**TEST PERFORMANCES**

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- Schirmer tear test
Phenylephrine equine dysautonomia (grass sickness) test
Alpha-2 adrenoreceptor agonist test for Horner's syndrome
Blondheim test for myoglobin in urine
Several part pages have been deliberately left blank to allow the reader to add any useful comments/tips which he/she observes during execution of the procedures themselves and any new helpful tips which are learned along the way! New tests and new interpretations are being developed and these should be learned by reference to current journals.

**TEST PERFORMANCES**

**Bromosulphothalein clearance test (BSP clearance)**

**Procedure**
1. Starve the horse for 12 hours.
2. Insert a jugular catheter in one side (using full aseptic precautions).
3. Collect two initial clotted blood samples (10 mL) at 30-minute intervals from catheter (flush before and after and discard first 5 mL of blood).
4. Inject 1 g BSP intravenously (approximates to 2.2 mg/kg) in solution into the other jugular vein (off the needle).
5. Collect 10 mL clotted blood samples at 1-minute intervals for 5–10 minutes from the jugular catheter.
6. Determine BSP concentrations and plot clearance against time (check with laboratory for technical ability to perform this – never do the test and then try to find a suitable laboratory!).

**Comment**
Do not inject and sample from same jugular vein! There is a risk that concentrated dye would be samples in one or more of the test samples.

**Result**

Normal value for BSP clearance ($T^{1/2}$) = 125–200 sec

Alterations from normal show a delay in clearance of BSP from the blood beyond the normal. Delays may be mild to severe and reflect the loss of liver function. This test has largely been superseded by simple estimation of serum bile acids.
Comment

BSP retention can be determined following administration of accurately calculated doses of BSP (5 mg/kg) from one sample taken 15 minutes after intravenous administration of the dye. Normal animals should have a negligible percentage of the initial concentration remaining at this time.
Glucose/xylose absorption test

Procedure
1. Starve the horse for 12 hours before and during test.
2. Insert (with aseptic precautions) IV cannula with tap/plug. Flush carefully with heparin saline before and after each sampling, taking care to discard the first 5 mL of blood.
3. Collect blood samples into fluoride oxalate anticoagulant at 30 minutes and 60 minutes prior to start (STAT samples) (best if unstressed) (in-dwelling IV cannula).
4. Administer 1 g glucose/kg bodyweight as 20% solution by stomach tube.
5. Take blood samples (using fluoride oxalate) immediately after glucose administration and at 30-minute intervals for 3 hours.
6. Determine blood glucose concentration of each sample and plot concentration against time (−60 to +180 minutes) to produce a graph.
7. Calculate relative maximum concentration (base level is the mean of the first three samples, i.e. those taken before any glucose absorption) and the time of peak concentration.

Comments
- Accuracy of time is important! Either sample accurately at 30-minute intervals or record the precise time of sampling relative to the start of the test for later plotting.
- Xylose absorption test is identical but uses 5 g xylose per kg body weight given as 5% solution by stomach tube. Xylose is difficult to estimate, but is not affected by endogenous glucose metabolism so is theoretically more accurate.
- Check with lab before sending samples (fluoride oxalate anticoagulant is essential).

Result
Normal glucose/xylose absorption = peak at 90–120 min at 2× basal concentration

Alterations from normal indicated by poor (low) peak value or prolonged time to peak (or both). Flat or delayed peak curves are suggestive of failure of small intestinal absorption of glucose.
**Fig. 2.2** Blood glucose values (mmol/L) for normal glucose absorption (♦–♦) and an abnormal absorption curve (●–●) obtained from a case of diffuse intestinal lymphosarcoma.

**Comments**

- Feeding and stress should be avoided during the test.
- Water must be available at all times during the test.
- There is merit in sampling at the time a catheter is placed (30 minutes before the test starts) because nasogastric intubation may be resented and cause a stress-related elevation in the initial (stat) sample taken at time zero. The resting value can be taken as the mean of the first two pre-treatment samples.
- Sampling every 30 minutes is the gold standard, but sampling every hour from the 120 minute point can reduce the number of samples taken without having a material effect on the test interpretation.
Sodium sulphanilate clearance test
This test measures the normal renal clearance (tubular function) of sodium sulphanilate from the blood after intravenous injection. The test is seldom performed in practice having been largely replaced by other kidney function tests including clearance ratios. The gold-standard test for renal function is the inulin clearance test, because this compound has no significant protein binding in plasma. Also it is freely diffused across the renal glomerulus and has no material tubular resorption or secretion.

