

Submission Guidelines and Test Protocols

General Information

Proper sample collection and handling are essential to accurate test results.

- A. Do not send primate samples.**
- B. Do not send whole blood for any test procedure.
- C. Do not send samples in serum-separator tubes (polymer-gel-tubes). This causes hemolysis of samples and the gel is known to precipitate drugs.
- D. Label sample containers adequately
 - a. Owner's name
 - b. Animal's name
 - c. Test desired
 - d. Time of collection (baseline, post-stim, etc.)
- E. For serum samples:
 - a. Clot at room temperature, centrifuge within 1 hour, remove serum and freeze.
 - b. Alternatively, store blood samples in the refrigerator to clot for 2 to 4 hours, centrifuge, remove serum and place in non-additive tube and freeze.
- F. Packaging samples for shipment
 - a. Ship samples in insulated container with adequate cold packs and insulating materials (peanuts, paper), being careful to pack to avoid leakage or breakage (plastic tubes are recommended).
 - b. Ship samples Monday-Thursday by overnight delivery service (UPS, FedEx, DHL).
 - c. Do not send immediately prior to a holiday!
 - d. Be sure to **include a brief history** of the case, and especially **include the sex status, age of the animal, and any current medications.**

Thyroid Function

(All samples should be *serum*; do not send in polymer gel tubes)

* Thyroid function tests (T3, T4, TSH) are run daily

- A. T3 and/or T4 – DOG, CAT, HORSE, RABBIT, ALPACA**
 - a. Prepare 0.5 ml of serum (sufficient for both tests).
 - b. Consider requesting the combined T4/TSH assay for dogs.
- B. Post Pill T4 (monitoring thyroid hormone treatment)**
 - a. Collect serum (0.5 ml) post-pill at 4-6 hrs.
- C. T3 Suppression**
 - a. Collect basal serum sample for T4. Administer liothyronine (T3 or Cytobin™) at an oral dose of 25 µg 3x/day for 7 doses.
 - b. Collect sample for T4 on morning of 3rd day, 4 hrs after 7th dose of liothyronine.
 - c. **NOTE:** Request T3 determination also, if there is concern for owner compliance or drug intake by the cat.
- D. Canine TSH**
 - a. Prepare 0.5 ml of serum.
 - b. Send sample cold on an ice pack by overnight delivery.

Adrenal Function

(All samples should be *serum*; do not send in polymer gel tubes. Pre-test fasting not required)

* Cortisol assays are run daily

A. Cortisol – ACTH Stim Test * – DOG, CAT

- For dogs, collect baseline serum sample (1.0 ml) then give synthetic ACTH (Cortrosyn™) at 5 µg/kg IV (preferred) or IM and collect post-ACTH serum sample (1.0 ml) 1 hr later.
- For cats, collect baseline serum sample (1.0 ml) then use Cortrosyn™ as for dogs, but give 25 µg/kg IM (maximum dose of 125 µg/cat). Collect post-ACTH serum sample at 60 minutes.
- If using ACTH gel, give 2.0 IU/kg IM. Collect post-ACTH serum sample 2 hrs later.
- * Cortrosyn™ (versus compounded ACTH) is the preferred ACTH for use in testing since quality is consistent.

B. Cortisol-Dex Supp/ACTH Stim Test – DOG

- Collect baseline serum sample (1.0 ml). Administer dexamethasone at 0.1 mg/kg (IV).
- Take a post-dex serum sample 4 hours later and 8 hours later.
- Following the 8 hours post-dex collection, perform the Cortisol-ACTH Stim Test as above.

C. Cortisol-Low Dose Dex Supp Test – DOG

- Collect baseline serum sample (1.0 ml).
- Administer dexamethasone at 0.01 mg/kg (IV).
- Collect Post-Dex serum samples at 4 and 8 hrs (1.0 ml).

D. Cortisol-High Dose Dex Supp Test – DOG

- Collect baseline serum sample (1.0 ml).
- Administer dexamethasone at 0.1 mg/kg (IV).
- Collect Post-Dex serum samples at 4 and 8 hrs (1.0 ml).

E. Cortisol-Post-Trilostane Pill (monitoring trilostane treatment)

- Collect baseline serum sample (1.0 ml).
- Administer trilostane pill with food.
- Collect serum sample (1.0 ml) 3 hours post-pill.

F. Cortisol-ACTH Stim Test Post-Trilostane (monitoring trilostane treatment)

- Administer trilostane pill with food.
- Four to 6 hours after pill, collect baseline serum sample (1.0 ml) and inject Cortrosyn™ as listed above (A).
- Collect post-ACTH serum sample (1.0 ml) 1 hour after injection.

G. Aldosterone- ACTH Stim Test – NEUTERED DOG, NEUTERED CAT

- Perform ACTH stimulation test as listed above (A). For this analysis, collect 0.5 ml serum for baseline sample and 0.5 ml serum for post-ACTH sample.

H. Adrenal Panel for Dogs and Cats (ACTH Stim) -Test for congenital adrenohyperplasia-like syndrome (dogs, cats), or Alopecia-X, Atypical Cushing's Syndrome

Note: All Adrenal function tests use serum samples.

* Adrenal panels are batch-run weekly

- Collect baseline serum sample (2.0 ml).
- Allow sample to clot then centrifuge and separate serum into non-additive tube.
- Administer the ACTH Stim test as above (A). Collect Post-ACTH serum sample (2.0 ml) at 1 hr, if Cortrosyn™ used for stimulation (collect post-ACTH sample at 2 hrs, if gel-ACTH is used for stimulation).
- Allow sample to clot then centrifuge and separate serum into a non-additive tube. (NOTE: If samples are grossly hemolyzed, check with lab before sending).

