



Equine Pituitary Pars Intermedia Dysfunction (PPID) / Cushing's Testing

The most commonly used tests for diagnosis and monitoring of equine PPID are: combined baseline tests for endogenous ACTH and Insulin; the TRH-response test measuring ACTH; and the Dexamethasone-suppression test (DST) measuring Cortisol. Normal results for the ACTH/Insulin combination test, TRH-response:ACTH, or DST:Cortisol do not preclude the presence of a pituitary adenoma, but at least one of these test results will usually be indicative of the abnormal condition. Post-treatment follow-up can include monitoring clinical signs, and testing ACTH and/or Insulin, either individually or combined.



Endogenous ACTH Testing

1. **EDTA Plasma** is specifically needed for ACTH testing. Draw blood into an EDTA collection tube (lavender *or* purple-top tube). Gently invert the specimen several times to mix blood and anti-coagulant and then chill immediately by placing in either an ice bath or a refrigerator.*
2. Separate plasma from cells by centrifugation as soon as possible and within 4 hours after collection.
3. After centrifugation immediately transfer plasma sample to a vial (*plastic preferred*) suitable for frozen storage and shipping.
4. Frozen storage of the sample is recommended, especially if time before shipping to the laboratory will be 12 hours or greater.
5. For accurate ACTH testing, the sample should be received at least "cold or partially thawed." Sending frozen plasma with frozen cold packs using an overnight or express courier service will usually ensure the sample is received chilled.

Note: Excitement, exercise, and/or severe illness can elevate ACTH concentration, while time of day or feeding have no effect.

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

Insulin Testing

Serum or EDTA plasma can be used for insulin testing. The *plasma* sample collected for ACTH can also be used for insulin testing, following the endogenous ACTH processing above. For insulin testing with serum samples:

1. Draw the blood specimen into a "plain" red-top collection tube.*
2. Allow whole blood adequate time to clot prior to centrifugation to ensure sufficient yield and avoid fibrin formation – this may take place at room temperature for up to 1 hour or refrigerate specimen if longer time is needed (within 2 - 4 hours).
3. After centrifugation, transfer the serum into a vial suitable for shipping or frozen storage. Frozen sample storage is recommended if the time before shipping will be 12 hours or more. The sample should arrive chilled for Insulin testing.

Note: Pregnancy, large grain meals, and severe illness may cause elevations in insulin concentrations.

*Avoid use of serum collection tubes with additives (*i.e.* separator gels, clot activators, inhibitors, etc.) due to potential assay interference.

TRH-Response: ACTH Testing

The TRH-response test is used for diagnosing Cushing's syndrome in horses. Pituitary adenoma cells seem to lose receptor specificity for hypothalamic-releasing hormones. In most cases, corticotrophs (ACTH-producing cells) are abnormally stimulated by TRH (thyrotropin-releasing hormone), causing increased ACTH production by the pituitary pars intermedia.

1. Collect a Pre (or baseline) blood specimen into a purple-top (EDTA) tube following the endogenous ACTH procedure above.
2. Inject 1 mg of TRH intravenously (see Note below for TRH information).
3. Collect an additional EDTA blood specimen at exactly 10 minutes (necessary) and 30 minutes (not necessary) after injection.
4. Process and submit multiple plasma samples *together*, following the endogenous ACTH processing above.

Note: TRH is presently available as a *compounded product* through Wedgewood Pharmacy. The Wedgewood Pharmacy product is called "Protirelin".

Dexamethasone-Suppression Test (DST): Cortisol Testing

1. Collect a Pre (or baseline) blood specimen into a plain red-top collection tube for serum as in Insulin testing above.
2. Inject 20 mg of dexamethasone intramuscularly. Either *dexamethasone sodium phosphate* or Azium® (*dexamethasone with polyethylene glycol*) can be used.
3. Collect a second blood sample approximately **19 hours** later and process similarly to the baseline (pre) sample.
4. Submit the paired serum samples together, optimally shipped so they are received chilled as in Insulin testing above.