Procedure
1. Place indwelling intravenous catheter (under full aseptic precautions).
   - Plug/tap and flush with heparin saline.
2. Discard first 5 mL of blood obtained through catheter.
   - Collect 10-mL blood sample (plain tube).
   - Flush catheter with heparin saline.
3. Empty the bladder by catheterisation.
4. Administer 10 mg/kg Na sulphanilate aqueous solution by slow intravenous injection.
   - Flush catheter with heparin saline.
5. Sample immediately (time 0) and at 30-minute intervals for 2 hours into plain (clotting) tubes.
6. Determine sodium sulphanilate concentration in serum samples.
7. Calculate T½.

Comments
- Check with laboratory before performing the test.
- This test is seldom performed and has largely been replaced by estimations of creatinine.

Result
Normal value for sodium sulphanilate clearance = 35–45 min
Abnormal clearances are associated with some types of renal tubular disease and reflect a loss of cell function. The test has largely been superseded by other clearance tests (especially creatinine) (see below).
Assessing the immunological status of foals

The foal is born immunologically naïve and has no effective immunity at birth. Survival, and more specifically resistance to neonatal septicaemic infection, relies heavily upon effective passive transfer of immune globulins via the colostrum. In the first instance the quality of the available colostrum must be excellent and then the foal must obtain an effective volume of this within 1–3 hours of birth. The same mechanisms that are responsible for colostral (IgG) absorption are also the main portals of entry of infection into the blood stream and so early colostral ingestion and prevention of ingestion of infective bacteria form the cornerstones of neonatal medicine.

- Testing foals’ blood for circulating IgG at 20–24 hours has become routine in good-quality breeding farm management but testing at this stage allows very little (if any) time for corrective measures to be taken. By 12–18 hours the intestinal absorption of colostral antibody is virtually zero and so corrective measures taken after this stage must involve intravenous plasma.
- An effective test can be performed at 12 hours using a rapid testing method such as the zinc sulphate test, the latex agglutination test or the CITE tests.
- The value of a test performed at 12–24 hours can be immense. Whilst most septicaemic foals show signs of illness at 24–48 hours of age, the extent of infection can be critical at 12–24 hours.

Zinc sulphate turbidity test

The zinc sulphate turbidity test is a simple test that can be very useful. It measures the approximate concentration of IgG in serum or plasma to establish the immune status of foals.

Procedure

1. Prepare 250 mg ZnSO₄·7H₂O in 1 L freshly boiled water (to remove CO₂).
2. Place 6-mL aliquots of the zinc sulphate solution into sealed 7–10 mL vacummed collection tubes (plain). It is useful to use a syringe attached to a fine needle, which is inserted through the bung into the vacuum (this leaves an air-free space over the solution and retains a slight vacuum in the tube). Tubes prepared in this way remain useful for several months without risk of carbon dioxide contamination and cloudiness.
3. Add 0.1 mL serum or plasma (collect with a 1 mL insulin syringe from the sedimented blood).
4. Mix by repeated inversion of the tube.
5. Wait 10 min (qualitative) or 60 min (quantitative).
6. Measure against calibrated barium sulphate standards or ‘printing test’ (see below).

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**Interpretation**
Qualitative result: Obvious turbidity developed in 10 min = positive result, i.e. adequate immunoglobulin content.

**Beware of possible CO₂ absorption into solution giving cloudiness!**
If you can JUST read the print through the zinc sulphate solution tube after adding the plasma the globulin level is approximately as shown opposite to each size of print below.

- Zinc sulphate turbidity test = <3 g/L
- Zinc sulphate turbidity test = 3–6 g/L
- Zinc sulphate turbidity test = 6–8 g/L
- Zinc sulphate turbidity test = 8–10 g/L
- Zinc sulphate turbidity test = >10 g/L

**Comments**
- Significant difference between serum and plasma especially in foals with elevated plasma fibrinogen (i.e. those which are sick, so misleading results might be obtained!).
- Subjective assessment by observing for obvious cloudiness/turbidity of solution after 10 minutes (equates to adequate passive transfer).
- Quick, accurate and cheap (commercial kits are available).
- Accuracy less in low levels (<4 g/L); tends to overestimate in this lower range.
- Easy ‘in field’ method using pre-prepared tubes with vacuum (avoids atmospheric carbon dioxide contamination).
- Useful to test mare’s serum at the same time (ideally turbidity should be same as, or greater than the mare’s serum).
- The test gives false-positive results with haemolysed serum.
- Can be quantified using spectrophotometer but for this it requires a properly standardised calibration graph.