- e. Send samples cold on ice packs by overnight delivery.
 - i. The following hormones will be assayed on baseline and post-ACTH samples: Cortisol, Estradiol, Androstenedione, 17- hydroxyprogesterone, Progesterone and Testosterone.

I. Adrenal Panel with Combined Dex Supp/ACTH Stim Test for Dogs

- a. Collect baseline serum sample (2.0 ml). Administer dexamethasone at 0.01 mg/kg (IV).
- b. Take a post-dex serum sample 4 hours later and 8 hours later.
- c. Following the 8 hours post-dex collection, administer synthetic ACTH (Cortrosyn™) at 5 µg/kg (IV), and collect post-ACTH serum sample (2.0 ml) 1 hr later. Use of synthetic ACTH (IV) is the preferred method however if using ACTH gel administer 2.0 IU/kg (IM) and collect post-ACTH serum sample (2.0 ml) 2 hrs later.
- d. This test will provide the same information as the adrenal panel with ACTH stimulation only however it will also provide information as to whether the hormones are suppressed by dexamethasone. With this approach, results from two separate testing procedures (dexamethasone suppression test and ACTH stimulation test) are achieved with one test.

J. Adrenal Panel for Ferrets -Test for adrenocortical disease in ferrets

- a. Collect baseline serum sample (0.5 ml).
- 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 17-hydroxyprogesterone. The assay is run each week.

K. Adrenal Panel for Rabbits (neutered rabbits only)

- a. Collect baseline serum sample (1 ml).
- b. Allow sample to clot then centrifuge and separate serum.
- c. Send sample cold on an ice pack by overnight delivery.
- d. Hormones analyzed are progesterone, 17-hydroxyprogesterone, androstenedione, testosterone, and cortisol.

Pituitary Function

(Sample should be EDTA plasma)

A. Endogenous ACTH – HORSE

- a. Collect 1 ml plasma on ice.
- b. Centrifuge sample immediately and transfer plasma to a plastic tube and freeze.
- c. Send sample on dry ice by overnight delivery.

Reproductive Hormones

(All samples should be *serum*; do not send in polymer gel tubes)

* Reproductive hormone assays are run weekly. Breeding progesterone is run same day as submitted; please notify laboratory personnel in advance of sample submission

A. Estradiol*, Progesterone*, or Testosterone* - DOG, CAT

- a. Collect 0.3 ml of serum for each.
- b. Allow sample to clot then centrifuge and separate serum into non-additive tube.
- c. Send sample cold on an ice pack by overnight delivery.
- d. * If analyzing sample for all 3 hormones send 0.5 ml serum.

B. Testosterone – hCG Stim – For cryptorchidism detection in the horse.

- a. Collect baseline serum sample (1.0 ml) in the morning hours (a.m.).
- b. Administer hCG at 10,000 units total dose (IV). Collect Post-hCG serum sample (2.0 ml) 72 hours later (a.m.).

- c. Allow samples to clot then centrifuge and separate serum.
- d. Send samples cold on ice packs by overnight delivery.
- C. Testosterone - hCG stimulation - For completeness of castration in dogs and cats.**
 - a. Collect baseline serum sample (1.0 ml).
 - b. Administer hCG at 50 IU/kg IM.
 - c. Collect Post-hCG serum sample at 2 hours.
 - d. Allow samples to clot then centrifuge and separate serum into non-additive tube.
 - e. Send samples cold on ice packs by overnight delivery.
- D. Progesterone – hCG Stim -For detecting ovarian remnant in dogs* or cats***
 - a. Collect baseline serum sample (0.5 ml).
 - b. Administer 50 IU of hCG/kg I.M.
 - c. Collect post hCG samples at 7 and 21 days.
 - d. Allow sample to clot then centrifuge and separate serum into non-additive tube.
 - e. Send samples cold on ice packs by overnight delivery.
 - f. * Perform test when in estrus.
- E. Progesterone – hCG Stim – For detecting ovarian remnant in rabbits**
 - a. Collect baseline serum sample (0.5 ml).
 - b. Then administer 50 IU hCG IV.
 - c. Collect serum sample (0.5 ml) 4 days later.
 - d. Allow samples to clot then centrifuge and separate serum into non-additive tube.
 - e. Send samples cold on ice packs by overnight delivery.
- F. Testosterone – hCG Stim – For detecting retained testicular tissue in rabbits**
 - a. Collect baseline serum sample (0.5 ml).
 - b. Then administer 1500 IU hCG IM.
 - c. Collect serum sample (0.5 ml) 3 hours later.
 - d. Allow samples to clot then centrifuge and separate serum into non-additive tube.
 - e. Send samples cold on ice packs by overnight delivery.
- G. Anti-Müllerian Hormone (AMH) – for detecting ovarian or testicular tissue in dogs or cats**
 - a. Collect baseline serum sample (0.5 ml).
 - b. Allow sample to clot then centrifuge and separate serum into non-additive tube and freeze.
 - c. Send sample on ice packs by overnight delivery.
 - d. Sample can be collected at any stage of reproductive cycle.

Pancreatic Function

(Sample should be serum; do not send in polymer gel tubes)

A. Insulin – HORSE

- a. Collect 1 ml serum.
- b. Allow sample to clot then centrifuge and separate serum into a non-additive tube.
- c. Send sample cold on an ice pack by overnight delivery.