

TEST SERVICE UPDATE: Equine Insulin Testing – 634

Introduction

Historically, Phoenix Lab submitted samples for equine insulin testing to an outside reference laboratory that uses the Immulite 1000 analyzer. We recently acquired the Immulite 2000 XPI, the most updated and current immunoassay analyzer on the market. After extensive testing and validation of equine insulin on the 2000 XPI and subsequent consultations with our pathology group and Dr. Rothschild, a leading equine internal medicine consultant, we are now able to offer equine insulin testing in-house at Phoenix Lab. As a result of our validation process, we found that the results from the more advanced 2000 XPI analyzer correlated closely to the Immulite 1000, but with a slight positive bias (results ran slightly higher). To account for this difference, we have established new normal reference interval utilizing data from 132 healthy horses, which are similar to other published reference intervals but lower than the Immulite 1000. Please keep this in mind when comparing insulin results prior to September 2018. Also, note that hemolyzed samples may give falsely low results and icteric and lipemic samples may give erroneous results. **Hemolysis, lipemia and icterus should be avoided.**

Reference Intervals

New Insulin reference interval

Horse: <2.0-22.7 μ U/mL

Prior Sendout Insulin reference interval

Horse: 4.0 – 45 μ U/mL

Clinical Assessment

Insulin and insulin dysregulation: Insulin is the hormone that allows for glucose to enter various cells in the body. After a meal or when glucose concentrations are high (sometimes secondary to stress/high cortisol, medications etc), insulin is released by the pancreas. Abnormalities of insulin metabolism include hyperinsulinemia and insulin resistance, and are collectively referred to as *insulin dysregulation*. Insulin dysregulation is a key component of *equine metabolic syndrome*, a collection of endocrine and metabolic abnormalities associated with the development of laminitis in equine species, and may or may not be associated with Equine Cushing's disease. Testing for both of these conditions is recommended depending on the age and clinical signs present. In cases where both are present, these must be treated individually so that insulin dysregulation can be successfully corrected.

Dr. Nicholas Frank has an excellent in depth review of Equine Metabolic Syndrome, Equine Cushing's disease/Pituitary Pars Intermedia Dysfunction (PPID) and insulin dysregulation (2008): Guide to Insulin Resistance and Laminitis for Equine Practitioners publication: http://www.lloydinc.com/media/filer_private/2018/01/17/guide_insulin_resistance_08_2.pdf

For a more concise summary with practical guidelines on sampling and result interpretation, the equine endocrinology group (multiple equine experts, including Dr. Frank) has an excellent and updated (2017) consensus/review paper on the Tuft's website titled: Recommendations for the Diagnosis and Treatment of Equine Metabolic Syndrome'. <https://sites.tufts.edu/equineendogroup/files/2016/11/2016-11-2-EMS-EEG-Final.pdf>.

Another nice, concise review paper from 2013 can be found at:
<https://onlinelibrary.wiley.com/doi/full/10.1111/evj.12169>.

Diagnosis of Insulin dysregulation: As with all laboratory testing, results need to be interpreted in light of clinical signs, current health status and any medications that the horse may be on. If insulin dysregulation is suspected, you can start with baseline/resting insulin concentrations. It is ideal that the horse be sampled in the morning and the blood drawn prior to any stressors (including if there is active laminitis). They should be off pasture and fed no grain for 4 -12 hours (see Table I and Table II) and no tranquilizers given. Note that all insulin samples are run with glucose at no extra cost. For the insulin test, a tiger top tube or red top tube is required. For an accurate glucose result, a grey top tube with at least 1 ml of blood is ideal (these can be obtained from Phoenix at no cost). If not using a grey top tube, the serum needs to be separated from the red cells within 20-30 minutes of collection. Your serum sample must be free from hemolysis (place the tube in a rack, in a chilled cooler, but not directly on ice, or in a rack in a refrigerator), lipemia and icterus. If there is going to be a submission delay of >24 hours, the serum should be frozen to preserve the insulin.

