



## Canine Adrenal & Pituitary Function Tests

### ACTH-Response Test measuring Cortisol

This test may be used for diagnosing Addison's disease and Cushing's syndrome. It should be used to evaluate adrenocortical function after treatment for Cushing's syndrome with o,p'-DDD (mitotane, Lysodren<sup>®</sup>) or Trilostane<sup>®</sup> and to diagnose iatrogenic Cushing's syndrome. ACTH-Response tests to monitor Trilostane<sup>®</sup> treatment should be completed 4-6 hours after Trilostane<sup>®</sup> administration.

1. Draw a baseline blood sample in a plain red-top tube for serum.
2. Inject 0.25 mg Cortrosyn<sup>®</sup> or 5 ug/kg intramuscularly or 2.2 IU/kg of ACTH Gel intramuscularly.
3. Collect samples 1 hour after Cortrosyn<sup>®</sup> injection or 1 & 2 hours after compounded ACTH Gel preparations.



### Low-Dose Dexamethasone-Suppression Test (LDDST) measuring Cortisol

This is the preferred test for the initial diagnosis of Cushing's syndrome. Severe illness, certain medications, etc. may yield inconclusive results.

1. Draw a baseline blood sample in a plain red-top tube for serum.
2. Inject **0.015** mg/kg of dexamethasone intramuscularly or intravenously.  
*Either dexamethasone sodium phosphate or Azium<sup>®</sup> (dexamethasone with polyethylene glycol) can be used.*
3. Collect additional blood samples at **6** and **8** hours.

### High-Dose Dexamethasone-Suppression Test (HDDST) measuring Cortisol

This test, combined with results from the LDDST or ACTH-response test, is used to differentiate between pituitary-dependent hyperadrenocorticism and adrenocortical tumor(s).

1. Draw a baseline blood sample in a plain red-top tube for serum.
2. Inject **0.1** mg/kg of dexamethasone intramuscularly or intravenously.  
*Either dexamethasone sodium phosphate or Azium<sup>®</sup> (dexamethasone with polyethylene glycol) can be used. Dexamethasone sodium phosphate is recommended for the high-dose DST. If Azium<sup>®</sup> is injected intravenously, do so very slowly.*
3. Collect additional blood samples **6** and **8** hours later.

### Sample Collection and Processing for Cortisol Testing

Adrenal function tests should be started in the morning to avoid possible circadian fluctuations in cortisol.

1. Draw all blood samples into plain red-top tubes and allow blood to clot at room temperature for approximately one hour. If more time is needed prior to centrifugation, specimen can be stored in the refrigerator for 2 – 4 hours.
2. Centrifuge samples at a speed and time that allow for adequate separation and sample yield.
3. After centrifugation draw off serum and transfer to a tube or vial suitable for frozen storage and/or shipping.
4. Frozen storage is recommended for serum samples if time before shipping will be 12 hours or more.
5. Optimally, ship samples frozen with cold packs. A frozen sample is not necessary for hormone stability, but does better ensure arrival of a chilled sample for more accurate test results.

**Note:** Do not use serum separator tubes as these gels/clot activators can falsely elevate results.

### Endogenous ACTH

This test can be helpful in differentiating pituitary-dependent hyperadrenocorticism from adrenocortical tumor. Dogs with increased endogenous ACTH concentrations are more likely to have pituitary-dependent disease. Adrenal tumors are usually independent of pituitary control.

1. Draw an EDTA specimen sample (purple-top tube). Fill tube to capacity (if possible) and invert several times to mix EDTA with blood.
2. Immediately centrifuge sample to separate plasma from cells. Transfer plasma to a plastic tube or vial and freeze promptly after centrifugation is completed. Canine samples must be stored in plastic.
3. Ship frozen sample via **overnight** courier with cold packs. Samples must be received at least cold or partially thawed to ensure accurate test results but frozen samples are recommended and preferred.

**Note:** Canine ACTH is very unstable in whole blood samples. It is most affected by heat and time spent on red blood cells.