Local labs, supporting
Local vets, nationwide.

Gribbles Veterinary
Veterinary Handbook 2015
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Avian and reptilian diagnostics

HAEMATOLOGY AND BIOCHEMISTRY

Recommended testing and sample requirements:

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<th>Tube</th>
<th>Sample Volume</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haematology</td>
<td>Lithium Heparin (preferred)</td>
<td>Minimum 300μL</td>
<td>Packed cell volume Estimated white cell count</td>
</tr>
<tr>
<td></td>
<td>(or EDTA) + 2x air-dried</td>
<td></td>
<td>White cell differential</td>
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<tr>
<td></td>
<td>smears</td>
<td></td>
<td>Morphology of red and white cells</td>
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<td></td>
<td>Thrombocyte assessment</td>
</tr>
<tr>
<td>Biochemistry*</td>
<td>Lithium Heparin Plain</td>
<td>Minimum 400μL</td>
<td>Liver disease</td>
</tr>
<tr>
<td></td>
<td>(also accepted)</td>
<td></td>
<td>Renal disease</td>
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<td></td>
<td></td>
<td></td>
<td>General</td>
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<td></td>
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<td></td>
<td>AST GLDH</td>
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<td></td>
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<td></td>
<td>Bile acids</td>
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<td>Uric acid</td>
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<td></td>
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<td></td>
<td>Total protein</td>
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<td></td>
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<td></td>
<td>Glucose</td>
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<td>Calcium</td>
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<td></td>
<td>Phosphorus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CK</td>
</tr>
</tbody>
</table>

* For small volume samples please indicate biochemistry analytes in order of priority.

Interpretation of Biochemistry

As interpretation of avian chemistry results differs from mammalian chemistry, a brief explanation of the various tests is included as follows:

Liver and Muscle

AST and Bile Acids are the most sensitive indicators of liver disease in birds.
- AST is not liver specific and can be increased in any septic or inflammatory condition, muscle disease and with certain antibiotics and steroids.
- GLDH is present within hepatocyte mitochondria and is considered the most specific indicator for hepatocellular damage in birds.
  - GLDH also has high activity in renal tissue in birds but most of the enzyme is excreted directly into urine and never reaches the blood.
- Serum bile acid concentration is a reliable indicator of liver function.
  - Requires serum as heparin interferes with the assay.
  - Feeding can increase bile acids up to 1.6-4.5 x the normal reference range therefore fasting samples are preferred - pigeons, ostriches and some parrots lack a gall bladder so fasting is not needed in these species.
  - Amazon parrots normally have slightly higher bile acids than other companion avian species
  - Low bile acid concentrations are common in birds with microhepatica, poor feather formation, and an overgrown malformed beak.

*Generally, a bile acid concentration > 100 μmol/l is considered abnormal and > 75μmol/l is suspicious for hepatic insufficiency.*
CK activity increases with muscle damage and this, along with AST and GLDH, is used to differentiate muscle from liver disease.

The following tests are NOT recommended as indicators of liver disease in birds:

- ALT and GGT occur in many different tissues and in some species enzyme activity is below the sensitivity of many analysers.
- ALP activity can be increased due to inflammation therefore is considered nonspecific.
- Bilirubin concentrations increase inconsistently in cases of liver disease and some birds (chickens) cannot form bilirubin.

Renal

Uric Acid is the most reliable test of renal disease.

- It requires more serum or plasma than the other tests (a minimum of 50μl) so is often done last.
- Age, diet, sex, and recent feeding may affect results especially in raptors where postprandial levels can increase to twice normal for up to 8 hours.
- Elevations are seen with severe dehydration and renal disease.
- It can be used as a prognostic indicator for gout - the solubility of uric acid in plasma in birds is around 600 μmol/l - levels higher than this (1500 – 2500 μmol/l) will lead to precipitation in joints.

Urea and creatinine are not useful tests for monitoring for renal disease.

- Urea can be useful in assessing hydration status - concentrations of 0.4 – 0.7 mmol/L are considered normal, but up to 10-15x increase can be seen in dehydration.
- Creatinine is not synthesised by birds - most muscle breakdown products are excreted as creatine rather than creatinine.

Other Tests

Protein concentrations in serum are generally lower in birds than mammals.

- In most avian species levels range between 20-40 g/L (some spp. are as low as 15 g/L)
- Plasma concentrations will be approximately 1-2g/l higher than serum concentrations due to the presence of fibrinogen.
- Glucose is not utilised by avian erythrocytes therefore concentrations in serum are much more stable in birds.
- Levels will fall slowly over 24-48 hours if the serum stays on the clot.
- If there is a delay in the sample reaching the laboratory, either spin and separate the sample or use a fluoride oxalate (grey top) tube.

*The glucose reference range in birds (11.2-27.7 mmol/L) is generally higher than in mammals*

- Stress and postprandial levels can cause transient increases to 24.9 – 33.3 mmol/L.
- Conditions such as egg yolk peritonitis and renal carcinoma can also cause similar increases.
- Borderline hypoglycaemia is common in cockatoos and probably of no clinical significance.

Calcium measured in serum includes albumin bound and free forms therefore concentration varies with albumin concentration.

- Oestrogen induces hypercalcaemia therefore calcium concentration in serum increases approximately 4 days prior to ovulation.
- Corticosteroids decrease total calcium.
- As in mammals dehydration and some tumors can increase calcium concentrations.
African Grey Parrots have a described idiopathic hypocalcaemia (a unique form of hypoparathyroidism in which calcium is not properly released from bone).

AVIAN URINALYSIS

- Urine collection can be aided by giving water by a crop tube – the bird will often urinate shortly after.
- Avian urine has a concentrated white to off white uric acid component and clear watery component – the watery fraction is assessed.

<table>
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<th>pH</th>
<th>Ranges from 6.0-8.0 (diet related)</th>
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<td>Protein</td>
<td>Trace amounts normal</td>
</tr>
<tr>
<td>Glucose</td>
<td>Trace amounts normal in some species. Will occur if blood glucose is &gt;33.3 mmol/L.</td>
</tr>
<tr>
<td>Ketones</td>
<td>Negative in normal birds. Increased with diabetes &amp; increased fat metabolism</td>
</tr>
<tr>
<td>Specific gravity</td>
<td>1.005-1.020</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>Not normally present. Biliverdin is the major bile pigment which does not react with the mammalian urine dip stick</td>
</tr>
<tr>
<td>Blood</td>
<td>Negative or trace</td>
</tr>
<tr>
<td>Nitrate</td>
<td>Unreliable in birds</td>
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<tr>
<td>Urates</td>
<td>White, pale yellow and pale grey are normal</td>
</tr>
<tr>
<td>Liver disease</td>
<td>Urates are yellow to green</td>
</tr>
<tr>
<td>Acute lead poisoning</td>
<td>Urates brown or blood tinged</td>
</tr>
<tr>
<td>Sediment*</td>
<td>RBC &amp; WBC: normal &lt;3/HPF Epithelial cells: Normally none present Casts: Presence is associated with renal disease Bacteria: False positive with faecal contamination of urine (can proliferate in transit)</td>
</tr>
</tbody>
</table>

* A fresh or recently refrigerated sample is required. Prolonged storage causes lysis of cells.

SPECIFIC AVIAN CYTOLOGY AND MICROBIOLOGY

Collection and submission of coelomic fluids and routine aspirates is as described for other species (see routine cytology section).

Crop Aspirate/Wash

Indicated if there is vomiting, regurgitation not associated with courtship, delayed crop emptying or other suspected disorders of upper alimentary tract.

- Make multiple air-dried direct smear slides at the time of sampling
- Submit any fluid collected in EDTA for cytology and a plain tube for culture

Choana

- Indicated in cases of choanal ulceration/erosion
- Samples can be obtained by swabbing gently with pre-moistened swab (sterile saline) - avoid touching the feathers around the vent
- Make multiple air-dried direct smears
Sinus Aspirate in Psittacines
- Indicated in cases of facial swelling, nasal discharge or other upper respiratory tract disease.
- Make multiple air-dried direct smear slides at the time of sampling
- Submit any fluid collected in EDTA for cytology and a plain tube for culture

Air Sac Samples
- Sampling is indicated in suspected chlamydial, bacterial or fungal infections of air sacs
- Make multiple air-dried direct smear slides at the time of sampling
- Submit any fluid collected in EDTA for cytology and a plain tube for culture

Faeces
- Sampling is indicated if birds have gastrointestinal signs or are doing poorly.
- Tests available include faecal worm egg count, coccidial oocysts, Giardia parasites and faecal culture

AVIAN SEROLOGY/PCR

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<td>*Psittacine Beak and Feather Disease PCR</td>
<td>Blood or blood feather</td>
</tr>
<tr>
<td>*Polyoma virus PCR</td>
<td>Blood or blood feather</td>
</tr>
<tr>
<td>*Mareks disease PCR</td>
<td>Blood or blood feather</td>
</tr>
<tr>
<td>Sexing</td>
<td>Blood spot on filter paper or blood feather</td>
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* can be done individually or as part of the Avian Panel

Chlamyphila (Chlamydia) psittaci

*Chlamyphila psittaci* infection can be the cause of fever, anorexia, lethargy, diarrhoea, excretion of green to yellow urates and occasionally shock and death in birds. Infection can be associated with conjunctivitis, enteritis, pericarditis, air sacculitis, sinusitis, coelomitis, hepatitis and splenitis. The importance of this infection in birds is enhanced by its zoonotic potential. *C. psittaci* infection has been demonstrated in over 460 bird species with the highest infections rates reported in psittacine birds and pigeons. Survivors of infection can become asymptomatic carriers. Transmission is from close proximity to another infected bird. The bacteria are shed in nasal secretions and faeces – faecal shedding is intermittent and can be activated by any cause of stress. The organism can survive in the environment for several months if protected by organic debris. Predator or scavenger species can become infected through consumption of the carcass of an infected bird. Nest transmission is possible through regurgitation feeding and via biting/blood-sucking arthropods.

Human infections (psittacosis) occur most commonly from inhaling aerosolized organisms from urine, respiratory secretions or dried faeces. Beaks to mouth contact, a bite from an infected bird or handling the plumage of an infected bird are other possible sources of infection. Appropriate protective equipment should be used if performing a post-mortem on an infected bird.

Psittacine Beak and Feather disease (PBFD)

PBFD is caused by beak and feather virus which is from the family *Circoviridae*. Infection with this virus causes chronic feather dystrophy and loss, beak deformities, occasionally immunosuppression and death. Death in chronic cases is often due to secondary infections associated with immunosuppression. There are acute and peracute forms which can cause sudden death. Parrots that are known to be particularly susceptible to PBFD include, Cockatoos, Ringneck parakeets, Eclectus Parrots and Lovebirds but all parrot breeds should be considered at risk. Any psittacine bird with chronic feather loss should be tested for this infection.
Polyoma virus (Budgerigar Fledgling Disease)
Polyoma virus in birds can be seen clinically as sudden death, neurological signs, abdominal distension (hepatomegaly or ascites), petechial to widespread haemorrhage or failure of development of normal feathers. All psittacine birds can be infected however fledgling and juvenile birds are most susceptible. Chronic, subclinical infection with intermittent viral shedding is common in birds that recover from acute infection. Infection is most commonly due to contact with another infected bird – the virus is shed in feather dander and faeces. The virus can remain stable in the environment for long periods.

Marek’s disease
Caused by Gallid herpesvirus-2, Marek’s disease is a highly infectious, wide spread infection in chickens. The viral incubation period is 4-6 weeks and flock mortality is reported to be 10-50%. Infection is most common in young flocks. Clinical signs include hind limb or wing paralysis, dyspnoea, depression, weakness, tumors on feather follicles, blindness and a withered comb. Most clinical signs are associated with the development of T cell lymphoma and the infiltration of lymphocytes into tissues and nerves. Infection with Marek’s disease also causes immunosuppression and increases susceptibility to secondary infections. The virus is shed in the feather dander and droppings or any secretions. The virus can persist for long periods in poultry yards.

AVIAN TOXICOLOGY
Heavy metal intoxication is not uncommon in caged and wild birds. Caging material and paint can be source of lead or zinc if chewed or ingested.

