days prior to testing such that the medication does not interfere with the assay results.

**Method:**

**NOTE:** Method has not been validated for plasma samples so must collect serum in a plain red top tube (do not use SST tubes).

1. Collect 1ml of blood in a red top tube. This is the baseline sample and should be labeled "pre".
2. Dogs: 5 ug/kg cosyntropin (Cortrosyn®) IV to a maximum of 250 ug (1 vial) per dog. Collect at least 1 mL of blood in a red top tube 1 hr. post injection. Label "1hr post".  
For monitoring trilostane or mitotane therapy in dogs: a dose of 1 ug/kg Cortrosyn® IV may be used, instead of 5 ug/kg.<sup>3</sup> The ACTH stimulation test should be initiated 4-6 hr. post pill (treatment)
3. Cats: 125 ug cosyntropin (Cortrosyn®) IV (1/2 vial) per cat. Collect at least 1 mL of blood in a red top tube at 1 hr. post injection. Label "1hr post"

**Note:** The reference intervals used by our laboratory are based on the use of Cortrosyn® and we recommend using this. If a formulation other than Cortrosyn® is sought for use in an ACTH stimulation test the attending clinician is responsible for researching dosages and protocols for such formulations. When other formulations are used, interpretation may be difficult. Please list the formulation used in the clinical history on the submission form.

**Interpretation:** If the test is being used to diagnose hypoadrenocorticism (which is the main purpose of the ACTH stimulation test) the typical response for an Addison's patient is to have a "flatline" response where no stimulation of cortisol output is observed. Some dogs in the early stages of Addison's will have a subdued cortisol response where some response is noted, however the "post" cortisol value is below the anticipated target range for what would be considered a normal response. Testing for Addison's should be performed promptly, prior to any administration of glucocorticoids. If glucocorticoid administration must be given, then it is recommended that dexamethasone be used for a short period (1-2 days) as this drug will not directly interfere with the cortisol assay and it has a short half-life. As it is a glucocorticoid, dexamethasone supplementation can affect the pituitary-adrenal axis and for this reason if longer periods of supplementation happen to have been the case (i.e. 4 or *more days*), a *1-2-day period* (or longer if very long periods of supplementation have occurred) where the drug is not given is recommended such that if adrenal glands are functioning they have a chance to recover. It is not known to what degree glucocorticoid administration will affect the adrenal response to the ACTH test on an individual basis (it depends on the dosage, duration and type of drug used); though supplementation can certainly affect the adrenal response on the test.

If the test is being used to diagnose hyperadrenocorticism, the typical positive response is characterized by an exaggerated response where cortisol levels are higher than the normal



reference interval (values can vary widely). The sensitivity of ACTH stim test for dogs with PDH is 80 -83% while it is 57 -63% for the dogs with FAT. Specificity ranges from 59 0 93% (Feldman

and Nelson's Canine and Feline Endocrinology, 4<sup>th</sup> Edition). **Note that** post ACTH-stimulation cortisol values can be elevated (sometimes significantly) above the reference interval in dogs that do not actually have hyperadrenocorticism and that this can be due to stress associated with several different disease processes. This can obscure the significance of results of a given ACTH stimulation test, highlighting the importance of limiting testing to patients that already have clinical features and laboratory findings that are strongly supportive of hyperadrenocorticism if this diagnosis is sought. LDDST does not have 100% diagnostic accuracy however the sensitivity and specificity are high, and this test is typically recommended for the initial diagnosis of hyperadrenocorticism.

If the ACTH stim test is being used for monitoring response to therapy for a previously diagnosed hyperadrenocorticism patient the target ranges vary depending on the medication being used.

When monitoring trilostane therapy, ideally one would like to see a post ACTH cortisol of 30-150nmol/L (Feldman and Nelson's Canine and Feline Endocrinology, 4<sup>th</sup> Edition).

According to Feldman and Nelson's Canine and Feline Endocrinology 4<sup>th</sup> edition the goal of therapy with Mitotane (o,p-DDD [Lysodren®]) is to have the dog become clinically normal. This goal can typically be achieved when the post-ACTH cortisol concentrations are between 30-150 nmol/L.

**Urine cortisol:creatinine ratio:** This test has been used as an early screening test for hyperadrenocorticism where the true value of the test is a "negative" value where the ratio is  $<10 \times 10^{-6}$ . This would suggest that the patient does not have Cushing's and that no further testing for the disease would be required. It is very important to note that a ratio of  $>10 \times 10^{-6}$  merely indicates that further testing for Cushing's is necessary and that the "positive" value is by no means definitive for Cushing's since stress associated with many different disease processes can yield a ratio of  $>10 \times 10^{-6}$ . The best use of the test is to rule out adrenal hyperfunction as by the time the clinical index of suspicion warrants consideration of Cushing's in the differential list, the ratio would be expected to be elevated anyhow and the clinical utility of the test is questionable.

### **Frequently asked questions:**

What tube do I use for collection? – *Red top tube only (SST, heparin and EDTA tubes are not acceptable).*

Does the serum have to be separated from packed cells prior to shipping? - *Yes.*

Does the patient need to be fasted? - *No, however try not to feed within an hour prior to sampling to help avoid a lipemic sample.*