



The Liphook Equine Hospital Laboratory

How to diagnose Cushing's disease in the horse.

The recognition of equine Cushing's disease (ECD) has evolved over the last 10-20 years from once being regarded as a rarity to now being recognised as almost a 'normal ageing feature' of horses. We are presented with ever increasing numbers of suspect ECD cases which the owner requires us to investigate and diagnose with a view to long-term treatment. There are however significant problems with diagnostic methods which lead to considerable confusion over the choice of test and interpretation of results. The following is intended to be a summary of current opinion on diagnostic testing for ECD.

<u>Simple tests</u>	<u>Dynamic tests</u>
Serum glucose	Dexamethasone suppression test
Serum cortisol	TRH stimulation test
Serum insulin	ACTH stimulation test
Serum ACTH	Combined ACTH stimulation/dexamethasone suppression
Urinary corticoid:creatinine ratio	Combined dexamethasone suppression/TRH stimulation

1. Simple tests

There are few causes of **resting hyperglycaemia** (normal 3.4-6.5 mmol/l) in the horse other than ECD so, if present, this finding is fairly specific. However, most horses with ECD have serum glucose concentrations within the reference range so this makes the test very insensitive. Other causes of hyperglycaemia to be aware of include acute stress, α_2 agonist sedatives and a cereal-based feed within the past 2-3 hours. Hence the testing of blood glucose should preferably be done first thing in the morning before feeding and also the stressful effects of problems such as transportation or acute laminitis should also be considered. In any case it would be preferable to reconfirm the presence of resting hyperglycaemia before placing too much importance on its presence.

Resting **serum cortisol** (normal 50-175 nmol/l) is of little use in suspected ECD cases. Published reports are as likely to have found high, low or normal resting cortisol in ECD cases. Some reports have suggested there is loss of the normal diurnal cortisol rhythm in ECD cases and it has been suggested that 2 blood samples taken 8 hours apart can be compared and used to aid diagnosis - the theory being that in normal horses with a normal diurnal rhythm the cortisol concentration will differ in the two samples by >30%. ECD cases may however show similar cortisol levels in both samples. This is a simple test which requires no stimulation testing although must be interpreted with caution due to other extraneous effects on endogenous cortisol production. Clearly stress, pain (e.g. laminitis) and diet have a big effect on cortisol secretion and will influence the results. Results of this test would be more reliable in a pain-free case not receiving hard feed during for at least 4 hours before the test period.

Resting **serum insulin** (normal 5-36 μ iu/ml) has become more popular recently and can be useful but again should be interpreted with great care. Many horses with Cushing's disease are indeed found to have elevated resting insulin concentrations due to cortisol-induced antagonism of insulin and also the pro-secretory effect of CLIP (one of the pituitary derived products in ECD cases). However, diet and stress also have profound effects on insulin secretion and these should be carefully considered. A hard feed will elevate insulin levels for up to 5 hours. We have seen insulin levels as high as 250 μ iu/ml in non-Cushingoid horses with painful conditions such as colic and it is likely the pain of laminitis could have a similar effect. Thus insulin is not a suitable test for ECD horses with active lamellar pain and also is best measured first thing in the morning before feeding.

Resting **serum ACTH** (normal < 7 pmol/l) is a very sensitive and specific test for ECD but is greatly limited by the stability of ACTH in blood samples. ACTH is adsorbed by glass so blood must be taken in plastic tubes. The test must then be performed immediately or the sample must be kept frozen on its way to a laboratory. These problems severely limit the practical use of what might otherwise be a very useful test.

Urinary corticoid : creatinine ratio (normal $\leq 20 \times 10^{-6}$) in urine samples collected first thing in the morning has been used by some. This is a simple test with reasonable reliability although results will be affected by the same influences as discussed above with serum cortisol and also may not be reliable in dilute samples of urine which are commonly found in ECD cases (due to polydipsia/polyuria).

2. Dynamic tests

The **overnight dexamethasone suppression test** is the most accurate test to have been reported in the literature. The procedure involves measuring baseline cortisol at about 4–5 pm; injecting 40 µg/kg dexamethasone iv or im (10 ml of 2mg/ml solution per 500kg); followed by a post stimulation cortisol at about 11–12 am next day (approx. 19 hours later). Normal horses show marked suppression of cortisol (<25 nmol/l) in the post stimulation sample whereas ECD cases show incomplete suppression. In a large study in USA with post mortem confirmation of ECD this test was highly accurate. It may be overstating the real situation to say it is completely accurate but it does seem to be the best available test. Importantly reports of precipitating or worsening laminitis following the test are very rare although this potential problem should be discussed with the owner first.

The **TRH stimulation test** is primarily used when concerns over the use of dexamethasone suppression exist (i.e. laminitis). The procedure involves measuring baseline cortisol; injecting 1 mg TRH iv (NB. 1 mg TRH costs approx. £100); followed by a post stimulation sample about 30 minutes later. Normal horses usually show no real difference between the cortisol concentrations in both samples whereas ECD cases tend to show elevation of cortisol in the 2nd sample (at least 20% rise but usually much greater). The test gives reasonable reliability but there are significant numbers of false positive and false negative results.

The **ACTH stimulation test** is too unreliable for clinical use. It is reported that ECD cases show a post stimulation cortisol of >420 nmol/l and/or >3 x baseline at 2 hours following ACTH administration. A positive result could be considered supportive of the diagnosis of ECD but is not at all definitive and of questionable value.

Combination tests exist including **combined ACTH stimulation–dexamethasone suppression test** and **combined dexamethasone suppression–TRH stimulation test**. None of these tests have been properly validated with post mortem confirmation of diagnosis in tested horses and also there are important theoretical concerns in the accuracy of combination tests which may well simply magnify the inaccuracies in the individual tests. Therefore it is difficult to justify the use of such tests.