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<th>Metal</th>
<th>Live bird sample</th>
<th>Dead bird sample</th>
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<tr>
<td>Lead</td>
<td>Minimum of 200 μL blood in a lithium heparin tube or EDTA.</td>
<td>Fresh liver and kidney (as much as available)</td>
</tr>
<tr>
<td>Zinc</td>
<td>Minimum 200 μL blood in lithium heparin or plain tube – do not use EDTA as it chelates the zinc</td>
<td>Fresh liver, kidney or pancreas (minimum 100g of tissue)</td>
</tr>
</tbody>
</table>

AVIAN VIRUS ISOLATION
Species: Avian
Specimen: Swabs, fresh tissue, serum or blood
Container: Red top tube for sera, EDTA tube for blood, virus transport media for swabs (contact the laboratory to source these from the referral laboratory), tissues in sterile pottles

Collection protocol: Venepuncture, post mortem, swab of lesions
Special handling/shipping requirements: Ship all samples chilled
General information about the disease: This will vary depending on the disease

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sample</th>
<th>Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Caecal tonsil</td>
<td>Sterile pottle</td>
</tr>
<tr>
<td>Avian encephalomyelitis (AE)</td>
<td>Serum, brain</td>
<td></td>
</tr>
<tr>
<td>Egg drop 76</td>
<td>Serum</td>
<td>Plain tube</td>
</tr>
<tr>
<td>Fowl pox</td>
<td>Lesion, serum</td>
<td>Sterile pottle, plain tube</td>
</tr>
<tr>
<td>Disease</td>
<td>Sample Type</td>
<td>Media Type</td>
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<tr>
<td>---------------------------------------------</td>
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</tr>
<tr>
<td>Infectious bronchitis (IB)</td>
<td>Trachea, caecal tonsil, kidney, serum</td>
<td>Sterile pottle, plain tube</td>
</tr>
<tr>
<td>Infectious bursal disease (IBD)</td>
<td>Brain, bursa, serum</td>
<td>Sterile pottle, plain tube</td>
</tr>
<tr>
<td>Infectious laryngotracheitis (ILT)</td>
<td>Trachea, larynx, serum</td>
<td>Sterile pottle, plain tube</td>
</tr>
<tr>
<td>Infectious anaemia</td>
<td>Liver</td>
<td>Sterile pottle</td>
</tr>
<tr>
<td>Tenosynovitis avian reovirus</td>
<td>Tendons, caecal tonsil, serum</td>
<td>Sterile pottle, plain tube</td>
</tr>
<tr>
<td>Viral arthritis</td>
<td>Synovial fluid, hock joint</td>
<td>Sterile pottle, plain tube</td>
</tr>
</tbody>
</table>

**General information about when this test is indicated:**
This test is undertaken by specialist referral government laboratories and would require pre-arranging transport media and notification of the laboratory to expect it.

**Comparison with other related tests:**
Check if an ELISA test is available to test for the virus either as an antigen or antibody.

**References**

Biochemistry

ALKALINE PHOSPHATASE (ALP)
Species: Dog, Cat, Avian. Not widely used in large animals
Specimen: Plasma or Serum
Container: Heparin or red top tube
Collection protocol: Fasted sample preferred.
Special handling/shipping requirements: None.
General information about the disease:
Intrahepatic and extrahepatic cholestasis, endocrine and neoplastic disorders, and others.
General information about when this test is indicated:
An indicator of osteoblastic activity in all species and cholestasis in most species. (In birds, it is primarily associated with osteoblastic activity, not useful for hepatobiliary disease). Several isoenzymes, but some have short half-lives and contribute little to serum ALP. Placental isoenzyme can be detected in late pregnancy in cats. Bone isoenzyme is associated with increased serum ALP in juvenile animals, bone remodelling and (probably) feline hyperthyroidism. Liver isoenzyme has a short half-life in cats (6 hours), compare with dogs (70 hours), so magnitude of increase in feline disease is lower. In dogs exogenous or endogenous steroidogenic hormones stimulate both liver and glucocorticoid isoenzymes and the largest increases in serum ALP are seen in cholestatic disorders and glucocorticoid excess, as well as some tumours. Phenobarbital induces ALP activity in the dog. In cats phenobarbitone and glucocorticoids have little effect, but hepatic lipidosis can be associated with marked increases. Major differentials: Endocrine, cholestasis, neoplasia, breed related, drug effect.
Comparison with other related tests:
Parallels, but more sensitive than GGT in canine hepatobiliary disease.

ALANINE AMINOTRANSFERASE (ALT)
Species: Dog, Cat
Specimen: Plasma or Serum
Container: EDTA, heparin or red top tube
Collection protocol: Fasted sample preferred.
Special handling/shipping requirements: None.
General information about the disease: Liver (hepatocyte) injury
General information about when this test is indicated:
An indicator of damage to liver cells. Also found in kidney and in cardiac and skeletal muscle. Present in hepatocyte cytosol with higher concentrations in periportal cells. Magnitude in serum correlates to number of cells affected but cannot be used to assess prognosis. Some animals with severe liver disease may have normal serum ALT due to lack of viable hepatocytes. Half-life in dogs controversial but levels usually rise rapidly within 24-48 hours and resolve over 2-3 weeks if no further injury, but pattern is highly variable.
Increases seen with Phenobarbital treatment. Major differentials: Circulatory disturbances (including anaemia), hepatotoxicity, infection, inflammation, endocrine associated hepatopathy, neoplasia, drug effect.

**Comparison with other related tests:** More liver specific than AST

## ASPARTATE AMINO TRANSFERASE (AST)

**Species:** All  
**Specimen:** Plasma or serum  
**Container:** EDTA, heparin or red top tube  
**Collection protocol:** Fasted sample preferred.  
**Special handling/shipping requirements:** None.

**General information about the disease:** Liver (hepatocyte) or muscle injury

**General information about when this test is indicated:**  
An indicator of damage to muscle (skeletal or cardiac) or liver cells. Also found in kidney. Present in cytosol and mitochondria. Increased significantly by muscle activity (e.g. seizure, or even exercise), in which case CK is also usually raised unless the injury is already resolving. In hepatocytes, found in higher concentrations in the periacinar zone. In dogs with hepatocellular injury increases tend to parallel ALT. If ALT (or GD in large animals) not raised, look for extrahepatic source. Short half-life in cats, so even small increases may be significant. Major differentials: Muscle: trauma or necrosis. Liver: circulatory disturbances (including anaemia), hepatotoxicity, infection, inflammation, neoplasia

**Comparison with other related tests:** See above

## BILE ACIDS (BA)

**Species:** Dog, Cat, Horse, Birds  
**Specimen:** Serum, heparinised plasma  
**Container:** red top tube, gel tube, heparin  
**Collection protocol:**  
- Do not perform this test in an animal with increased bilirubin or in dogs with bilirubinuria – BA will be increased in these animals  
- Sample 1 or single pre-prandial should be a fasted sample, avoid haemolysis  
- Sample 2 obtain 2h after feeding a small amount of food to avoid sample lipaemia, taking care to avoid sample haemolysis  
  
  Note: Lipaemia causes a false increase in BA and haemolysis causes a false decrease in BA

**Special handling/shipping requirements:** Separating serum from the clot is ideal, but not always possible

**General information about the disease:** None

**General information about when this test is indicated:**  
- Used to assess liver function in cases where liver disease or failure, or portosystemic shunt are suspected
Most animals with either congenital or acquired portosystemic shunt will have marked increases in post-prandial bile acids.

Pre-prandial values may be higher than post-prandial values in healthy animals, because of a recent meal, catching sight or scent of food, or delayed gastric emptying.

Horses do not have a post-prandial increase in bile acids, but bile acids may be mildly increased in anorexic horses.

In parrots with BA repeatedly in excess of 200 umol/L, liver biopsy is warranted.

**Comparison with other related tests:** No

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**CHOLESTEROL**

**Species:** All, including birds

**Specimen:** Plasma or Serum

**Container:** EDTA, heparin or red top tube

**Collection protocol:** Fasted sample preferred.

**Special handling/shipping requirements:** None.

**General information about the disease:**
Used mainly to assist with assessment of liver function and some endocrinopathies.

**General information about when this test is indicated:**
A sterol lipid found in animal tissue which can be absorbed from diet or made in liver (and other tissues). In fasting samples most will have been made by hepatocytes. Hypercholesterolaemia can result from increased production, reduced lipolysis/processing of lipoproteins, and some other processes. Hypocholesterolaemia can result from reduced production and some other processes. Liver diseases may result in increases or decreases in cholesterol, depending on the pathogenesis.

**Major differentials:**
Increase - Postprandial, nephrotic syndrome, hypothyroidism, diabetes mellitus, excess glucocorticoids/hyperadrenocorticism, pancreatitis, idiopathic hyperlipidaemia (breed related), and cholestasis.

Decrease - Hepatic dysfunction/shunt, low fat diet, protein losing enteropathy/Gi disease/malabsorption, hyperthyroidism, hypoadrenocorticism.

**Comparison with other related tests:** N/A

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**CREATININE**

**Species:** All (but not very useful in birds/reptiles)

**Specimen:** Plasma or serum

**Container:** EDTA, heparin or red top tube

**Collection protocol:** Fasted sample preferred.

**Special handling/shipping requirements:** None.
General information about the disease:
Used as a convenient, though poorly precise/sensitive, indicator of renal function/Glomerular filtration rate (GFR)

General information about when this test is indicated:
Creatinine is formed from muscle creatine at a relatively constant daily rate, not as affected by extra-renal factors as urea. Minor dietary and postprandial effects (red meat consumption increases and eating a meal decreases serum urea). Excreted almost exclusively through glomerular filtration in kidneys. Patient may experience significant reduction in functional nephron number/GFR (>75%) before serum creatinine is significantly increased. Reductions in GFR from prerenal, renal, or post-renal causes cannot be distinguished as all may increase serum creatinine. Post-renal causes (e.g. lower urinary tract obstruction) are usually associated with the largest and fastest increases. Decreased results are rarely clinically significant, though animals with lower muscle mass tend to have lower creatinine.

Major differentials: Reduced renal perfusion (dehydration, shock, cardiovascular disease) urinary tract obstruction or rupture, renal disease.

Comparison with other related tests:
Tends to parallel serum urea in renal disease or reduced renal perfusion. Affected less by extra-renal factors. Should be interpreted in conjunction with urinalysis.

FRUCTOSAMINE

Species: Dog, cat
Specimen: Serum
Container: Red top tube

Collection protocol:
• Fasted sample, particularly if other biochemical tests will be run
• Levodopa at therapeutic doses can cause a false increase in fructosamine
• Hyperthyroid cats may have decreased serum fructosamine

Special handling/shipping requirements: No

General information about when this test is indicated:
Fructosamine is used for the diagnosis and monitoring of diabetes mellitus in cats and dogs. It is particularly useful in cats, where stress hyperglycaemia can be marked and may lead to glucosuria and interference with the performance of blood glucose curves during therapy.

Generally fructosamine concentrations provide a reflection of blood glucose concentrations during the previous 1-3 weeks but it is important to note that other conditions may affect this analyte. Examples include hypoalbuminaemia/hypoproteinaemia, hyperlipidemia, azotemia and in cats, hyperthyroidism.

Serum fructosamine may also be useful for demonstrating prolonged hypoglycemia in animals presenting with a suspicion of an insulinoma/beta cell tumour. Further workup to exclude other causes of hypoglycaemia and demonstrate inappropriate insulin secretion however would still be required to confirm a diagnosis of insulinoma. Used to monitor response to insulin therapy

Comparison with other related tests: NA
GAMMA GLUTAMYL TRANSFERASE (GGT)

Species: All
Specimen: Plasma or serum
Container: EDTA, heparin or red top tube
Collection protocol: Fasted sample preferred.
Special handling/shipping requirements: None.

General information about the disease:
Cholestasis/biliary disease (especially intrahepatic), colostrum ingestion.

General information about when this test is indicated:
Membrane bound glycoprotein associated with bile ducts/canaliculi. Serum increases associated with intrahepatic bile duct obstruction and biliary disease (e.g. sporidesmin toxicity in ruminants). Glucocorticoids stimulate production in dogs, similar to ALP, but anticonvulsants such as phenobarbital have only mild effects. Produced abundantly by mammary gland with high levels in colostrum (not in the horse). Neonates of most species (not cats) have high serum levels. This test has been used to assess adequacy of colostral transfer in some species (e.g. in calves it has been suggested that levels should be >600 U/l at 1 day, >400 at 3 days, >130 at 5-10 days and >65 at 10-15 days)

Major differentials: cholestasis, biliary disease, colostrum ingestion, endocrine.

Comparison with other related tests:
More useful than ALP for detecting cholestasis in large animals (horses, ruminants) due to narrower reference intervals.

GLUTAMATE DEHYDROGENASE (GDH, GLDH)

Species: All but most commonly used in large animals (horse, ruminants)
Specimen: Plasma or Serum
Container: EDTA, heparin or red top tube
Collection protocol: Fasted sample preferred (in companion animals).
Special handling/shipping requirements: None.

General information about the disease: Liver (hepatocyte) injury, especially acute.

General information about when this test is indicated:
An indicator of damage to liver cells. Mitochondrial leakage enzyme in cytoplasm. In humans more activity in centrilobular than periportal hepatocytes. More liver specific than AST. Generally, less sensitive than GGT for detecting liver damage in horses but has a better positive predictive value. Magnitude in serum generally correlates to number of cells affected but cannot be used to assess prognosis. Half-life approximately 14 hours in bovine. Increases sometimes with Phenobarbital treatment. Major differentials: Circulatory disturbances (including anaemia), hepatotoxicity, infection, inflammation, endocrine associated hepatopathy, neoplasia, drug effect.

Comparison with other related tests:
More liver specific than AST and generally, less sensitive than GGT for detecting liver damage in horses but has a better positive predictive value.

