Indications for testing:

1. Screening/diagnosis of hyperadrenocorticism or hypoadrenocorticism
   The ACTH stimulation test may be performed at any time of day. Patients should ideally be fasted UNLESS the test is for monitoring of treatment for hyperadrenocorticism.

2. Monitoring Trilostane or Mitotane therapy
   Animals on Trilostane or Mitotane therapy should receive their normal morning medication with a small amount of food. The ACTH stimulation test should be performed 4-6 hours after dosing.

3. In suspected Addisonian crisis
   The ACTH stimulation test should ideally be performed before administration of therapeutic corticosteroids. When rescue dexamethasone is given prior to diagnostic testing, the ACTH stimulation test should ideally be completed within 3 hours to avoid potential confounding of results by corticosteroid-mediated suppression of ACTH, which may lead to adrenal atrophy within several hours. Other forms of corticosteroids will interfere with the cortisol assay and should not be used.

Collection protocol:

- Collect a resting blood serum sample for a basal cortisol concentration and label as “0 hour”. Allow tube to clot. If possible separate the serum from the red cells by centrifugation. If a plain tube has been used for the assay, centrifuge and transfer the serum from the collection tube into a plain (red top tube). Do not syringe into tube. Store at 4 degrees.

- Inject synthetic ACTH (Synacthen®) 250ug I/V (or I/M) regardless of bodyweight. If Synacthen® Depot is used, inject 250 ug I/M

Continued on next page
Canine ACTH-Stimulation Test

- Collect a second serum sample at time-points indicated below. Follow separation instructions as above.
  - Synacthen®: Collect at 1 hour post-ACTH.
  - Syncathen® Depot: Collect at 2 hours post-ACTH. An optional 3-hour post sample may be sent, additional charges apply.*
  - Label tubes with appropriate time point.
- Refrigerate samples until they can be transported to the laboratory. Samples should be tested within 48 hours of collection.*

**Note on the use of the Synacthen® depot**

The ACTH stimulation protocol reference ranges and Trilostane monitoring guidelines are based on soluble Synacthen®. There is currently no published consensus regarding use of Synacthen®-Depot with existing ACTH stimulation protocols in dogs suspected of hyperadrenocorticism, or being monitored with therapy for hyperadrenocorticism. Initial studies show maximal adrenal stimulation between 2-4 hours after Synacthen®-Depot administration in normal dogs and dogs with hyperadrenocorticism (n=10)1,2, with higher peak cortisol than soluble Synacthen®. There are no studies of Synacthen®-Depot use in dogs being monitored on Trilostane or Mitotane therapy. We recommend avoiding use of Synacthen®-Depot for monitoring tests where possible, as Trilostane monitoring and dose adjustment guidelines are based on soluble ACTH. If depot is used, consider submitting 2 and 3 hr post-ACTH samples to most accurately measure maximal adrenal output. Additional timepoints will incur additional charges. Interpretation must always consider clinical signs and results of other testing. Synacthen®-Depot may be used interchangeably with soluble Synacthen® for diagnosis of hypoadrenocorticism and iatrogenic hyperadrenocorticism.

**References**