**Note:**
- Haemolysis gives distorted (falsely elevated) results.
- Serum is used rather than plasma as distorted results may be obtained if the plasma fibrinogen is elevated. Serum is slower to obtain than plasma. Rapid testing could be delayed therefore.
- Alternative sodium sulfite turbidity test is easy and cheap but is probably too unpredictable for everyday use. Furthermore there is no need to use it if the other tests are available.
Single radial immunodiffusion (SRID)

- Specific immunoglobulin assay performed in agar gel.
- Kits are commercially available. Similar kits are available for IgM estimation.
- Home/practice prepared kits are less predictable and must be calibrated.
- The kit can also be used to quantify colostrum quality using whey fraction (minimal acceptable levels 100 g/L of unfractionated/unseparated colostrum).
- Plasma or serum can be used.
- Not applicable stableside as the test needs 24 hours; compromised foals may be dangerously low for 24 hours longer than they might otherwise.
- Accurate (wide range from 0–30 g/L), quantitative, but expensive.
- Useful accurate confirmation test.

Electrophoresis and biochemical protein estimation

- Total serum protein can be accurately determined by the Biuret reaction or using a refractometer but this is an unreliable indicator of globulin status because of the wide range of ‘normal’ concentrations of total protein in pre-suckle foals.
- Individual plasma proteins (α₁/₂, β₁/₂, γ globulins and albumin) determined from electrophoresis and scanning by densitometer provide a very accurate estimation but the process is time consuming and needs specialist equipment.
- A combination of laboratory estimation and electrophoresis can be a useful confirmation of ‘stable side’ results obtained from other methods.

Latex agglutination

- Polystyrene particles (latex beads) used to visualize the reaction between antibody and specific protein.
- Specific equine immunoglobulin (IgG) antiserum coated onto latex reagent and mixed with sample under test.

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4 Aglutinade Equine Colostrum Test® (Ab-Ag Laboratories, 11 Victoria Street, Littleport, Cambs, CB6 1LU, UK).
5 ICN Immunobiologicals, PO Box 1200, Lisle, Illinois 60532, USA.
6 VMRD Inc., PO Box 502, Pullman, WA 99163, USA.
7 Veterinary Immunogenic Ltd, USA and Penrith, UK.
Positive agglutination occurs within 15 minutes with quantitative capability.

Some kits are only useful for plasma and others only serum. ENSURE THAT THE CORRECT SPECIMEN IS USED.

**Comments**
- Very expensive commercial kits available⁹.
- Technically simple and rapid.
- High accuracy around or below 4 g/L, but above this it is poor (may miss marginal deficiencies around 6 g/L).
- Not affected by haemolysis and whole blood can be used in some kits.
- Can be performed stable side or in stud office.

**Concentration immunoassay technology test (CITE test)**
- This is a rapid (8–10 minutes to completion) and reliable test that can easily be performed with practice facilities¹⁰. It does require accurate pipetting (suitable pipettes are included in the commercial pack).
- The instructions on the commercial kits must be followed accurately.

**Note:**
Accuracy is close to the definitive methods. Standards provide reliable quality and technical controls. The test is relatively expensive but its convenience makes this a minor consideration. The test is less affected by haemolysis than most others.

**Glutaraldehyde coagulation**
- Chemical (analytical) grade 25% glutaraldehyde solution is used and this forms an insoluble precipitate when mixed with basic proteins¹¹,¹². The test is rather crude, but it is very inexpensive and very reliable. Any haemolysis may result in overestimated IgG. The method should be quality checked against the SRID test (above).
- Prepare a 10% solution of glutaraldehyde in deionised (pure) water.
- Add 50 µL glutaraldehyde to 5 mL of serum and start clock.
- Measure time for clotting of serum to develop.

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⁹Haver-Lockhart, Shawnee, Kansas, USA.
¹⁰IDEXX Ltd, Portland ME; USA and UK.
Interpretation

- Clotting in less than 10 minutes = IgG >8.0 g/L.
- Clotting in 60 minutes = IgG concentration 4.0–8.0 g/L.

Immunoturbidometry

Immunoprecipitate measured by optical instruments. These are accurate and rapid but unnecessarily complicated for practical situations. Their main use lies in research and quality control/calibration of other methods.

Remember:

- There is no transplacental immune globulin transfer so the foal is immunologically naive at birth.
- Passive transfer is the most significant (only) source of immune globulin in the newborn foal.
- Fetus has some immunological competence from 6 months gestation but only starts to produce significant antibodies at birth. It takes >14 days to produce any effective active humoral protection and establishment of normal cellular responses may take longer than this.
- The mare concentrates IgGs into udder in last 3 weeks of gestation in preparation for colostrum production.

Assessment of colostrum quality

- Colostrum should be checked at the earliest possible stage and in any case before the foal has nursed. A 5-mL aliquot is usually all that is required.
- The physical appearance of the secretion will often provide a good guide of quality. Thick, sticky yellow secretion is likely to be good quality.
- Gentle warming to 65°C or so will cause good-quality colostrum to clot strongly. This test can easily be performed in a teaspoon held over a lighter flame for a few seconds.
- Good-quality colostrum should have a specific gravity of >1.060 and contain at least 300 g/L of IgG.
- The specific gravity of the colostrum can be measured with a colostrometer13.
- Recent developments in these enable the use of small volumes (5–10 mL) of colostrum.