**UREA**

**Species:** All (but less useful in birds/reptiles)

**Specimen:** Plasma or Serum

**Container:** EDTA, lithium heparin or red top tube

**Collection protocol:** Fasted sample preferred.

**Special handling/shipping requirements:** None.

**General information about the disease:**

Used as a traditional but poorly precise/sensitive indicator of renal function/Glomerular filtration rate (GFR)

**General information about when this test is indicated:**

Most of the urea produced by the body is excreted through the kidneys. Reduced GFR increases serum urea (BUN), but BUN is also affected by many extrarenal factors. Increased dietary protein, upper GI bleeding, and increased protein catabolism can increase BUN, and conversely low protein intake and reduced hepatic function can reduce it. Also affected by tubular flow rate (so hydration status is relevant). Patient may experience significant reduction in functional nephron number/GFR (>75%) before serum urea is significantly increased. Reductions in GFR from prerenal, renal, or postrenal causes cannot be distinguished as all may increase serum urea.

Major differentials: Increase: Reduced renal perfusion (dehydration, shock, cardiovascular disease, hypoadrenocorticism), urinary tract obstruction or rupture, renal disease. Extrarenal (dietary protein, GI haemorrhage, catabolic state)

Decrease: Hepatic dysfunction/shunt, protein malnutrition/malabsorption, fluid therapy, polydipsia.

**Comparison with other related tests:**

Tends to parallel serum creatinine in renal disease or reduced renal perfusion. When only urea is increased look for extrarenal cause. Should be interpreted in conjunction with urinalysis.

**REFERENCE(S):**


Thrall M.A. *Veterinary Hematology and Clinical Chemistry* 2006
Coagulation

COAGULATION SCREEN

Includes prothrombin time (PT), activated partial thromboplastic time (APTT), Thrombin time (TT) and platelet count.

Species: All species

Specimen: Whole blood

Container: Citrate (blue top)

Collection protocol:

Collect blood with a minimum of trauma so coagulation cascade is not triggered, preferably from the jugular so a good flow is ensured. Fill the tube to the level indicated on the blood tube.

Special handling/shipping requirements:

Samples should reach the laboratory within 18 hours of collection, but the sooner the better. They should be kept at or below room temperature.

General information about the disease:

A coagulation screen will help to differentiate Vitamin K antagonism, inherited coagulopathies, DIC and other rare causes such as severe hepatic disease where there is decreased clotting factor formation.

General information about when this test is indicated:

Where there is unexplained haemorrhage, access to anticoagulant compounds (eg Vit K antagonists), and suspected inherited coagulopathies. Also include an EDTA blood sample for a routine CBC and blood smears as described above.

FACTOR VIII (HAEMOPHILIA A)

Species: Dogs

Specimen: Whole blood

Container: Citrate (blue top)

Collection protocol:

Collect blood with a minimum of trauma so coagulation cascade is not triggered, preferably from the jugular so a good flow is ensured. Fill the tube to the level indicated on the blood tube.

Special handling/shipping requirements:

Deliver to laboratory as soon as possible because it has to be spun and frozen as soon as possible.

General information about the disease:

Suspected in young dogs with history of haemorrhage and in which other causes have been ruled out.

General information about when this test is indicated:

Where there is unexplained haemorrhage in young dogs and where other causes have been ruled out.
VITAMIN K ANTAGONISM (WARFARIN, BRODIFACOUM TOXICITY)

Species: Dogs and cats
Specimen: Whole blood
Container: Citrate (blue top)

Collection protocol:
Collect blood with a minimum of trauma so coagulation cascade is not triggered, preferably from the jugular so a good flow is ensured. Fill the tube to the level indicated on the blood tube.

Special handling/shipping requirements: Keep cool and send to laboratory as soon as possible

General information about the disease:
A full coagulation screen will help to rule out some causes of haemorrhage. PT is increased first but both PT and APTT may be increased TT is normal. Platelets may be mildly to moderately decreased as a result of consumption/haemorrhage.

It may take up to 48-72 hours for clotting times to be increased.

Measure PT 2-3 days after stopping Vit K therapy to ensure it is safe to stop treatment. If anticoagulant is still within the body times will still be increased.

Warfarin may cause haemorrhage for up to one week.

Brodifacoums may cause haemorrhage for at least 2 weeks.

General information about when this test is indicated:
Where there is suspected ingestion of rat baits containing these compounds. Where there is unexplained haemorrhage.

Comparison with other related tests:
Liver, urine and EDTA blood may also be screened at a referral laboratory for the presence of the toxic compounds.

VON WILLEBRANDS DISEASE

Species: Dogs
Specimen: Whole blood
Container: Citrate (blue top)

Collection protocol:
Collect blood with a minimum of trauma so coagulation cascade is not triggered, preferably from the jugular so a good flow is ensured. Fill the tube to the level indicated on the blood tube.

Special handling/shipping requirements:
Deliver to laboratory as soon as possible because it has to be spun and frozen as soon as possible.

General information about the disease:
This is the most common inherited disorder of haemostasis. It is a defect of primary haemostasis and has a high prevalence in some breeds but may be found in other breeds. Von Willebrands factor is produced in endothelial cells and is used in the formation of the platelet plus and aids in the incorporation of fibrin into the platelet plug. There are 3 types namely types I, II and III with type I being the most common.

**General information about when this test is indicated:**
Where there is unexplained haemorrhage in young dogs and where other causes have been ruled out.

**Comparison with other related tests:**
Genetic tests for carriers of VWD are available in the following breeds:

Type I – Australian Terrier, Bernese Mountain Dog, Doberman, German Pinscher, Kerry blue Terriers, Labradoodles, Manchester Terriers, Papillon, Standard Poodles, Welsh Corgis, White Swiss Shepherd breeds.

Type II - German Shorthaired Pointers, German Wirehead Pointers

Type III – Scottish Terriers, Shetland Sheepdogs

Genetic diagnosis involves a swab of epithelial cells from the inside of the mouth/cheek, see section on genetics.
CYTOLOGY

GENERAL GUIDELINES

Cytology is the examination of cells collected from body fluids or fine needle aspiration (FNA) or impression smears from solid tissue lesions. It is a relatively non-invasive technique with the added advantage of requiring no or minimal sedation in the majority of patients. The turnaround time is more rapid than that for histopathology, with results generally available within one working day. With good sample quality (i.e., adequate numbers of intact cells that are well spread out) clinical description and history, cytology can provide a definitive diagnosis or give useful information with regard to further testing modalities, or treatment options.

Sites and tissues well suited to cytological examination include:

- Cutaneous and subcutaneous masses - Masses of spindle cell origin (i.e., sarcomas) are generally less exfoliative than round cell or epithelial masses and architecture is required to differentiate subtypes
- Nasal exudates and masses
- Bronchial and tracheal washes
- External ear canal
- Lymph nodes
- Synovial fluid
- Pleural and peritoneal fluids
- CSF taps - Being mindful that if the lesion is caudal to the cisterna magna, then a lumbosacral puncture is recommended. Cells are very fragile, and special handling is recommended (see below)
- Prostatic aspirates/washes
- Vaginal smears
- Blood smears
- Bone marrow
- Liver - Diffuse processes (e.g., infiltrative tumours, fatty changes) are better than solitary masses (these may even be difficult to diagnose on histology)
- Intra-abdominal / thoracic masses - Ultrasound guided generally gives better result
- Urine / masses in the urinary bladder
- Spleen - Non-aspiration technique may be best

Sites less well suited to cytological examination, requiring greater attention to sample collection technique, or absolutely requiring biopsy for diagnosis of malignant potential:

- Kidney - Blood filled organ, unless a diffuse process is present (e.g., lymphoma), these samples are often non-diagnostic
- Mammary gland – specifically in canine, histology is preferable. Architecture is required for diagnosis of malignancy in dogs (because invasion cannot be assessed by cytology), but in cats cytology can be diagnostic because most feline mammary neoplasms are malignant
- Lung - Often unrewarding because it’s a blood filled organ, and it is difficult to tell one disease process from another. For example, cells from lung lobe torsion can look very similar to those from a carcinoma on
FNA cytology

- Skin tumours on nose / ear tips e.g., squamous cell carcinoma - Deep scrapings are required
- Oral masses - Often demonstrate surface inflammation / infection and changes in epithelial cells that reflect inflammation – if incisional biopsy is not possible, FNA or scraping must be deep
- Conjunctival scrapes - If not deep enough or not enough material on slide, these samples can be non-diagnostic. Using a moistened cotton bud on the lesion and gently rolled onto the slide can work very well
- Rectal mucosal scrapes
- Firm fibrous masses - Architecture is often an absolute requirement for determining whether these are scar tissue, the edge of a cyst, granulation tissue, or a neoplasm. If the latter, which specific subtype of fibrous neoplasm. Therefore, incisional / excisional biopsy will often be required. If that is not possible, then multiple needle passes may assist cell exfoliation.
- Cystic/Fluid filled skin lesions - May be cell-poor and therefore not have enough cells to permit a diagnosis. Aiming for the wall of the cyst in addition to the centre may help. However, incisional or excisional biopsy is generally required because architecture is the key to understanding many of these cystic processes.

Things to avoid:

- Exposure of cytological material to formalin – including close exposure in the operating theatre, or being packaged in the same bag as the formalin-filled pottle. Formalin prevents proper staining and often alters cells beyond recognition.
- Ensure that cytological preps are not packed with formalinised tissue for transportation to the lab, as formalin fumes also affect smears. Wrap cytology smears separately from histology containers. Preferably place them in different plastic bags.
- Sufficiently dried smears should be placed in clean, dry slide holders - **Do not use flame or heat fixation** – rapid air drying (ideally with a fan, or vigorous arm flapping) is sufficient
- **Do not refrigerate smears at any time** - The cells will lyse when condensation forms on the slide after removal from the fridge
- Fluids however, should be refrigerated (never frozen) if there is delay in despatch. Make a smear of fluids before refrigeration if there is any delay in despatch
- Urine should be refrigerated (never frozen) if there is a delay in despatch
- CSF – should be processed within 4h of collection – if this is not possible, then adding autologous serum will help preserve cellular integrity (EDTA alone does not do this) e.g., if there is ~90 uL of CSF, then add 10 uL of serum (2-3 drops; see below)

**CYTOLOGY – SAMPLES AND COLLECTION**

**Fine Needle Aspirates (FNA)**

This technique is non-invasive, generally non-painful, quick, and simple. In contrast to histopathology, results are usually available within one working day. FNA is suitable for any surface mass, lymph node or mass in a body cavity that can be palpated and immobilised (as noted above).

**Equipment Required**

- 5 mL or 10-12 mL syringes, 22-25 gauge 0.5-1.5 inch needles. Avoid larger needles as they result in greater blood contamination and “core biopsies” (too thick for cytological examination)
Clean glass slides and slide holders for transporting slides

EDTA (lavender top) and plain (serum – red/lemon top) tubes if fluid is obtained. If there is sufficient fluid, it is always useful to collect samples into both types of tubes. The EDTA sample for cytological examination and the serum/plain tube for culture or other specialized tests (if indicated).

**Collection Technique**

- Clipping / shaving of the area is usually unnecessary, but the site should be swabbed to remove dust and debris
- Localise the mass firmly with one hand and insert the needle attached to the syringe into the mass. Generally, aspirate from the centre of small masses, but aspirate large masses from periphery, as centre may be cystic or necrotic
- Apply gentle negative pressure (1/2 - 3/4 volume of syringe)
- Without completely exiting the mass, retract and redirect the needle 3 times with each aspiration attempt (gently releasing suction in between aspiration attempts as the needle is retracted and replaced)
- In most cases, fluid will not be visible within the syringe. The cells are in the needle and hub
- Tissues that are more likely to bleed such as the thyroid, spleen, suspected haemangiomas etc. are often best sampled by making multiple needle passes without a syringe attached. Cellular material is drawn into the needle by capillary action and the syringe is only used to expel the material onto a clean slide as noted above.
- When handling material from aspiration of the lymph nodes and bone marrow, apply as little pressure as possible to spread the aspirated material. Cells are fragile and rupture easily.
- If a bone marrow aspirate is planned, please speak with a Clinical Pathologist prior to sampling for additional advice and information
- Stop aspiration if any fluid / blood is noted in the syringe (if aspirating a solid mass)
- Release the vacuum before withdrawing out of the body / mass
- Once out of the mass, detach the needle from the syringe
- Draw air into the syringe and reattach the needle
- Gently expel the contents of the needle close to the frosted end of a clean glass slide. If the material is quite liquid, make a smear the same way as for a blood film. Make several smears if possible.
- If the material is thick, make a squash preparation by gently placing a clean slide on top of the material at right angles to the other slide.
  - Use just enough pressure to smear the material out. Too much pressure will cause cellular disruption
  - Pull the second slide along *gently*, keeping the right angle position and then *gently* pull the two slides apart, without lifting the slides directly apart. *Be gentle.*
- Dry material rapidly with a fan, hair dryer on ‘cool’ setting, or by flapping slides vigorously
- When submitting to the laboratory, it is not necessary to fix the smears. It is always a good idea to stain at least one of the smears, and examine to see if there is sufficient intact material on the preparation. Please also submit this smear.
- Prepare a few smears (e.g., 3-4) rather than just one – this allows for examination of more material, and enhances the diagnostic success rate.
o Label smears (with pencil) on frosted end of the slide, on the correct side of the smear, to prevent it from being wiped off in error. (Permanent markers come off in the alcohol used for fixing).

o Send the slides to the lab in slide containers, which are then packaged in bubble wrap to prevent breakage.

Impression smears

These are made when there is a solid piece of tissue available, e.g., biopsy specimen, solid core from a nasal flush etc.