Resting insulin concentrations - usually only identifies the severely affected horses

- 1) Table I - From the 2016 recommendations of the Equine Endocrine group
<https://sites.tufts.edu/equineendogroup/files/2016/11/2016-11-2-EMS-EEG-Final.pdf> (no grain or lush grasses within 4 hours and hay is OK):

Insulin ($\mu\text{U}/\text{mL}$)	Interpretation
< 20 $\mu\text{U}/\text{ml}$	Non-diagnostic/normal
20-50 $\mu\text{U}/\text{ml}$	Suspicious for insulin dysregulation; consider dynamic testing or retesting at a later date
> 50 $\mu\text{U}/\text{mL}$	Diagnostic for insulin dysregulation

- 2) Table II - From Dr. Frank's paper (2008)
http://www.lloydinc.com/media/filer_private/2018/01/17/guide_insulin_resistance_08_2.pdf, (not on grain, no grain or lush green grass for 12 hours; hay is OK)

Glucose (mg/dL)	Insulin ($\mu\text{U}/\text{ml}$)	Interpretation
<100	<20 (<30 if on hay)	Normal – no evidence of insulin resistance. If insulin resistance is suspected based on clinical impression, consider retesting at another time or performing a dynamic insulin test (see below) ***
<100	>20	Normoglycemia with hyperinsulinemia. Horse suffers from compensated insulin resistance and increased risk for laminitis ***
<100	>100	Normoglycemia with marked hyperinsulinemia Severe compensated insulin resistance, high risk for laminitis ***
>100	>20	Rule out that the horse was recently fed grain or fresh grasses Horse is losing its ability to regulate glucose, because pancreatic insufficiency is developing Transitioning from compensated to uncompensated insulin resistance and high risk of laminitis DEFINITELY Test for PPID
>120	<20	Glucose concentrations are unregulated and pancreatic insufficiency has developed. There is uncompensated insulin resistance with a high risk of laminitis Check urine glucose for evidence of type II diabetes mellitus and DEFINITELY test for PPID

***** Horses with Equine Cushing's Disease/PPID may or may not have accompanying insulin dysregulation. Thus, the fact that there is no insulin dysregulation does not mean the horse does not have Equine Cushing's disease/PPID. In all cases where the patient is over 10 years of age and has any clinical sign of polyuria/polydipsia, unusual shedding and/or sweating patterns, is lethargic or poor doing, has recurrent hoof abscesses as well as several other potential indicators of the disease it is recommended they also be tested for it via the PRE and POST ACTH-TRH stimulation test.**

Dynamic Insulin Testing

There are many dynamic insulin test methods being done today. Recent papers show that despite the different methods, the results often correlate well. **It is important to consider the risks of administering exogenous insulin to horses prone to laminitis. There are a few studies demonstrating that insulin administration even in normoglycemic horses induced the onset of laminitis in a significant number of them.**

1. **Oral sugar test** – Fast 3-8 hours, give 0.15 ml/kg body weight corn syrup via PO syringe and collect blood at 60-90 minutes then measure insulin and glucose.
Normal insulin <45 uU/ml.
Insulin dysregulation >45 uU/ml
2. **In-Feed Oral Glucose test** – fast overnight then feed 0.5- 1.0 g/kg body weight dextrose powder in a non-glycemic feed. Collect blood at 2 hours post and measure insulin and glucose. Insulin dysregulation is indicated if the insulin is:
>68 uU/ml if feeding 0.5 g/kg body weight
>85 uU/ml if feeding 1 g/kg body weight
3. **Insulin Tolerance Test** – feed, collect blood at time 0 and give 0.01 IU/kg body weight regular (soluble) insulin IV. Collect blood 30 min later. Measure glucose at time 0 and 30 min. Consider only in horses with high glucose
Normal = >50% decreased in baseline glucose at 30 min
Insulin resistance = < 50% decrease in baseline glucose at 30 min
4. **Combined glucose-insulin test (CGIT)** – best performed at a referral hospital when the horse is allowed to acclimatize to the surroundings for at least 24 hours and placement of the IV catheter. “Pre” samples are collected for glucose and insulin and then 150 mg/kg body weight of 50% dextrose solution is infused and immediately followed by 0.1 U/KG regular insulin (see Dr. Frank's paper if you are attempting this on your own). Blood glucose concentrations are then measured with a hand held glucometer at 1, 5, 15, 25, 35, 45, 60, 75, 90, 105, 120, 135 and 150 min post infusion. At 45 min post infusion a second sample to measure insulin is also taken.

Insulin resistance is diagnosed when blood glucose concentration remains above baseline for more than 35 min or if the serum insulin concentration is >100uU/ml at 45 min. The time taken for the blood to return to baseline is also recorded.

If you have any questions, or would like to speak to our equine internist, Dr. Rothschild, please call 1-800-347-0043.