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Colostral quality can also be measured quantitatively using a variety of commercially available kits\textsuperscript{14} including:

\textbf{Colostrocheck:}

- Samples clotting within 3 minutes of mixing with reagents in tube have >60 g/L IgG.
- Samples clotting 3–10 minutes after mixing have 40 g/L IgG.

These test kits are simple to perform and help to identify mares with poor-quality colostrum. Owners can easily perform them stable-side.

\textbf{Phytohaemagglutinin skin test for T-lymphocyte function}

The test should be performed within 4 days of birth in animals suspected of having poor immunity. It can equally be performed in older horses but of course the value of the test and the implications to the owner are less easily accepted at a later stage.

\textbf{Method}

1. Aseptic preparation of a clipped area of skin on the side of the neck.
2. Measurement of skin thickness using accurate (tuberculin testing or fat thickness calipers).
3. Inject 50 mg PHA in 0.05 mL phosphate buffered saline intradermally using 1 mL tuberculin syringe and a 25 G \times 1 cm needle. Using the same technique inject the same volume of phosphate buffered saline at an adjacent site (this acts as a negative control).
4. Measure skin thickness at the two sites after 24 hours (some swelling is usually present in normal foals after 4 hours).

\textbf{Interpretation}

- Positive results produce obvious lumps (usually + 2–10 mm skin thickness at the site).
- ≤0.6 mm is negative and suggests failure of T-lymphocyte responses. Negative results usually have no lump at all.

\textbf{Note:}

- This test can be performed \textit{in vitro} on samples of blood collected into lithium heparin anticoagulant.
- The laboratory must be contacted before sending the samples to ensure that they have the test functional.
- Of course, an \textit{in vitro} test is not subject to the variations of an \textit{in vivo} system and both methods have advantages and disadvantages.

\textsuperscript{14}GAMMA-CHECK-C ® (Veterinary Immunogenics Ltd, Carlton Hill, Penrith, Cumbria, UK).
Cryptorchidism test

Procedure
1. In horses <2 years old and donkeys of any age.
2. Obtain a clotted blood sample (plain tube).
3. Administer 6000 IU human chorionic gonadotrophin intravenously.
4. Obtain a second clotted blood sample 30–120 min later.
5. Samples are submitted for testosterone analysis.

Note:
In horses ≥3 years old, a single sample of clotted blood submitted for oestrone sulphate analysis is sufficiently accurate.

Table 2.1 Expected values for cryptorchidism test

<table>
<thead>
<tr>
<th></th>
<th>Testosterone (nmol/L)</th>
<th>Oestrone sulphate (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before hCG*</td>
<td>After hCG*</td>
</tr>
<tr>
<td>Entire male</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cryptorchid</td>
<td>0.3–4.3</td>
<td>1.0–12.9</td>
</tr>
<tr>
<td>Gelding</td>
<td>0.15–0.3</td>
<td>0.05–0.19</td>
</tr>
</tbody>
</table>

*Human chorionic gonadotrophin/luteinising hormone.

Water deprivation test

■ Water deprivation tests are used to identify renal failure and to differentiate psychogenic polydipsia from neurological (central) and nephrogenic (renal) diabetes insipidus.
■ These tests MUST NOT be performed in horses that are azotaemic or which show evidence of any dehydration.
■ It is essential that an accurate bodyweight is taken and tested during the procedure.

Procedure
1. Take a routine blood sample and submit for routine haematology, total protein and albumin and urea and creatinine.
2. Weigh the horse accurately.
3. Remove water from the stable in the evening (so that the tests can be performed on the following morning). Sometimes feed is removed but this is not likely to affect the test materially. Concentrate and salty foods must not be given, however.
4. Empty the bladder by catheterisation and determine urine specific gravity.
5. Weigh the horse and obtain urine and blood samples at 4–8-hour intervals.

**Note:**
The test should be stopped if the horse shows an ability to concentrate urine (SG rising above 1.025) or if weight loss reaches 5% or if any overt evidence of azotaemia or dehydration develops.

**Interpretation**
- Normal horses show rapid urine concentration with SG rising above 1.025.
- Horses with psychogenic polydipsia also show normal concentration ability.
- A low or suboptimal SG suggests diabetes insipidus or renal medullary washout (see below).
- If urine does not concentrate at 24 hours to SG >1.025 an extended modified (partial water deprivation test is advisable) (see below).