- Use a saline moistened swab (sterile saline, not water, as water causes osmotic rupture of cells) to remove superficial debris. If the tissue is bloody, blot with clean paper towel to remove blood first
- Gently touch the surface to a clean slide. Do this several times so that there are up to five imprints per slide.
- Dry the slide rapidly by air drying or use a hair dryer on the ‘cool’ setting. This technique can be used for surface lesions but it is not as useful as a FNA for these as the imprint will most often contain just superficial inflammatory cells and the true lesion may not exfoliate.
- NB: if a culture is desired transport medium can be used to protect swab contents, but once the swab has contacted transport medium, that swab cannot be used to make cytology slides.

Scrapings

Some tissues or masses do not exfoliate well on impression smears made from incisional biopsies due to their firm consistency e.g., spindle cell tumours, squamous cell carcinomas, and bone lesions. In cases where impression smears do not result in material on a slide, the scraping technique can provide a diagnostic sample. The cut surface of the tissue is gently scraped with a scalpel blade and the accumulated material is transferred onto a slide and then spread as a squash preparation (see Fine Needle Aspiration section). Dry as above.

Swab Smears

Used for vaginal smears, fistulous tracts, ears, etc. Moisten swab with sterile saline (not water). Transfer cells by gently rolling swab along surface of slide.

NB: if a culture is desired transport medium can be used to protect swab contents, but once the swab has contacted transport medium, that swab cannot be used to make cytology slides.

Body Fluids

Cytological examination of body fluids can provide useful information if an organ within the body cavity is diseased. It also helps determine the cause for any increased volume of cavity fluid e.g., ascites.

The body fluids most commonly evaluated are pleural, peritoneal, cerebrospinal, and synovial. Collection techniques are described in clinical pathology or some internal medicine textbooks.

For all fluids, it is important to:
1. Collect most of the fluid into EDTA tubes (purple top) for cytology, as this prevents clotting and preserves cells to some degree.
2. Aseptically place a few drops into a serum tube (plain tube / red/lemon top) for culture (EDTA inhibits microbial growth). Or use blood culture bottle.
3. Always make a few smears from the fluid immediately after collection. Cells are never as well preserved as when they are first collected.

CSF Collection

For collection techniques, please refer to any recently published anaesthesia or internal medicine textbook.

In small animals, this is generally collected from the cisterna magna, and from the lumbar area in large animals. If a lesion is suspected between the cisterna magna and the lumbar area, then a lumbar puncture is recommended, even in small animals (recognising that this can be difficult to achieve).

CSF Sample Handling

CSF should drip gently from the needle to be collected in a sterile red top vacutainer. If a trail of blood appears, discard the first few drops of CSF to collect as clear a sample as possible. If there is sufficient CSF (>0.75 mL), the sample should be aliquoted into 2-3 red top tubes, one of which will be used for microbiological examination. Alternately, one aliquot can be transferred to a blood culture bottle, which is available from Gribbles.

If the sample cannot be delivered to the Gribbles laboratory within 30 minutes to 1 hour, then autologous serum should be added to one of the tubes to preserve cells because they degenerate rapidly due to the low protein content of CSF. EDTA alone is not an adequate preservative.

Procedure for adding protein to CSF (example calculation):

1. The solution should be in a 9 parts CSF to 1 part serum ratio. For example, if you have 1 mL (or 1000 µL) CSF:

   Calculation: \[
   \frac{90 \times 1000 \mu L}{100} = \text{Amount of CSF}
   \]

   Calculation: \[
   \frac{10 \times 1000 \mu L}{100} = \text{Amount of serum (adjust according volume you have)}
   \]

2. Mix by very gently inverting 5 to 10 times.

Store all CSF tubes at 4°C and ship (protected by paper towel) on ice. If there will be a very prolonged delay in shipment, make a smear as well. Air dry smears very rapidly using a small fan, hair dryer on “cool” setting, or by flapping vigorously.

Pleural, Peritoneal, Pericardial Fluid Sample Handling

Generally, collecting samples into both an EDTA and a red top (or lemon top) tube is sufficient. Making 1-2 direct preparations of fluid (as you would make a blood film) and air drying rapidly will help the Clinical Pathologist determine things like sampling-associated haemorrhage versus pre-existing haemothorax or haemoadenotum.
Synovial Fluid Collection

Cytological and microbiological examination of synovial fluid is extremely helpful in differentiating septic, immune-mediated, and degenerative joint diseases. As well as in cases of lameness, collecting joint fluid is also useful in cases of pyrexia of unknown origin and in suspected immune-mediated diseases, even in the absence of lameness.

Strict aseptic technique is needed and the necessity for sedation or anaesthesia is dependent on the animal and the joint involved.

The carpi and hock joints are commonly affected in immune-mediated diseases.

When collecting synovial fluid, the order of priorities for handling the specimen is:

1. Make 1-2 smears (as you would a blood smear) and **air dry rapidly**
2. With remaining fluid, place 3-4 drops into blood culture medium or red top (or lemon top) tube for possible future microbiological culture. There may be a 50% decrease in the culture rate if the sample is received in a red top container compared with blood culture bottles.
   - Blood culture bottles can be ordered from Gribbles
3. If any fluid still remains, place it into EDTA for cell counts and assessment by the Clinical Pathologist

Nasal Cavity Sampling

It may be difficult to get a diagnostic sample from a suspect lesion in the nasal cavity. Nasal swabs often do not yield satisfactory material as they cannot be inserted far enough or are not abrasive enough to yield representative samples of deep mucosal lesions. A diagnosis may occasionally be obtained if a causative agent such as Cryptococcus is present.

Flush and direct aspiration can be used to obtain diagnostic material from deeper lesions.

NB: if a culture is desired transport medium can be used to protect swab contents, but once the swab has contacted transport medium, that swab **cannot be used to make cytology slides**.

Bone Marrow Examination

Examination of bone marrow is an adjunct to haematology and is indicated in a number of different circumstances. These include:

- Pancytopenia (anaemia + neutropaenia + thrombocytopenia)
- Persistent, unexplained, non-regenerative anaemia
- Persistent, unexplained neutropaenia with no left shift
- Persistent, unexplained thrombocytopenia (rule out DIC first, can perform even with platelets as low as 10 x10^9/L, tends not to cause problems so long as secondary haemostasis is adequate)
- Leukocytosis, especially if there are atypical cells in the blood
- Atypical cells or immature cells
- Hyperproteinaemia due to a monoclonal gammopathy (or polyclonal if ehrlichiosis is a possibility due to travel history of the animal, but assess lymph nodes first)
- Hypercalcaemia with no other identifiable cause
- Pyrexia of unknown origin
- Clinical staging of neoplasia
- Monitoring effects of chemotherapy
- Evaluation of iron stores (not in horses, cats, neonatal animals)

**Bone Marrow Aspiration – Dogs, Cats, Horses, Cattle**

As with all of these techniques, a good, current internal medicine textbook will have all the information you require for obtaining bone marrow cores and aspirates, but following are a few extra points and tips.

A concurrent CBC is required for a proper assessment of bone marrow. Bone marrow core biopsies and cytology very much complement one another. Individual cell morphology is largely a mystery on histology, so that is why cytology is required.

Consider adding sodium bicarbonate to the lidocaine (immediately prior to injection) to minimize pain (e.g., 0.9 mL lidocaine and 0.1 mL sodium bicarbonate. Do not increase sodium bicarbonate beyond 10% as this will cause precipitation of lidocaine, mix immediately prior to use).

“Rinsing” the syringe(s) to be used for aspiration with sterile EDTA can help prevent clotting (take up some sterile EDTA and expel it, enough will remain in the syringe).

Syringe contents can be expelled onto a petri dish held at an angle to drain off excess blood. Individual particles can be suctioned up using a pipette, transferred to a glass slide, from which pull apart smears can be made. Otherwise, excess blood can be allowed to drain down a glass slide and then a squash preparation can be made. Finally, a regular “blood film making” technique can be used to make both feather edge and “stop edge” preparations. The latter involves stopping the pushing slide so a line of material results. This concentrates cells of interest (e.g., if lymphoma is suspected) and minimises breakage that can occur with the feather-edge preparation technique.

Dry slides rapidly with a small fan, a hairdryer set on “cool”, or by flapping vigorously.

It is good to stain one in house and to look at it to see if it has cells. If not, consider re-sampling while the patient is still in clinic.

Please remember to package the bone marrow core in formalin separately from cytology slides – formalin alters the cells and sometimes ruins the sample completely.

**BONE MARROW ASPIRATES**

**Species:** Any

**Specimen:** Bone marrow

**Container:** EDTA bone marrow, direct smears of bone marrow, EDTA blood

**Collection protocol:**
Collect using standard techniques with appropriate sedation/anaesthesia/analgesia. The iliac crest and humeral head are preferred sites for dogs; iliac crest and proximal femur for large cats; proximal femur for smaller cats and neonates; and a sternal vertebra in horses and ruminants. It is always recommended to obtain a needle aspiration and core biopsy when sampling. Make a few direct smears of the aspirated material and place the remainder in an EDTA tube. Bone marrow aspirates/biopsies must be interpreted along with a concurrent CBC so make sure to send in EDTA blood as well. The CBC is included in the bone marrow cytology fee. Also, a thorough clinical history is essential.

**Special handling/shipping requirements:**
Do not ship with formalin (or wrap and bag separately) as the formalin fumes prevent cellular uptake of differential stains rendering the slides non-diagnostic.

**General information about the disease:** See general cytology section

**General information about when this test is indicated:**

Required to diagnose causes of unexplained anaemias, cytopenias, presence of abnormal cells within the peripheral blood e.g. leukemias, and unusual conditions affecting platelets.

When a bone marrow aspirate is sent to a laboratory send an EDTA blood sample for a concurrent CBC even if there are prior haematology results. This is because the best interpretation possible can be made only when both are carried at the same time. The blood picture is very dynamic and may vary from day to day.

**Comparison with other related tests:**

When sampling bone marrow, it is recommended to obtain both an aspirate as well as a biopsy for histopathology.

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**FLUID-FILLED MASS ASPIRATES**

**Species:** Any

**Specimen:** Aspirated material, fluid in ETDA (for cytology) and red or lemon top tube/pottle (in case culture is required)

**Container:** Glass slides, slide container, EDTA (for cytology only as EDTA is bacteriostatic), red or lemon top tube/pottle (culture)

**Collection protocol:**

See “Cytology – solid mass aspirates”. Try to aspirate both solid areas and fluid-filled areas of the mass. With any fluid, always send a direct smear with the remainder of the fluid in EDTA (for cytology) and red or lemon top tube/pottle (for culture, if required)

**Special handling/shipping requirements:**

Do not ship with formalin (or wrap and bag separately) as the formalin fumes prevent cellular uptake of differential stains rendering the slides non-diagnostic.

**General information about the disease:** N/A

**General information about when this test is indicated:** N/A

**Comparison with other related tests:** N/A

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**FLUIDS**

**Species:** Any

**Specimen:** Effusions, joint fluid, washes, CSF

**Container:** EDTA (for cytology only as is bacteriostatic), red or lemon top tube/pottle for culture, direct smears

**Collection protocol:**

Collect fluid using sterile techniques with appropriate sedation/pain management. It is always advisable to make direct smears of the fluid at the time of sampling and to dry them rapidly with a fan or flapping motion.
The remainder of the fluid should be submitted in an EDTA tube (cytology) as well as a red or lemon top tube or pottle (should culture be required). EDTA assists in preservation of cells for cytology but is bacteriostatic so not ideal for culture. CSF samples must be sent to the lab as soon as possible, preferably protected and on ice.

**Special handling/shipping requirements:**
Do not ship with formalin (or wrap and bag separately) as the formalin fumes prevent cellular uptake of differential stains rendering the slides non-diagnostic.

**General information about the disease:** N/A

**General information about when this test is indicated:** N/A

**Comparison with other related tests:** N/A

Refer to samples types and collection for more information.

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**LYMPH NODE ASPIRATES**

**Species:** Any

**Specimen:** Aspirated material

**Container:** Glass slides, slide container

**Collection protocol:**
21-22 gauge needle alone (“woodpecker” technique). A smaller gauge needle may lyse the cells (neoplastic lymphocytes are quite fragile) and a large needle may result in too much blood contamination. Expel the aspirated contents onto the top 1/3 of a clean glass slide. Then take another slide, gently place it on top. Do not apply pressure, but use only the weight of the slide to spread out the material. Gently pull the slides along each other lengthwise until they separate. This will result in two slides with a nice monolayer of cells. Aspirate multiple lymph nodes. Submandibular lymph nodes tend to be more reactive (as they are draining the oral cavity) so it is not recommended to aspirate only these nodes.

**Special handling/shipping requirements:**
Do not ship with formalin (or wrap and bag separately) as the formalin fumes prevent cellular uptake of differential stains rendering the slides non-diagnostic.

**General information about the disease:** N/A

**General information about when this test is indicated:** N/A

**Comparison with other related tests:**
Immunocytochemistry can be performed on submitted cytology slides if they are of good quality (monolayer, not too many lysed cells, etc.).

