



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

The most commonly used tests for the 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. There are presently no reference ranges for post-TRH (ACTH) levels after treatment has started, but it should be lower than when originally tested.



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 sample (refrigerator/cooler/etc.).
2. Separate plasma from cells within 4 hours after collection. After centrifugation transfer plasma sample to a vial (*plastic preferred*) suitable for frozen storage and shipping.
3. Refrigerate sample if shipping same day, freeze if shipping the following day or later. ACTH is stable at least 6 months in the freezer.
4. The sample should ideally be received cool or cold. Sending frozen plasma with frozen cold packs using an overnight courier service will usually ensure the sample is received chilled and is recommended especially in summer months.

Note: Medication should be given as normal if monitoring treatment. Excitement, exercise, and/or severe illness can elevate ACTH concentration. ACTH is very unstable in whole blood and serum samples. It is most affected by time spent on cells and heat. Do NOT send whole blood.

Insulin Testing

The plasma sample collected for ACTH can also be used for insulin testing **or** serum can be collected as detailed below.

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.
4. Refrigerate sample if shipping the same day, freeze if shipping the following day or later. Insulin is stable frozen for at least 5 years.

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. TRH is presently available as a compounded product through Wedgewood Pharmacy. The Wedgewood Pharmacy product is called "Protirelin". There are presently no reference ranges for post-TRH (ACTH) levels after treatment has started, but it should be lower than when originally tested.

1. Collect a Pre (or baseline) blood specimen into a purple-top (EDTA) tube.
2. Inject 1 mg TRH intravenously (IV) for horses >250 kg; inject 0.5 mg TRH IV for horses and ponies <250 kg.
3. Collect an additional EDTA blood specimen at exactly 10 minutes (necessary) and 30 minutes (optional) after injection.
4. Process and submit multiple plasma samples *together*, following the endogenous ACTH processing/collection procedure above. Please ensure samples are labeled appropriately: *i.e.* are labeled pre and post and as plasma.

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.