**Comments**
- Renal medullary washout is due to excessive drinking in the absence of pathology and is due to the loss of osmotic gradient within the renal tubules. In this case it is possibly better to perform a partial water deprivation test. Water intake is restricted to 40–45 mL/kg/day for several days, fed in small volumes frequently through the day. This will usually restore the gradient and the urine SG will rise to >1.025 and the associated polydipsia will usually resolve.
- Increase in SG >1.025 suggests psychogenic polydipsia while failure to concentrate >1.025 suggests diabetes insipidus.
- An ADH response test is then indicated (see below).

**Vasopressin (antidiuretic hormone) challenge test**
This test is used to distinguish neurogenic diabetes insipidus from nephrogenic diabetes insipidus.

**Procedure**
1. The bladder is emptied by catheterisation.
2. Bodyweight is recorded.
3. Blood and urine samples are obtained prior to the start of the test.
4. EITHER: 0.2 IU of exogenous ADH (vasopressin: pitressin synthetic) is administered IM; OR: 60 IU ADH is administered IM every 6 hours over a 24-hour period.
5. Urine and blood samples are taken every 4–6 hours and bodyweight checked.
Interpretation

- In normal horses and those with central diabetes insipidus, urine SG should increase to over 1.025 within 4–12 hours of the start of the test.
- Horses affected with nephrogenic diabetes insipidus show no or little response to the ADH indicating that the renal tubular resorption of water is incapable of responding to the hormone.

Renal clearance ratios

1. Creatinine clearance ratio
   This is proportional to GFR if renal function is normal.

Procedure

1. Collect total 24-hour urine output.
2. Determine creatinine concentration and volume of urine.
   Creatinine clearance ratio, glomerular filtration rate (assuming normal kidney function). GFR is traditionally measured by inulin clearance (a starch-like polymer of fructose which passes readily through the renal glomerular membrane and which is neither secreted nor absorbed by the renal tubules) or by 24-hour endogenous creatinine clearance.

   Creatinine clearance ratio = \frac{[\text{Creatinine}]_{\text{SERUM}}}{[\text{Creatinine}]_{\text{URINE}}} \times 100\%

   (NORMAL = 1.0–2.0 mL/kg/min)

2. Electrolyte clearance ratio

Procedure

1. Obtain simultaneous venous blood (clotted) and urine (preferably by catheterisation).
2. Submit for creatinine and electrolyte clearances.
3. Remember that urinary creatinine may be expressed in mmol/L rather than µmol/L, as it will be for serum! (If you forget this, the results will look wildly out!)
4. The same principle can be applied to any metabolite including cortisol for pituitary-adrenal disorders such as equine Cushing's disease.

   Clearance [X] (%) = \frac{[\text{Creatinine}]_{\text{SERUM}}}{[\text{Creatinine}]_{\text{URINE}}} \times \frac{[X]_{\text{URINE}}}{[X]_{\text{SERUM}}} \times 100\%
### Table 2.2  Normal clearance ratios

<table>
<thead>
<tr>
<th>Ion</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>0.1–1.0%</td>
</tr>
<tr>
<td>K⁺</td>
<td>15–65.0%</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>1.5–3.5%</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>0.0–0.5%</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>0.05–1.5%</td>
</tr>
</tbody>
</table>

**Comments**

- Simultaneously obtained urine and plasma samples can be used to obviate the need to collect 24-hour urine output. However, the accuracy of the analysis is dependent on normal renal function.
- If renal function is not normal the test is invalid (renal creatinine clearance ratio not normal).
- Values may show diurnal variation – results should be used as a guide rather than an absolute indicator.
- Diet has been shown to be a major influence on the results and so interpretation can be very difficult.

### 3. Excretion factors

\[
\text{Calcium excretion} = 15 \text{ µmol/mosmol (micromoles per milliosmole)} \\
\text{Phosphate excretion} = 15 \text{ µmol/mosmol}
\]

\[
\text{Urine concentration (mmol)} \times 0.04 \\
\text{Urine specific gravity} - 0.997
\]
Diagnostic tests for pituitary adenoma/Cushing’s disease in horses/ pars intermedia dysfunction (PID)

**Note:**
Diagnosis can also be made from:

1. INSULIN estimation (>100 µmol/mL) and no response to IV glucose challenge.  
2. URINARY CORTISOL/INSULIN clearance ratios (compared to creatinine).
   - A diagnosis of pituitary adenoma should only be made when the clinical features are suggestive and clinical pathology is confirmatory/supportive.
   - A genuine hypothalamic induced pituitary adenoma is extremely unlikely in horses under 12–15 years of age.

**ACTH stimulation test**

**Procedure**
1. A plain (clotted) blood sample is obtained 30 minutes before and immediately before intravenous administration of 100 IU synthetic adrenocorticotrophic hormone (Synacthen, Ciba, UK).
2. A plain (clotted) blood sample is obtained 2 hours after the injection.
3. Samples are submitted for cortisol assay.