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**NASAL EXUDATES AND MASSES**

**Species:** Any

**Specimen:** Nasal discharge, aspirated material

**Container:** Glass slides, slide container, swab for culture

**Collection protocol:**
Place the nasal discharge on a glass slide. Then take another slide, gently place it on top. Applying a small amount of downward pressure to spread out the mucoid material, pull the slides along each other lengthwise until they separate. This will result in two slides with a nice monolayer of cells. If there is a nasal mass, fine needle aspiration is recommended (see “solid mass aspirates”).

**Special handling/shipping requirements:**
Do not ship with formalin (or wrap and bag separately) as the formalin fumes prevent cellular uptake of differential stains rendering the slides non-diagnostic.

**General information about the disease:** N/A

**General information about when this test is indicated:**
Cytology from nasal exudates often reveals septic purulent inflammation which is consistent with rhinitis but could be secondary to an underlying disease (fungal infection, foreign body, viral infection, tumour, etc.). Deep swabs from the nasal cavity, especially if any white plaques are observed, can reveal fungal hyphae (e.g., *Aspergillus*).

**Comparison with other related tests:**
Chronic nasal discharge/epistaxis cases can be a bit of a diagnostic challenge. A definitive diagnosis may require a combination of imaging, cytology, rhinoscopy, histopathology, and culture.

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**SOLID MASS ASPIRATES**

**Species:** Any

**Specimen:** Aspirated material

**Container:** Glass slides, slide container

**Collection protocol:**
21-22 gauge needle alone (“woodpecker” technique) or with a 3-6cc syringe attached for aspiration. A smaller gauge needle may lyse the cells and a large needle may result in too much blood contamination. Round cell tumours and epithelial tumours typically exfoliate readily whereas mesenchymal tumours (e.g., sarcoma) do not. If the mass feels firm and deeply adhered to underlying tissue, then use a syringe for aspiration. Expel the aspirated contents onto the top 1/3 of a clean glass slide. Then take another slide, gently place it on top. Do not apply pressure, but use only the weight of the slide to spread out the material. Gently pull the slides along each other lengthwise until they separate. This will result in two slides with a nice monolayer of cells.

**Special handling/shipping requirements:**
Do not ship with formalin (or wrap and bag separately) as the formalin fumes prevent cellular uptake of differential stains rendering the slides non-diagnostic.

**General information about the disease:** N/A

**General information about when this test is indicated:** N/A

**Comparison with other related tests:** N/A
IMMUNOCYTOCHEMISTRY (CD3)

Species: Dog

Specimen: Smears (unstained or stained with Wright’s stain – Diff Quik renders smears unsuitable). Submit multiple (e.g. 3-5) smears so that the best can be selected for testing, since smear thickness, cellularity and cell preservation are important in their final interpretation.

Container: Slide holder.

Collection protocol: As per normal cytology sample collection methods.

Special handling/shipping requirements: As apply generally to shipping of cytology smears.

General information about the disease: Not applicable.

General information about when this test is indicated:

CD3 is part of the T-cell receptor, the critical part of T-cells responsible for recognising antigens presented by other cells. Therefore, this antibody recognises lymphocytes of T-cell lineage (including CD4+ and CD8+ cells).

This antibody is used to confirm and characterise diagnoses of T-cell lymphoma or leukaemia, and to help rule out lymphoma or leukaemia in cases of poorly differentiated “round cell” tumours. It is typically used in conjunction with a B-cell antibody (CD20).

This is helpful because the prognosis and treatment for different forms of lymphoma varies according to their classification. There are low-grade T- and B-cell lymphomas that may have a fair to good prognosis, medium-grade B-cell lymphomas that are often chemoresponsive, and high-grade T-cell lymphomas that may be poorly chemoresponsive and may have a poor prognosis.

Comparison with other related tests:

IMMUNOCYTOCHEMISTRY (CD20)

Species: Dog

Specimen: Smears (unstained or stained with Wright’s stain – Diff Quik renders smears unsuitable). Submit multiple (e.g. 3-5) smears so that the best can be selected for testing, since smear thickness, cellularity and cell preservation are important in their final interpretation.

Container: Slide holder.

Collection protocol: As per normal cytology sample collection methods.

Special handling/shipping requirements: As apply generally to shipping of cytology smears.

General information about the disease: Not applicable.

General information about when this test is indicated:
CD20 is a surface molecule found on B-lymphocytes, which plays a role in their differentiation into plasma cells.

This antibody is used to confirm and characterise diagnoses of B-cell lymphoma or leukaemia, and to help rule out lymphoma in cases of poorly differentiated “round cell” tumours. It is typically used in conjunction with the T-cell antibody CD3.

This is helpful because the prognosis and treatment for different forms of lymphoma varies according to their classification. There are low-grade T- and B-cell lymphomas that may have a fair to good prognosis, medium-grade B-cell lymphomas that are often chemoresponsive, and high-grade T-cell lymphomas that may be poorly chemoresponsive and may have a poor prognosis.

**Comparison with other related tests:**

Endocrinology – Adrenal testing

DIAGNOSIS AND MONITORING OF HYPERADRENOCORTICISM – GENERAL GUIDELINES

Hyperadrenocorticism may arise either from an adenoma usually involving the pars distalis and very occasionally the pars intermedia of the pituitary gland, or an adenoma or carcinoma of the adrenal gland. Hormones, usually ACTH from the pituitary gland, then cause hyperplasia of the adrenal glands. Functional adrenal tumours secrete excessive amounts of cortisol independent of pituitary control.

The diagnosis of hyperadrenocorticism generally involves three steps.

1. Clinical examination and general blood tests, which are used to rule in/out other possible differentials.
2. Carrying out one or more of the screening tests for hyperadrenocorticism. These are the low dose dexamethasone suppression test, the ACTH stimulation test, or the urinary cortisol:creatinine ratio.
3. Differentiating pituitary dependent hyperadrenocorticism from adrenal tumours with the high dose dexamethasone suppression test or an endogenous ACTH assay. This step is not always needed.

Refer to each individual test listing for test protocols.

Low Dose Dexamethasone Suppression Test - Dogs

The low dose dexamethasone suppression test is considered to be the most reliable screening and diagnostic test for hyperadrenocorticism. In normal dogs and cats dexamethasone suppresses ACTH secretion which reduces cortisol production and release.

It has slightly greater sensitivity than the ACTH stimulation test (i.e. there will be fewer false negatives) but there will be more false positives (it has lower specificity). Combined data from studies shows:

For pituitary dependent hyperadrenocorticism
- Using <40 nmol/L as the cut-off, then 94% of cases failed to suppress
- Using <50% of baseline as the cut-off, then 77% of cases failed to suppress

For adrenal tumours
- 100% of cases failed to suppress regardless of criteria

For dogs with nonadrenal illness
- 55% failed to suppress at 8 hours (NB. These dogs were not suspected of having hyperadrenocorticism)

For healthy dogs
- 100% suppressed at 4 and 8 hours

Test interpretation:

Suppression is defined as a > 50% decrease in cortisol at 8 hours. Pituitary-dependent hyperadrenocorticism (PDH) may show a decrease of cortisol to <40 at 3 hours, and subsequent escape from suppression to >40 at 8 hours. Absence of this pattern does not allow differentiation between PDH and adrenocortical tumors.
In cats the low dose dexamethasone suppression test is better than both the ACTH stimulation test and urinary cortisol to creatinine ratio as a screening test but is not very effective as a discrimination test for diagnosing Cushings disease.

For diagnosis of Cushings disease the 3-4 hour sample is not needed but it may be useful for differentiation of pituitary dependent Cushings from adrenal tumours. The low dose dexamethasone suppression test can differentiate between pituitary dependent hyperadrenocorticism and adrenal tumours in up to 60% of cases.

The low dose dexamethasone suppression test is of no value for diagnosing hypoadrenocorticism, iatrogenic hyperadrenocorticism or for monitoring response to treatment.

False positives (lack of suppression) can occur with:

- Non-adrenal illness such as diabetes mellitus, renal failure, liver disease; clinically ill animals with concurrent disease should be treated before testing for Cushings. The more severe the non-adrenal illness, the greater the likelihood of having exaggerated cortisol results.
- Exogenous glucocorticoid drugs such as prednisolone which will be measured as cortisol by the assay
- Bathing, hospitalisation or other stress-inducing procedure
- Anticonvulsant medication

**ACTH Stimulation Test - Dogs**

This is a useful screening test for hyperadrenocorticism but not quite as sensitive as the low dose dexamethasone screening test (there will be more false negatives with the ACTH stimulation test compared with the low dose dexamethasone suppression test). Combined data from a number of studies shows that:

- 30% of dogs with pituitary tumours give an exaggerated response on the ACTH stimulation test while 30% give a borderline response.
- 60% of dogs with an adrenal tumour give an exaggerated response
- 15% of dogs with nonadrenal illness give an exaggerated response

This test is diagnostic for hypoadrenocorticism and for iatrogenic hyperadrenocorticism and is the test of choice for monitoring effectiveness of treatment of hyperadrenocorticism. The test can be carried out at any time of the day without any special preparation.

**ACTH Stimulation Test - Cats**

Hyperadrenocorticism is a rare disease in cats. It is most commonly seen in insulin resistant diabetics and associated with hyperglycaemia but can be a difficult diagnosis to confirm. Like all endocrine disease, it should be diagnosed first on the basis of clinical signs. Very few cats with Cushings have increased serum AP and ALT concentrations compared to dogs. Urine is not often dilute and the cats are rarely PU/PD.

The peak increase in cortisol after ACTH administration occurs more rapidly in cats than dogs. The sensitivity is low and affected animals often have normal results and it is recommended by some authors that the ACTH stimulation test should not be used to diagnose Cushings in cats.

Test interpretation:

**DOGS**

- Post-ACTH cortisol values >550 nmol/L usually indicate hyperadrenocorticism.
- Post-ACTH cortisol values of 470-550 nmol/L are equivocal.
- Post-ACTH cortisol values <470 nmol/L suggest normal adrenocortical function.
Note that in 15% of pituitary-dependent hyperadrenocorticism (PDH) cases and almost 50% of cases due to adrenocortical neoplasia there is no significant elevation of Post-ACTH cortisol values. Failure of cortisol levels to increase significantly in a dog with typical clinical and laboratory features of Cushing’s syndrome does not eliminate this syndrome from the diagnosis. Cortisol values in dogs with iatrogenic hyperadrenocorticism mimic those with hypoadrenocorticism.

Anticonvulsant therapy (phenobarbitone, primidone, phenytoin) may cause an elevated post-ACTH cortisol value. Non-adrenal illness that causes stress, such as diabetes mellitus and renal failure, may also result in an exaggerated response to ACTH.

**CATS**
- Post-ACTH cortisol values >420 nmol/L indicate hyperadrenocorticism.
- Post-ACTH cortisol values between 360 and 420 nmol/L are equivocal.
- Post-ACTH cortisol values <360 nmol/L suggest normal adrenocortical function.

### Urinary Cortisol:Creatinine Ratio

This is a useful screening test for canine hyperadrenocorticism as a low (normal) result makes it unlikely (approximately 90% sensitivity). It is therefore useful in those cases where hyperadrenocorticism is unlikely but needs to be definitely excluded. The test has low specificity, (there are a lot of false positives) so that further tests are needed to confirm that a high result is due to hyperadrenocorticism. It is thought that the test has similar sensitivity and specificity in cats but there are few published reports.

The urinary cortisol to creatinine ratio may be used as a screening test on urinary samples collected at home by the owners. Patients with suspicious results may then be tested with the low dose dexamethasone test.

- **Ratio** < 10 x 10^6 Cushings ruled out
- **Ratio** 10 – 15 x 10^6 Equivocal result, retest
- **Ratio** > 10 x 10^6 Cushings may be present

### High Dose Dexamethasone Suppression Test - Dogs

This test is used to help determine if the hyperadrenocorticism is pituitary dependent or due to an adrenal tumour. It should be used only after a diagnosis of hyperadrenocorticism has been made, using either the low dose dexamethasone test or ACTH stimulation tests. The high dose dexamethasone suppression test has no value in cases of hypoadrenocorticism or iatrogenic hyperadrenocorticism. This test is gradually being phased out by clinicians in favour of the more specific endogenous ACTH assay.

**Test interpretation:**

Suppression is defined as a concentration of cortisol at 4 or 8 hours that is < 50% of the baseline cortisol concentration, or a cortisol concentration at 4 or 8 hours that is < 40 nmol/l.

In dogs, 25% of animals with pituitary adenoma do not suppress, approximately 60% of animals with pituitary adenomas have a cortisol < 50% of the baseline and approximately 40% of animals have cortisol concentrations < 40 nmol/l. Failure of suppression does not allow differentiation between PDH and adrenal tumours.

Dogs with adrenal tumours do not suppress ; both 3 and 8 hour cortisol values are >50% of the baseline value

### High Dose Dexamethasone Suppression Test - Cats
Two different protocols are described, one carried out in the clinic and one carried out at home by the owners.

Suppression is described as a ratio that is < 50% of the baseline.

Cats that suppress have pituitary adenoma; cats that do not suppress may have either pituitary adenoma or an adrenal tumour.

**Monitoring of hyperadrenocorticism**

Target cortisol values for Cushingoid dogs being treated with mitotane or ketoconazole are 30-100 nmol/L for both the baseline and post ACTH samples.

