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ACTH, insulin and glucose testing at Liphook Equine Hospital – for PPID/IR diagnosis – from EU countries plus Norway, Liechtenstein and Switzerland.

Blood for ACTH testing can be collected at any time of day and at any time of the year – testing ACTH between August and October can give the greatest differentiation between PPID and normal horses. There is no need to starve the horse before collecting blood for ACTH testing UNLESS blood for insulin and glucose testing is also being collected – see below.

Blood for insulin and glucose testing should be collected after controlling carbohydrate intake as this greatly affects the results. This can be done by starving the horse for approx. 6 hours – generally the easiest way to do this is to stable the horse, give a small feed of low NSC/soaked hay around 10 pm and arrange for the vet to collect the blood the following morning between 8 and 10 am. (See below for Oral Glucose Test).

In advance of vet visit:

Order chiller pack from Liphook Equine Hospital Laboratory (see below for contact details). Freeze chiller pack blocks in a domestic freezer – ensure the chiller blocks are thoroughly frozen prior to packing and dispatching the blood.

For all tests - samples from Europe should be frozen before dispatch and therefore must be centrifuged before separation – do not freeze whole blood or blood with any cells remaining – the blood must be correctly centrifuged before freezing.

Plasma ACTH (for PPID)

Collect blood into EDTA (purple) tube – plastic or glass tubes are acceptable. Ensure the EDTA tube is filled to the line and gently invert the tube approx. 8 times to mix. (NB At least 0.5 ml of <u>plasma</u> is required).

Chill/refrigerate within 3 hours, ideally immediately (but do not freeze until separated) – for transport to the vet's surgery for centrifugation the tube can be carried in a picnic chiller bag/box with frozen chiller blocks but avoid direct contact between chiller blocks and blood tube as freezing may occur.

Centrifuge to separate plasma (from blood cells) – this should be done as soon as possible but is not that important as long as the sample is chilled within 3 hours & kept chilled.

Decant at least 0.5 - 1 ml plasma into a separate tube and freeze. Ensure tube is clearly labeled (with "EDTA plasma" for "ACTH" & horse's name).

Liphook have been offering 2 ACTH tests for the price of 1 to assess pulsatility (check current offers Liphook). In this case, collect the second sample of blood into a separate EDTA tube (as above) 10 minutes after the first sample. Label clearly (1st sample/2nd sample) and ensure the 2 tubes of plasma are also labeled.

Serum insulin (for insulin resistance and PPID) (can only be run on horses not currently in pain from active laminitis):

Starve for 6 hours (see above).

Collect blood into dry (red top) tube.

Allow to clot (for an hour or so, ideally chilled (but NOT frozen until after serum has been decanted off), if possible keep tube upright.

Centrifuge.

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Decant at least 1 ml serum into a separate tube and freeze. Ensure tube is clearly labeled (with "**Serum**" for "insulin" & horse's name)

Plasma glucose (for insulin resistance and PPID)

Take simultaneously with insulin sample above after starving for 6 hours.

Collect into oxalate-fluoride (grey-topped) tube. Ensure the OxF tube is filled to the line and gently invert the tube approx. 8 times to mix.

Keep tube chilled and upright if possible before centrifugation.

Centrifuge.

Decant at least 1 ml plasma into a separate tube and freeze. Ensure tube is clearly labeled (with "OxF plasma" for "glucose" & horse's name)

Oral Glucose Test (OGT)

It is not unusual for horses that are insulin resistant to have resting insulin concentrations in the normal range, especially after fasting or when on a low NSC diet. Therefore a stimulation test may be required to measure the insulin response to a glucose challenge.

Starve the horse for approx. 6 hours – stable the horse, give a small feed of low NSC/soaked hay around 10 pm. Next morning give a small low NSC feed (Dengie HiFi Molasses Free or rinsed soaked sugar beet would be ideal) mixed with 1g per kg bodyweight glucose or dextrose powder (so 500g glucose/dextrose for a 500kg horse – NB half this quantity may now be used, i.e. 0.5 g glucose per kg bodyweight). Blood samples for insulin and glucose should be collected 2 hours later as above, but ensure Liphook is informed that these are OGT and not resting samples. Insulin > 85 μ IU/ml is diagnostic of insulin resistance following the OGT.

Dispatching the samples

Print and enclose or attach a copy of DEFRA's import licence – this could speed up the passage of your samples through border inspections:

http://liphookequinehospital.co.uk/wp-content/uploads/2012/03/impgen-2011-01.pdf

Complete the submission form and enclose in pack If owner is submitting the submission form, please ensure the horse's vet's email or fax details are given so that the results can be copied to him/her. http://liphookequinehospital.co.uk/wp-content/uploads/LEH-submission-form-2012-2.pdf

Enclose payment or telephone to make payment.

Place frozen plasma and serum tubes into the "Cool Transport Container", surround with frozen chiller blocks and dispatch to Liphook in the chiller pack, using 24 hour delivery where possible. If not using Liphook's chiller pack, please ensure you package the blood according to HSE P650 requirements: http://liphookequinehospital.co.uk/wp-content/uploads/2012/03/Packing-regulations.pdf

If you have any queries, please contact the Liphook Equine Hospital Laboratory direct:

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Forest Mere, Liphook, Hampshire

GU30 7JG

Tel: 01428 729509 Fax: 01428 722263

Email: lab@theleh.co.uk

Website: http://liphookequinehospital.co.uk/equine-laboratory/