**Interpretation**
- Normal horses show a 60–80% elevation in serum cortisol at 2 hours after injection.
- Most but not all cases of pituitary-dependent hyperadrenocorticism show much higher increases.
- Interpretation is not always easy when the resting cortisol concentrations are very high.

**Note:**
There is a theoretical risk of inducing laminitis with this test and so cases must be carefully selected to exclude those horses that might be prone to or have or have had laminitis.

**Dexamethasone suppression test**
The ability of a small dose of exogenous dexamethasone to suppress the secretion of ACTH from the pituitary gland is tested by this method.

**Procedure**
1. Plain (clotted) blood samples are obtained 30 minutes and immediately before intramuscular administration of 40 µg/kg dexamethasone intramuscularly.
2. Second and third clotted blood samples are obtained 4 hours and 24 hours later.
3. Samples are submitted for cortisol assay.

**Interpretation**
- Normal horses show a fall to 30% of the resting value of serum cortisol concentration. And the depression is maintained for at least 24 hours.
- In cases of pituitary-dependent hyperadrenocorticism the depression is much less (often negligible).
Note:
The test is a more reliable indicator of pituitary hyperadrenocorticism than the ACTH stimulation test.
There is a theoretical risk of inducing laminitis by the administration of the dexamethasone (even though the dose is very small – usually around 20 mg). The test should not be used in horses that have had laminitis or which are clinically affected at the time, or are otherwise prone to develop laminitis.

Combined ACTH stimulation test response and dexamethasone suppression test
Adrenocortical hypertrophy (for any reason, either primary or secondary) can be inferred from an exaggerated response to administration of adrenocorticotropic hormone (ACTH).

Procedure

Table 2.3 2-day method

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>Collect 10 mL clotted blood [CORTISOL] Inject 40 μg/kg dexamethasone (IM)</td>
</tr>
<tr>
<td>4 h</td>
<td>10 mL clotted blood [CORTISOL]</td>
</tr>
<tr>
<td>24 h</td>
<td>10 mL clotted blood [CORTISOL] Inject 1 mg ACTH IV OR Inject (1 IU/kg) ACTH gel IM</td>
</tr>
<tr>
<td>36 h</td>
<td>10 mL clotted blood [CORTISOL]</td>
</tr>
</tbody>
</table>

Interpretation for normal: 2-day test
- Cortisol falls to 30% of baseline in 4 hours and remains at 30% for 24 hours.
- Then 2–3× elevation by 8–12 h after injection of ACTH.

Table 2.4 1-day method

<table>
<thead>
<tr>
<th>Time</th>
<th>Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h</td>
<td>10 mL clotted blood [CORTISOL] Inject 10 mg dexamethasone IM</td>
</tr>
<tr>
<td>3 h</td>
<td>Clotted blood [CORTISOL]</td>
</tr>
<tr>
<td>5 h</td>
<td>Clotted blood [CORTISOL]</td>
</tr>
</tbody>
</table>
Interpretation for normal: 1-day test

- Cortisol depressed to 35% baseline by 3 hours.
- Cortisol rises to 2.5× baseline at 5 hours.

Comments

- Theoretical risks of laminitis are increased by combination of two compounds that may each have some role in causing laminitis.
- The test should probably not be performed if the patient has or has had laminitis or is liable to it by virtue of breed or management.
- The test requires a minimum period of 5 hours, but is usually conducted over 2 days to minimise the risks of laminitis.

Thyrotropin releasing hormone (TRH) response

This test is performed to investigate possible cases of pituitary adenoma (equine Cushing's disease). The test relies upon there being an aberrant response of the pituitary adenoma to TRH and the release of ACTH in response to this. The mechanism for the test is not well understood.

Procedure

1. Obtain a heparinised blood sample (10 mL).
2. Inject 1 mg thyrotropin releasing hormone (TRH) intravenously.
3. Obtain lithium heparin samples at time 0, 15, 30, 60 minutes.
4. Submit specimens for cortisol assay.
**Interpretation**

- Normal horses show minimal or absent responses.
- Horses affected with pituitary-dependent hyperadrenocorticism show an abnormal elevation of cortisol (often 50–100% increase). Some authorities accept elevations of 25% or more as being positive.

**Comments**

- There is a very small risk of laminitis induction. Cases should be selected accordingly to avoid this risk as far as practicable.
- There will usually be a simultaneous rise in plasma thyroid stimulating hormone (TSH) and in thyroid hormones (T₃ and T₄).
- Normal maximal cortisol level at 30 minutes. Positive result baseline cortisol elevated above 150 nmol/L with 40% elevation after TRH stimulation OR baseline level normal with >70% rise after stimulation.