For dogs on trilostane therapy the ACTH stimulation test should be performed 4 hours after the morning dose and interpreted in the light of the clinical history, physical exam, haematology and biochemistry (electrolytes) results. Current recommendations are:

- If the post-ACTH cortisol concentration is <50 nmol/L, the trilostane is stopped for 7 days and then re-introduced at a lower dose.
- If the post-ACTH cortisol concentration is 50 - 250 nmol/L, and clinical signs show significant improvement then continue on the current dose.
- Monitor those with values between 200 - 250 nmol/L for clinical signs of recurrence.
- If the post-ACTH cortisol concentration is >250 nmol/L, increase the morning dose.
- If after at least 28 days of therapy, clinical signs show very little or no improvement, concurrent illness is ruled out and the post-ACTH cortisol concentration is >200 nmol/L, increase the dose.
- If an ACTH stimulation test is performed at times other than 4 to 6 hours after trilostane, then the current recommendation is that the post-ACTH cortisol concentration falls between 50 - 250 nmol/L.


**DIAGNOSIS AND MONITORING OF HYPOADRENOCORTICISM – GENERAL GUIDELINES**

Tests available include:

- Electrolyte assessment
- ACTH stimulation test

**Dogs**

Primary hypoadrenocorticism (or classical Addison's disease) is a relatively uncommon but important disease of dogs. There is destruction of the adrenal gland leading to deficiency of both minercorticoids and glucocorticoids. The clinical signs can be non-specific and while the first clue that this disease is present is often an alteration in the sodium potassium ratio, some cases may be atypical and hyperkalaemia and hyponatraemia are not always present.

Other clinicopathological findings that may be present include hypoglycaemia, hypercalcaemia, hyperphosphataemia, mild nonregenerative anaemia, lymphocytosis, eosinophilia, prerenal azotaemia and dilute urine.
ACTH stimulation test

The diagnosis of hypoadrenocorticism is confirmed with an ACTH stimulation test.

Test interpretation:

- In hypoadrenocorticism baseline cortisol levels are usually <28 nmol/L and post-ACTH values are <28nmol/L.

As the electrolyte changes often present in these dogs can be life threatening and the stimulation test must be carried out before any treatment can be given, it is advisable to have Synacthen available at all times in the clinic. Note that steroid usage will impact on the results of the ACTH stimulation test making it difficult to interpret, but a single dose of dexamethasone may be used in life threatening situations.

Secondary hypoadrenocorticism occurs when there is a lack of ACTH secretion by the pituitary gland resulting in low serum cortisol concentrations and clinical signs are related to the low cortisol. Electrolyte concentrations will be normal because aldosterone secretion is usually not affected. An ACTH stimulation test will show a low baseline serum cortisol concentration and minimal response.

Cats

Hypoadrenocorticism is a rare disease in cats. The same ACTH protocol is recommended.

PITUITARY PARS INTERMEDIA DYSFUNCTION (PPID, “EQUINE CUSHING’S”) - GENERAL TESTING GUIDELINES

Pituitary pars intermedia dysfunction in horses is most often due to hyperplasia or an adenoma of the pars intermedia of the pituitary gland. An ACTH stimulation test has been described for horses but this is not recommended as a diagnostic test as it does not adequately differentiate normal horses from those with PPID.

Measurement of endogenous ACTH concentrations using seasonal reference intervals has largely superseded the overnight dexamethasone suppression test as the screening test of choice for PPID. However, the overnight dexamethasone suppression test may still be used outside of the autumn period (a high rate of false positives occurs with this test during the autumn) if so desired as both tests perform relatively similarly during the non-autumn period. The measurement of endogenous ACTH during the autumn however is likely to give the highest accuracy in diagnosis.

There is seasonal variation in the baseline serum ACTH concentrations in normal horses and ponies. Therefore appropriate seasonal reference intervals must be used in interpretation. Sensitivity is also an issue for both tests and retesting in 3-6 months (preferably during the autumn for endogenous ACTH) is recommended if testing in inconclusive despite a suspicious clinical picture.

ACTH STIMULATION TEST

Species: Canine and feline

Specimen: Serum

Container: Red top tube or gel separator tube

Collection protocol:
NOTE: Patients should be fasted UNLESS the test is for monitoring of treatment for hyperadrenocorticism. Animals on Trilostane therapy should receive their normal morning medication with a small amount of food and the ACTH stimulation test performed 4-6 hours after dosing.

DOGS:
1. Collect a resting blood serum sample (red top tube) for a basal cortisol concentration and mark it "0 hour" in the morning.
2. Allow tube to clot. If possible separate the serum from the red cells by centrifugation. If a plain tube has been used for the assay, centrifuge and transfer the serum from the collection tube into a plain (red top tube). Do not syringe into tube. Store at 4 degrees.
3. Inject synthetic ACTH (Synacthen) 250ug I/V (or I/M) regardless of bodyweight. If Depot Synacthen is used, inject 250 ug I/M.
4. Collect a second serum sample one hour later and label it 1 hour and follow separation instructions as above.
5. Place samples in the refrigerator until they can be transported to the laboratory.

CATS:
1. Collect a resting serum sample (red top tube) at zero hours and label it "0 hour".
2. Allow tube to clot. If possible separate the serum from the red cells by centrifugation.
3. If a plain tube has been used for the assay, centrifuge and transfer the serum from the collection tube into a plain (red top tube). Do not syringe into tube. Store at 4 degrees.
4. Inject synthetic ACTH (Synacthen) 125 ug I/V (or I/M).
5. Collect post ACTH blood sample at 30min, label as “30 min”. Follow separation instructions as above
6. Collect post ACTH blood sample at 60min, label as “60 min”. Follow separation instructions as above
7. Place samples in the refrigerator until they can be transported to the laboratory.

Special handling/shipping requirements: Samples should reach the laboratory within 24 hours of being collected.

General information about the disease: See “Diagnosis and monitoring of hyperadrenocorticism – general guidelines” and “Diagnosis and monitoring of hyperadrenocorticism – general guidelines”.

General information about when this test is indicated: Screening test for hyperadrenocorticism. Diagnosis of hypoadrenocorticism and iatrogenic hyperadrenocorticism. Monitoring the treatment of hyperadrenocorticism.

Comparison with other related tests: See “Diagnosis and monitoring of hyperadrenocorticism – general guidelines” and “Diagnosis and monitoring of hypoadrenocorticism – general guidelines”.

Gribbles VETERINARY

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ENDOGENOUS ACTH

Species: Horse
Specimen: EDTA plasma
Container: EDTA

Collection protocol:

1. Collect an EDTA blood sample any time of the day.
2. The sample must be chilled within 3 hours of collection, preferably immediately after collection.
3. If possible separate the plasma from the red cell pack. This can be done by centrifugation or by allowing gravity to sediment the red cells.
4. Freeze the separated plasma and send to the lab with gel ice packs.
5. If separation is not possible, wrap the chilled EDTA sample lightly in cotton wool and send with gel ice packs. NOTE it is very important that unseparated samples do not become frozen as the resulting haemolysis will invalidate the test results.

Special handling/shipping requirements: Yes, see above

General information about when this test is indicated:

Endogenous ACTH has largely superseded the overnight dexamethasone suppression test as the screening test of choice for the diagnosis of pituitary pars intermedia dysfunction (PPID, also known as equine Cushings syndrome). Testing can be performed at any time of the year although test performance is highest in the autumn. Both non-PPID and PPID horses display an increase in ACTH concentrations in the autumn (March, April, May) and therefore seasonal reference intervals appropriate for the time of year must be used.

BASAL CORTISOL

Species: Dog, cat
Specimen: Heparinised plasma or Serum
Container: Heparin or plain tube

Collection protocol: Collect as per other routine sampling/serum chemistries etc.

Special handling/shipping requirements: None.

General information about when this test is indicated: Basal cortisol is predominantly used as part of dynamic testing for hyperadrenocorticism or hypoadrenocortism when paired with additional samples collected after administration of ACTH (i.e. ACTH stimulation test) or dexamethasone (ie. dexamethasone suppression test). However it can be a useful stand-alone screening test to eliminate hypoadrenocorticism/Addison's disease from a differential list. Typically if basal cortisol concentrations are above 60 nmol/L, hypoadrenocortism is very unlikely. Note however if basal cortisol concentrations are low this neither confirms nor excludes hypoadrenocorticism. In these situations it is appropriate to proceed with an ACTH stimulation test to fully assess the adrenal reserve.

Horses: Basal cortisol is not useful for the diagnosis of Pituitary Pars Intermedia Dysfunction/Equine Cushings disease. Horses affected with this condition can have variable basal cortisol concentrations. Plasma ACTH or an overnight dexamethasone suppression test are the more appropriate screening tests for PPID.
DEXAMETHASONE TEST – LOW DOSE

Species: Canine and feline

Specimen: Serum

Container: Red top tube or gel serum tube

Collection protocol:

**DOGS**

1. Collect a resting serum sample (red top tube) at zero hours and label it "0 hour".
2. Allow tube to clot. If possible separate the serum from the red cells by centrifugation. If a plain tube has been used for the assay, centrifuge and transfer the serum from the collection tube into a plain (red top tube). Store at 4 degrees.
3. Inject dexamethasone sodium phosphate intravenously at a dose rate of 0.01 mg/kg of body weight.
4. Collect a serum samples at 3 hours post injection, label as “3hr” and follow separation instructions as above.
5. Collect a serum samples at 8 hours post injection, label as “8hr” and follow separation instructions as above.

**CATS**

The protocol currently recommended is to collect a serum sample (red top tube).

1. Collect a resting serum sample (red top tube) at zero hours and label it "0 hour".
2. Allow tube to clot. If possible separate the serum from the red cells by centrifugation. If a plain tube has been used for the assay, centrifuge and transfer the serum from the collection tube into a plain (red top tube). Do not syringe into tube. Store at 4 degrees.
3. Inject dexamethasone sodium phosphate intravenously at a dose rate of 0.1 mg/kg of body weight.
4. Collect a serum samples at 3 hours post injection, label as “3hr” and follow separation instructions as above.
5. Collect a serum samples at 8 hours post injection, label as “8hr” and follow separation instructions as above.

Note the higher dose for cats compared with dogs. This is because the cat's adrenals are normally more resistant to the effects of cortisol.

**Special handling/shipping requirements:** Send all samples to the laboratory within 12-24 hours of collection.

**General information about the disease:** See “Diagnosis and monitoring of hyperadrenocorticism – general guidelines”

**General information about when this test is indicated:** Screening for hyperadrenocorticism.

**Comparison with other related tests:** See “Diagnosis and monitoring of hyperadrenocorticism – general guidelines”
**DEXAMETHASONE SUPPRESSION TEST - OVERNIGHT**

**Species:** Equine

**Specimen:** Serum

**Container:** Red top or gel (serum separator) tube

**Collection protocol:**

1. Collect a baseline serum sample (red top tube) between 4-6pm
2. Administer dexamethasone at 40ug/kg IM (0.04mg/kg)
3. Collect a second serum sample at noon the next day (about 19 hours post-dexamethasone)
4. Submit both samples to the laboratory for cortisol analysis

**Special handling/shipping requirements:** Send all samples to the laboratory within 12-24 hours of collection.

**General information about the disease:** Hyperadrenocorticism in horses is most often due to hyperplasia or an adenoma of the pars intermedia of the pituitary gland. The dexamethasone suppression test is recommended for confirming hyperadrenocorticism. An overnight test is most frequently used. An ACTH stimulation test has been described for horses but this is not recommended as a diagnostic test as it does not adequately differentiate normal horses from those with hyperadrenocorticism.

There is seasonal variation in the baseline serum ACTH concentrations and also in the results of the dexamethasone suppression test (both in baseline and end results) in normal horses and ponies. There are also differences in the baseline and end stage serum cortisol concentrations between horses and ponies. Test results therefore should be interpreted with clinical signs.

**General information about when this test is indicated:** Diagnostic work up for suspected PPID

**Comparison with other related tests:** Endogenous ACTH has largely superceded the overnight dexamethasone suppression test as the screening test of choice for the diagnosis of pituitary pars intermedia dysfunction. ACTH testing can be performed at any time of the year although test performance is highest in the autumn. Both non-PPID and PPID horses display an increase in ACTH concentrations in the autumn (March, April, May) and therefore seasonal reference intervals appropriate for the time of year must be used.

**HIGH DOSE DEXAMETHASONE TEST**

**Species:** Canine and feline

**Specimen:** Serum

**Container:** Red top or gel (serum separator) tube

**Collection protocol:**

**DOGS**

1. Collect a resting serum sample (red top tube) at zero hours and label it "0 hour".
2. Allow tube to clot. If possible separate the serum from the red cells by centrifugation. If a plain tube has been used for the assay, centrifuge and transfer the serum from the collection tube into a plain (red top tube). Do not syringe into tube. Store at 4 degrees.
3. Inject dexamethasone sodium phosphate intravenously at a dose rate of 0.1 mg/kg of body weight.
4. Collect a serum samples at 3 hours post injection, label as “3hr” and follow separation instructions as above.

5. Collect a serum samples at 8 hours post injection, label as “8hr” and follow separation instructions as above.

CATS

Two different protocols are described, one carried out in the clinic and one carried out at home by the owners.

In clinic protocol: The dose used is 10x that used in the low dose dexamethasone test, ie 1.0mg/ kg bodyweight. The test is carried out as above with a baseline sample and samples obtained at 4 and 8 hours. Suppression is defined as a cortisol at either 4 or 8 hours that is < 50% of the baseline cortisol or a cortisol concentration at 4 or 8 hours that is < 40 nmol/l.

Cats with adrenal tumours do not suppress. Some cats with pituitary tumours may not suppress.

At home protocol:

This uses the urinary cortisol to creatinine ratio. This test is considered easier to perform and to interpret than the in clinic test. Diagnostic results for discriminating between pituitary adenoma and adrenal tumours also appear better than results from the in clinic method, although the low dose dexamethasone suppression test is better as a screening test than the cortisol to creatinine ratio.

The owner collects 2 consecutive morning urine samples. After collection of the second urine sample the owner then administers three oral doses of 0.1 mg/kg/dose of dexamethasone at 8 hourly intervals i.e. 8.00 am, 4 pm and midnight. On the third morning a final urine sample is obtained and all three urine samples are brought to the clinic.

The first 2 morning samples are used as a screening sample for Cushings and provide a basal sample. The third sample is used as a discriminatory test between pituitary adenoma and adrenal tumours. Suppression is described as a ratio that is < 50% of the baseline.

Cats that suppress have pituitary adenoma; cats that do not suppress may have either pituitary adenoma or an adrenal tumour.

DIABETC PATIENTS - Diabetic dogs - give half the regular morning dose of insulin and monitor blood glucose every 1 to 2 hours throughout the procedure.

Special handling/shipping requirements: Send all samples to the laboratory within 12-24 hours of collection.

General information about the disease: See “Diagnosis and monitoring of hyperadrenocorticism – general guidelines”

General information about when this test is indicated: Differentiation between pituitary or adrenal dependant hyperadrenocorticism.

Comparison with other related tests: See “Diagnosis and monitoring of hyperadrenocorticism – general guidelines”
URINARY CORTISOL: CREATININE RATIO

Species: Canine and feline

Specimen: Urine

Container: Pottle

Collection protocol:

1. Collect a morning urine sample
2. Submit sample with a request for urinary cortisol and creatinine concentrations.

Special handling/shipping requirements:

General information about the disease: This is a useful screening test for canine hyperadrenocorticism as a normal result makes a diagnosis of Cushings unlikely (approximately 90% sensitivity). It is therefore useful in those cases where hyperadrenocorticism is unlikely but needs to be definitely excluded. The test has low specificity, (there are a lot of false positives) meaning further tests are needed to confirm that a high result is due to hyperadrenocorticism. It is thought that the test has similar sensitivity and specificity in cats but there are few published reports.

The urinary cortisol to creatinine ratio may be used as a screening test on urinary samples collected at home by the owners. Patients with suspicious results may then be tested with the low dose dexamethasone test.

Ratio  < 10 x 10^6 Cushings is very unlikely
Ratio  10 – 15 x 10^6 Equivocal result, retest
Ratio  > 10 x 10^6 Cushings may be present

See also “Diagnosis and monitoring of hyperadrenocorticism – general guidelines”

General information about when this test is indicated: Screening for hyperadrenocorticism

Comparison with other related tests: See “Diagnosis and monitoring of hyperadrenocorticism – general guidelines”
Endocrinology – Thyroid function

DIAGNOSIS AND MONITORING OF HYPERTHYROIDISM – GENERAL GUIDELINES

Hyperthyroidism is the most common endocrinopathy of cats but is a rare disease in dogs. Most canine thyroid tumours are non-functional and the dogs are euthyroid or hypothyroid but occasional cases have been reported where the neoplastic cells are producing functional hormone. The presenting signs and the diagnostic tests are the same in both species.

Tests available include:

- Total T4
- Free T4
- T3 suppression test

**Total T4**

This is a measure of all T4 in the circulation and includes protein-bound and Free T4. Total T4 may be measured by radioimmunoassay (RIA) or enzyme immunoassay. Both of these tests are validated for use in dogs and cats, and studies show an excellent correlation between these two test methodologies.

Furthermore, the enzyme immunoassay has been shown to be less sensitive to interference from autoantibodies. Total T4 by enzyme immunoassay is precise, reliable and should be used as a baseline determination of thyroid status in dogs and cats. As with all endocrine disease, diagnosis is complicated by concurrent disease and use of medications, including certain antibiotics, corticosteroids and anticonvulsants. As Total T4 predominantly measures protein-bound thyroid hormone, it may be affected by conditions that impact on the patient’s serum proteins. Thus, interpretation of results should always take clinical history into account and may be aided by combining Total T4 with Free T4 and canine TSH as appropriate. Gribbles Veterinary Pathology offers an automated enzyme immunoassay for testing Total T4.

**Free T4**

Free T4 is a measure of metabolically active, unbound thyroid hormone and makes up approximately 1% of the circulating thyroid hormone concentration. Free T4 is that portion of the thyroid hormone that enters into cells to perform metabolic functions and exerts a negative feedback on pituitary production of TSH. Currently the only validated method for the determination of Free T4 in dogs and cats is a modified equilibrium dialysis method. This entails a preliminary dialysis stage followed by Free T4 determination by RIA.

Although Free T4 by equilibrium dialysis is less affected by non-thyroidal illness and autoantibodies than Total T4, it does have limitations, including: it is expensive; it is labour intensive; and periodically, reagents become unavailable in Australia resulting in lengthy delays in testing. Free T4 by equilibrium dialysis is not commonly used as an initial screening test for thyroid disease, but rather is best used in those patients whose Total T4 results appear not be in keeping with their clinical symptoms and where there is a suspicion that the Total T4 concentration is being affected by autoantibodies or concurrent non-thyroidal illness. However, in general, RIA methods are becoming less popular due to potential workplace hazards and access to these assays is likely to become more limited. Free T4 is rarely used in the diagnosis of hyperthyroidism given the sensitivity of total T4 in detecting this condition.
Hyperthyroidism in cats

Most hyperthyroid cats have total T4 concentrations significantly greater than the reference range. Confirming the diagnosis is easy in these cases. However, in a few cases a single T4 concentration may be only mildly increased or is at the high end of the reference range. This can occur if there is variable production by the gland or if there is concurrent non-thyroidal illness, which may be lowering the T4 concentration. In these cases, another serum sample should be taken in two to six weeks time. If the result is still equivocal, a T3 suppression test is recommended.

T3 Suppression Test

Principle: If triiodothyronine (T3) is given to a normal cat it will cause suppression of TSH release and hence suppression of serum T4 concentrations. Giving T3 to a hyperthyroid cat should have little effect on the serum T4 concentration as TSH production is already chronically suppressed. Note that a source of (triiodothyronine) T3 is no longer available so this test cannot be carried out unless an alternative source of T3 is found.

Therapeutic Monitoring of Hyperthyroid Cats

Adverse reactions are uncommon with anti-thyroid drugs but can occur. It is recommended that a CBC, serum chemistry, T4 and urinalysis be checked every 2 weeks for the initial 8-12 weeks of treatment. After that cats should be monitored every 3-6 months. To evaluate T4 concentrations the timing of the sample, relative to when the anti-thyroid drug is administered, is not critical.

Subnormal serum T4 concentrations can occur with short and long-term carbimazole (Neomecazole) treatment. Most of these cats will maintain serum T4 concentrations within reference range and clinical signs of hypothyroidism are not commonly recognised. Without clinical signs, there is usually no need to change the dosage.

DIAGNOSIS AND MONITORING OF HYPOTHYROIDISM - GENERAL GUIDELINES

Hypothyroidism in dogs

Hypothyroidism is the most common disease condition of the canine thyroid glands. Canine lymphocytic thyroiditis is believed to cause of about 50% of cases of canine hypothyroidism. Hypothyroidism does not occur until a large amount of the thyroid gland is non-functional, and sub clinical lymphocytic thyroiditis is present for some time before clinical signs of hypothyroidism emerge and total serum T4 and T3 concentrations decrease.

Clinical signs and clinical pathology changes in hypothyroidism can be non-specific. Where there is clinical suspicion, the first step in diagnosis involves a general biochemistry and haematologic screen and assessment of the total T4 concentration. Low serum total T4 concentrations are seen frequently, because total T4 measurements are included in many geriatric blood screens. A single low basal total T4 result is not diagnostic as T4 can be decreased by a number of chronic disease processes and drugs (the "sick euthyroid" state). Serum T4 concentrations fluctuate throughout the day and studies have shown that 50-60% of normal dogs have a T4 below the reference range at some time during the day. Likewise a basal T4 in the lower end of the reference range does not rule out the diagnosis.

To help confirm the diagnosis, measurement of free T4 and endogenous serum TSH concentrations are recommended. Details of each test are provided below.
Total T4

This is a measure of all T4 in the circulation and includes protein-bound and FreeT4. Total T4 may be measured by radioimmunoassay (RIA) or enzyme immunoassay. Both of these tests are validated for use in dogs and cats, and studies show an excellent correlation between these two test methodologies. Furthermore, the enzyme immunoassay has been shown to be less sensitive to interference from autoantibodies. Total T4 by enzyme immunoassay is precise, reliable and should be used as a baseline determination of thyroid status in dogs and cats. As with all endocrine disease, diagnosis is can be complicated by concurrent disease and use of medications, including certain antibiotics, corticosteroids and anticonvulsants. As Total T4 predominantly measures protein-bound thyroid hormone, it may be affected by conditions that impact on the patient’s serum proteins. Thus, interpretation of results should always take clinical history into account and may be aided by combining Total T4 with Free T4 and canine TSH as appropriate. Gribbles Veterinary Pathology offers an automated enzyme immunoassay for testing Total T4.

FreeT4

Free T4 is a measure of metabolically active, unbound thyroid hormone and makes up approximately 1% of the circulating thyroid hormone concentration. Free T4 is that portion of the thyroid hormone that enters into cells to perform metabolic functions and exerts a negative feedback on pituitary production of TSH. Currently the only validated method for the determination of Free T4 in dogs and cats is a modified equilibrium dialysis method. This entails a preliminary dialysis stage followed by Free T4 determination by RIA. Although Free T4 by equilibrium dialysis is less affected by non-thyroidal illness and autoantibodies than Total T4, it does have limitations, including: it is expensive; it is labour intensive; and periodically, reagents become unavailable in Australia resulting in lengthy delays in testing. Free T4 by equilibrium dialysis is not commonly used as an initial screening test for thyroid disease, but rather is best used in those patients whose Total T4 results appear not be in keeping with their clinical symptoms and where there is a suspicion that the Total T4 concentration is being affected by autoantibodies or concurrent non-thyroidal illness. However, in general, RIA methods are becoming less popular due to potential workplace hazards and access to these assays is likely to become more limited.

TSH Assay

Hypothyroidism leads to lack of feedback inhibition resulting in increased concentrations of serum TSH. Endogenous serum TSH concentrations are useful in determining if a dog with a low T4 is truly hypothyroid, i.e. differentiating true primary hypothyroidism from “sick euthyroid” syndrome in many but not all cases. In most cases, serum TSH will eliminate the need for doing TSH stimulatory tests. Serum TSH measurements are recommended only after an initial T4 result is low or low normal. If the hypothyroidism is secondary and a result of lesions within the pituitary gland or higher in the hypothalamus then TSH secretion will be reduced. In these cases serum TSH concentrations will be below the reference range.

The serum TSH measurement can be done on the same serum sample as the initial serum T4 measurement. Serum TSH concentrations should not be requested and interpreted alone but need to be interpreted with clinical signs and the concurrent serum T4 concentration. Depending on the study, 13-38% of hypothyroid dogs may have serum TSH concentrations within the reference range and serum TSH concentrations may be increased above the reference range in 7.6-18% of normal dogs. Where serum TSH and T4 concentrations are interpreted together there is good diagnostic accuracy. TSH results should be interpreted only in the light of clinical and laboratory evaluation for the presence of non-thyroidal illness.

Test Interpretation

From the two major studies on the diagnosis of hypothyroidism in dogs (Peterson et al 1997, Dixon & Mooney 1999) the following has been found:

Using total T4 alone then

- 0 - 11% of dogs which are actually hypothyroid will have a normal T4 concentration (false negatives)
• 18 - 25% of the low T4 results will be from normal dogs (sick euthyroid) (false positives)

Using TSH alone then

• 13 - 24% of dogs which are actually hypothyroid do not have the expected elevation in TSH (false negatives)

• 7 -18% of the elevated TSH results were from normal dogs (false positives)

Interpreting T4 and TSH together

• Using the two tests together still results in a significant number of true hypothyroid dogs not being diagnosed correctly as 13 -33% of the results in the above studies were false negatives. In these cases, where the clinical signs point to hypothyroidism it is ideal to do a TSH stimulation test; TSH can be difficult to obtain however and this is not routinely done.

• Interpreting the two tests together results in low numbers (2 - 8%) of false positives. This means that only a low number of normal/sick euthyroid dogs will be diagnosed incorrectly as hypothyroid.