**Table 2.5** The relative ease and specificity of the various tests that are used for the diagnosis of pituitary-dependent hyperadrenocorticism

<table>
<thead>
<tr>
<th>Test</th>
<th>Ease factor</th>
<th>Specificity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH stimulation</td>
<td>FAIR</td>
<td>MODERATE</td>
<td>Laminitis risk probably overstated</td>
</tr>
<tr>
<td></td>
<td>Total time 2 hours</td>
<td></td>
<td>Availability of ACTH</td>
</tr>
<tr>
<td></td>
<td>Single visit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexamethasone suppression</td>
<td>GOOD</td>
<td>GOOD</td>
<td>Laminitis risk probably overstated</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Delay to second sample</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Delay to third sample</td>
</tr>
<tr>
<td>Combined ACTH/DEX</td>
<td>POOR</td>
<td>GOOD</td>
<td>Laminitis risk probably overstated</td>
</tr>
<tr>
<td>suppression</td>
<td></td>
<td></td>
<td>Repeat samples required over 2 days or repeated samples over 3–6 hours</td>
</tr>
<tr>
<td>TRH stimulation</td>
<td>GOOD</td>
<td>GOOD</td>
<td>TRH hard to get</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Difficulty with natural hormones from bovine brain</td>
</tr>
</tbody>
</table>

Continued
Table 2.5  The relative ease and specificity of the various tests that are used for the diagnosis of pituitary-dependent hyperadrenocorticism  (Continued)

<table>
<thead>
<tr>
<th>Test</th>
<th>Ease factor</th>
<th>Specificity</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting cortisol concentration</td>
<td>EXCELLENT (single sample)</td>
<td>POOR (diurnal and other variations)</td>
<td>SAFE SINGLE TEST</td>
</tr>
<tr>
<td>Insulin</td>
<td>GOOD (urine and blood required)</td>
<td>POOR</td>
<td>Very safe/easy Best performed as an excretion test with cortisol in urine and blood Need to obtain urine sample at same time</td>
</tr>
</tbody>
</table>
Dynamic thyroid tests
Thyroid deficiency is very rare in horses and the test is seldom performed. Check with laboratory before start to ensure correct samples and timing.

Note that concurrent phenylbutazone may result in a significant reduction in estimated concentrations of $T_3$ and $T_4$.

1. Thyroid stimulation test
(i) Blood samples (plain or heparinised) are obtained 1 and 2 hours before the test begins and again at the start of the test. This provides an effective baseline.
(ii) 5 IU thyrotropin (TSH) (SIGMA Chemicals Ltd) administered IV or IM.
(iii) Plain (or heparinised) blood samples obtained at 1, 2, 3, 4, 6, 8 and 12 hours post-injection.

Interpretation
(a) NORMAL:
- Peak increase of $T_4$ of $2 \times$ base-line concentration at 4 hours post-injection.
- $T_3$ will peak between 1–3 hours at 2–4 times baseline concentration and then drops to less than $2 \times$ baseline by 6 hours.
- In foals the $T_3$ rise may be faster with doubling at 1–2 hours.
(b) In hypothyroidism the baseline level of $T_3$ is hardly affected by the TSH and the $T_4$ responds more slowly than in normal horses.

2. TRH stimulation test
(i) Initial blood samples at – 1 hour and time 0 provide the baseline.
(ii) Administer 0.5 mg (ponies) or 1 mg (horses) TRH IV.
(iii) Blood samples collected at 2 and 4 hours post-injection.

Interpretation
NORMAL:
- Twofold increase in $T_4$ by 4 hours and $T_3$ increases 3-fold by 2 hours.

Note:
- Most commercially available TSH, which is of reasonable price, is of BOVINE ORIGIN. THIS SHOULD NOT BE USED IN VIVO IN HORSES due to risk of transmitted CNS disorders and MUST NOT UNDER ANY CIRCUMSTANCES BE USED for horses intended for human consumption.
- Where practicable pharmaceutical grade (HUMAN origin or recombinant) TSH or thyrotropin releasing hormone may be used. THESE ARE VERY EXPENSIVE.
Schirmer tear test
Tear production in the horse is high compared to other species but the large majority of the aqueous component of the pre-corneal tear film is lost by evaporation from the surface of the eye. The Schirmer test provides information about the volume of tears that are being produced. The options are excessive tearing (as in painful or injured or infected eyes) or reduced (as in keratoconjunctivitis sicca).

Procedure
1. Either a standard Schirmer tear test strip or a 65 cm length (×4–6 mm wide) piece of Whatman Number 1 Filter Paper can be used. In the latter case a bend or a small notch is placed about 5 mm from one end.
   ■ The Schirmer test strip has a notch that should be placed level with the eyelid margin.
   ■ The fold on the homemade paper serves the same purpose and measurement is made from these points.
2. The paper is introduced into the lower conjunctival sac so that the mark/fold or notch is at the eyelid margin, behind the lower lid and the eye allowed to close.
3. The paper is left in situ for 1 minute exactly and the distance the wetness has advanced down the paper is measured accurately.
4. The result is expressed as mm/minute.

Interpretation
■ Normal tear production results in a distance of 12–28 mm/minute.
■ Less than 10 mm/min is consistent with inadequate tear production while values over 35 mm indicate excessive tear production.
■ Horses with obstructed tear ducts (in the absence of any conjunctival infection or inflammation) will usually have normal tear production.

The patency of the nasolacrimal duct is tested by instilling a dye (usually fluorescein) into the conjunctival sac and examining the floor of the ipsilateral nostril for the dye some 10–15 minutes later.
   (Note that this is much later than in many other species.)

Comment
Topical local anaesthetic is not used and medications should be withdrawn for some hours prior to the test.
Phenylephrine equine dysautonomia (grass sickness) test
The clinical diagnosis of equine dysautonomia (grass sickness) can be very difficult. However, the phenylephrine eye (ptosis reversal) test can be an effective diagnostic aid. The test is particularly useful in the field situation where sophisticated facilities may not be available.

Procedure
1. Prepare a 0.5% solution of epinephrine (adrenaline) by diluting 1 mL of a 10% solution with 19 mL sterile saline.
2. Assess the degree of ptosis (drooping of the upper eyelids) in both eyes by carefully viewing from the front.
3. Add 0.5 mL of the 0.5% solution to the conjunctival sac of one eye only.
4. Reassess the eyelash angle (degree of ptosis) in both eyes after 30 minutes.

Interpretation
Positive result (i.e. the horse has equine dysautonomia (grass sickness) if the angle of the treated eye is normalised, i.e. the eyelashes are lifted and the palpebral fissure is widened (the degree of ptosis is reduced) when compared to the untreated eye (see Fig. 2.4).

Comment
The test must not be performed on a horse that has received acepromazine or an alpha-2 adrenoreceptor agonists drug in the previous 6–8 hours.

Fig. 2.4a and b  Prior to application of the phenylephrine the eyelids of most grass sickness cases are uniformly drooped (ptosis) (a). Following application of phenylephrine to one eye the treated eyelid returns to a more normal position. The difference between the eyelid positions is usually very noticeable (b).
Alpha-2 adrenoreceptor agonist test for Horner’s syndrome

Horner’s syndrome is a rare condition in horses associated with impaired autonomic function in the region of the head and neck. The signs are less obvious in the horse than some other animals and usually there is sweating, ptosis, mydriasis and corneal dryness associated with the affected side. Intravenous injection of an alpha 2-adrenoreceptor agonist such as romifidine to an affected horse will cause the sweating signs to transfer from the affected side to the normal side.

Procedure

1. Identify the difference in temperature between the two sides of the horse’s face by careful palpation or with the help of a thermal camera.

2. Inject a sedative dose (75 µg/kg) of romifidine (Sedivet, Boehringer Ingelheim, UK) intravenously.

3. Wait 12–15 minutes.

4. Reassess temperature of the two sides in the same way.

Interpretation

Positive result (i.e. Horner’s syndrome is present) if the warmth and sweating of the affected side is reduced and there is increased sweating and warmth on the unaffected side.
Blondheim test for myoglobin in urine
Differentiation of myoglobin from haemoglobin or other abnormal pigments in urine can be difficult in practice. Differentiation is critical to diagnosis of conditions that have pigmenturia as a prominent clinical sign.

Method
STAGE 1: Differentiation between protein and non-protein pigments:
- Three millilitres of 3% sulfosalicylic acid is added to 1 mL of freshly voided or preserved urine (see below).
- The resultant solution is mixed and then centrifuged to separate any precipitate.

Interpretation
If the pigment is precipitated and the supernatant is clear or the colour of normal urine, the pigment is due to either haemoglobin or myoglobin (i.e. it is protein in nature).

STAGE 2: Differentiation between haemoglobin and myoglobin:
- Add 2.8 g of ammonium sulphate to 5 mL of fresh or preserved urine.
- This creates an 80% saturated solution; mix well.
- Centrifuge to separate any precipitate.

Interpretation
If filtrate still displays the abnormal colour myoglobin is present. If only the normal colour of urine is visible then the pigment was haemoglobin.

Note:
- Urine preservation is important and this can be achieved by ensuring the urine is at pH 7.0–7.5 (this is a normal pH for horse urine but it may be acidic when myoglobin is present). The urine is then stored in a sealed container at 4°C until analysis can be undertaken. This method permits the Blondheim test to be performed up to 2–3 months after collection and the test is valid even if there is bacterial contamination.
- Other tests can be used also, including spectrophotometric methods.