Tests for lymphocytic thyroiditis – thyroglobulin autoantibody test

The best test to diagnose this condition is considered to be the thyroglobulin autoantibody test. All dogs with thyroglobulin autoantibodies are considered to have lymphocytic thyroiditis and this test is both sensitive and specific for the condition. A dog may have antibodies present but may not yet be hypothyroid. It is recommended that dogs with thyroglobulin autoantibodies be monitored regularly for signs of hypothyroidism. Of 171 dogs monitored with thyroglobulin autoantibodies, 20% became hypothyroid in the following year.

Hypothyroidism in cats

There are few documented cases of naturally acquired hypothyroidism in cats and most cases are iatrogenic following treatment for hyperthyroidism. Hypothyroidism has been reported as a congenital defect in young kittens. Low T4 concentrations are seen frequently now that T4 is included in many geriatric blood screens. In the majority of these cases the low T4 will be due to the suppressive effects of other illness (“sick euthyroid“) and not due to hypothyroidism.

Hypothyroidism in Horses

True hypothyroidism is uncommon in horses, with only very occasional reported cases. Nevertheless, single serum T4 concentrations below the reference range are common. This is usually due to the presence of nonthyroidal illness or medication. The test recommended to further evaluate the hypothalamic-pituitary-thyroid axis in horses is the TSH stimulation test. In horses in which an initial low serum T4 concentration is seen, a second T4 measurement a couple of weeks later after medication has ceased or after recovery from an illness will usually reveal a normal value within the reference range.

Therapeutic Monitoring of T4 Concentrations in Dogs During Thyroxine Treatment

It is not necessary to routinely monitor serum T4 concentrations during treatment, as the most important parameter to measure is the response to treatment. Adequate therapy can be associated with T4 concentrations at the high end, or above the reference range.

This is a useful procedure in dogs under thyroid hormone therapy if:

• Clinical response to treatment has been poor
• Possible signs of thyrotoxicosis are present
• Dosage or product has been changed
• Other drugs that are known to alter serum T4 concentrations have been administered.
The common drugs include corticosteroids, anticonvulsants, nonsteroidal antiinflammatories, some antibiotics and insulin.

Protocol:

1. Continue therapy for 2 - 4 weeks before monitoring concentrations
2. Take a serum sample (red top tube) just prior to, and 6 hours after administration of levothyroxine regardless of the frequency of dosing
3. Send both samples to the laboratory for measurement of T4 concentrations.

To reassess a diagnosis of hypothyroidism via hormone assays in a dog receiving thyroxine supplementation, thyroxine should be discontinued for at least 4 weeks to let thyroid function return to normal.

While serum TSH concentrations are used in humans to assess the response to treatment, in dogs’ serum TSH is not considered useful, as high values are found in less than half of under-supplemented dogs and low values cannot be accurately measured.

**TOTAL T4 ASSAY**

**Species:** Canine, feline, equine

**Specimen:** Serum

**Container:** Plain (red top) or gel (serum separator) tube

**Collection protocol:** Standard venepuncture

**Special handling/shipping requirements:** Send samples to the laboratory within 12-24 hours of collection.

**General information about the disease:**

This is a measure of all T4 in the circulation and includes protein-bound and FreeT4. Total T4 may be measured by radioimmunoassay (RIA) or enzyme immunoassay. Both of these tests are validated for use in dogs and cats, and studies show an excellent correlation between these two test methodologies. Furthermore, the enzyme immunoassay has been shown to be less sensitive to interference from autoantibodies. Total T4 by enzyme immunoassay is precise, reliable and should be used as a baseline determination of thyroid status in dogs and cats. As with all endocrine disease, diagnosis is can be complicated by concurrent disease and use of medications, including certain antibiotics, corticosteroids and anticonvulsants. As Total T4 predominantly measures protein-bound thyroid hormone, it may be affected by conditions that impact on the patient’s serum proteins. Thus, interpretation of results should always take clinical history into account and may be aided by combining Total T4 with Free T4 and canine TSH as appropriate. Gribbles Veterinary Pathology offers an automated enzyme immunoassay for testing Total T4.

See “Diagnosis and monitoring of hypothyroidism” and “Diagnosis and monitoring of hyperthyroidism” for more information.

**General information about when this test is indicated:**

Screening for the presence of hyperthyroidism or hypothyroidism, and monitoring the treatment of these diseases.

**Comparison with other related tests:** See “Diagnosis and monitoring of hypothyroidism” and “Diagnosis and monitoring of hyperthyroidism”
**FREE T4 TEST**

**Species:** Canine and feline  
**Specimen:** 1 mL serum  
**Container:** Plain (red top) tube  
**Collection protocol:** standard venepuncture  
**Special handling/shipping requirements:** Send samples to the laboratory within 12-24 hours of collection.  
**General information about the disease:**

Free T4 is a measure of metabolically active, unbound thyroid hormone and makes up approximately 1% of the circulating thyroid hormone concentration. Free T4 is that portion of the thyroid hormone that enters into cells to perform metabolic functions and exerts a negative feedback on pituitary production of TSH. Currently the only validated method for the determination of Free T4 in dogs and cats is a modified equilibrium dialysis method. This entails a preliminary dialysis stage followed by Free T4 determination by RIA. Although Free T4 by equilibrium dialysis is less affected by non-thyroidal illness and autoantibodies than Total T4, it does have limitations, including: it is expensive; it is labour intensive; and periodically, reagents become unavailable in Australia resulting in lengthy delays in testing. Free T4 by equilibrium dialysis is not commonly used as an initial screening test for thyroid disease, but rather is best used in those patients whose Total T4 results appear not to be in keeping with their clinical symptoms and where there is a suspicion that the Total T4 concentration is being affected by autoantibodies or concurrent non-thyroidal illness. However, in general, RIA methods are becoming less popular due to potential workplace hazards and access to these assays is likely to become more limited.

See also “Diagnosis and Monitoring of Hypothyroidism”.

**General information about when this test is indicated:** See “Diagnosis and Monitoring of Hypothyroidism”

**Comparison with other related tests:** See “Diagnosis and Monitoring of Hypothyroidism”

**THYROID AUTOANTIBODY TEST**

**Species:** Canine  
**Specimen:** 1 mL serum  
**Container:** Plain (red top) tube  
**Collection protocol:** Standard venepuncture  
**Special handling/shipping requirements:** Samples should be transported to the lab within 12-24 hours  
**General information about the disease:**

Hypothyroidism is the most common disease condition of the canine thyroid glands. Canine lymphocytic thyroiditis is believed to cause of about 50% of cases of canine hypothyroidism. Hypothyroidism does not occur until a large amount of the thyroid gland is non-functional, and sub clinical lymphocytic thyroiditis is present for some time before clinical signs of hypothyroidism emerge and total serum T4 and T3 concentrations decrease.

The best test to diagnose this condition is considered to be the thyroglobulin autoantibody test. All dogs with thyroglobulin autoantibodies are considered to have lymphocytic thyroiditis and this test is both sensitive and specific for the condition. A dog may have antibodies present but may not yet be hypothyroid. It is recommended that dogs with thyroglobulin autoantibodies be monitored regularly for signs of
hypothyroidism. Of 171 dogs monitored with thyroglobulin auto-antibodies, 20% became hypothyroid in the following year.

**General information about when this test is indicated:** See “Diagnosis and monitoring of hypothyroidism”.

**Comparison with other related tests:** See “Diagnosis and monitoring of hypothyroidism”.

## THYROID STIMULATING HORMONE (TSH)

**Species:** Canine and feline  
**Specimen:** 1mL blood  
**Container:** Plain (red top) tube or gel serum separator tube  
**Collection protocol:** Standard venepuncture  
**Special handling/shipping requirements:** Standard  

**General information about the disease:**

Hypothyroidism leads to lack of feedback inhibition resulting in increased concentrations of serum TSH. Endogenous serum TSH concentrations are useful in determining if a dog with a low T4 is truly hypothyroid, i.e. differentiating true primary hypothyroidism from “sick euthyroid” syndrome in many but not all cases. In most cases, serum TSH will eliminate the need for doing TSH stimulatory tests. Serum TSH measurements are recommended only after an initial T4 result is low or low normal. If the hypothyroidism is secondary and a result of lesions within the pituitary gland or higher in the hypothalamus then TSH secretion will be reduced. In these cases serum TSH concentrations will be below the reference range.

The serum TSH measurement can be done on the same serum sample as the initial serum T4 measurement. Serum TSH concentrations should not be requested and interpreted alone but need to be interpreted with clinical signs and the concurrent serum T4 concentration. Depending on the study, 13-38% of hypothyroid dogs may have serum TSH concentrations within the reference range and serum TSH concentrations may be increased above the reference range in 7.6-18% of normal dogs. Where serum TSH and T4 concentrations are interpreted together there is good diagnostic accuracy. TSH results should be interpreted only in the light of clinical and laboratory evaluation for the presence of non-thyroidal illness.

**Interpretation:**

**Reference intervals:** Normal cTSH: 0.0 – 0.5 ng/mL

Normal cTSH and fT4 or tT4 is very likely to reflect the presence of normal thyroid function. Low fT4 or tT4 with increased cTSH in a patient with the appropriate history and clinical signs supports a diagnosis of primary hypothyroidism.

Other combinations of fT4, tT4 and TSH are hard to interpret and may be seen with hypothyroidism, presence of non-thyroidal illness or prior use of medication such as sulphonamides, glucocorticoids or anticonvulsants

See also “Diagnosis and monitoring of hypothyroidism”.

**General information about when this test is indicated:** See “Diagnosis and monitoring of hypothyroidism”

**Comparison with other related tests:** See “Diagnosis and monitoring of hypothyroidism”
PREGNANCY DIAGNOSIS – GENERAL GUIDELINES (ALL SPECIES)

Tests that can be used in different species at particular times during gestation to confirm pregnancy include:

**Cows**
Samples are collected at a time of expected oestrus if the animal is not pregnant
Day 19-22: serum progesterone

**Hinds**
Serum progesterone during seasonal nonbreeding period

**Alpacas and Llamas**
Day 7: serum progesterone to assess if ovulation has occurred
Day 21: serum progesterone to assess if pregnant

**Bitches**
Pregnancy can be confirmed by measuring concentrations of the hormone relaxin. Relaxin is produced by the placenta from about 21 days post mating. By day 28 the test is positive 80% of pregnant bitches. However, depending on factors such as breed, size of bitch, litter size and time of mating relative to the LH surge it may not become positive to 33 days then remains positive for the remainder of the gestation. Pseudopregnancy does not give a positive result, as there is no placenta. Relaxin levels decrease within 2-3 days of the loss of a viable pregnancy. Refer to relaxin test for specific test protocols.

**Queens**
There is no reliable blood test routinely available.

**Mares**
Day 19-22: serum progesterone
Day 40-100: serum PMSG
Day > 100-310: serum oestrone sulphate

**Pregnant Mare Serum Gonadotrophins (PMSG or MIP Test)**
PMSG is detectable in mares between days 40-120 of gestation when functional endometrial cups are present. Peak concentrations in most mares occur between days 60-80. Variation in levels between mares means that a negative test result before 60 days or after 90 days from breeding does not rule out pregnancy. The test is a latex agglutination test, with results reported as positive or negative. A positive result generally indicates pregnancy but false positives do occur if there has been foetal death after the endometrial cups have formed (i.e. after day 40).

**Oestrone sulphate**
This is the most reliable means of pregnancy diagnosis from approximately 100 to 310 days. Increased concentrations of oestrone sulphate indicate a viable foetus and concentrations will drop immediately if foetal
death occurs. Oestrone sulphate concentrations can decrease in late pregnancy and can give a negative result in mares in late pregnancy. A non-pregnant mare has a oestrone sulphate concentration of < 5 ng/ml. The concentration in a pregnant mare is typically > 20 ng/ml and generally around 100 ng/ml.

Progestosterone in Mares Later in Pregnancy
Measurement of progesterone is very method dependent. Using antibody assays high concentrations of progesterone are detectable throughout pregnancy but with other methods, the hormone is not detectable in the last half of gestation. Measuring progesterone is therefore not considered to be valuable for determining deficiencies during gestation or predicting time of parturition.

ANTI- MÜLLERIAN HORMONE (AMH)
Species: Canine, feline, equine
Specimen: 2 mL serum
Container: Plain (red top) or serum separator tube
Collection protocol: Standard venepuncture
Special handling/shipping requirements: Samples should be transported to the laboratory within 24 hours
General information about the disease: N/A
General information about when this test is indicated:
Determination of gonadal status of an animals in circumstances including:

- after desexing surgery to confirm complete removal of gonadal tissue
- supposedly desexed bitches and queens exhibiting signs of oestrus (confirmation of “ovarian remnant syndrome”)
- suspected cryptorchid males
- female cats and dogs with unknown desexing history, particularly stray animals

Anti-Müllerian hormone (AMH) is produced by the follicles of a sexually mature ovary and Sertoli cells in a sexually mature testes. After complete ovariectomy or castration, levels of AMH decrease significantly. Intact bitches and dogs, and cryptorchid animals, will have higher levels of AMH than completely desexed animals. A single serum test can differentiate these animals. After desexing, it is recommended to wait 7 days before testing to allow serum levels to decrease before testing to confirm ovariectomy was complete. Low levels of AMH indicate the animal is desexed, high levels indicate the animal has functional gonadal tissue.

Preliminary results suggest this test is reliable in differentiating mares with ovarian granulosa cell tumours from normal mares.

Comparison with other related tests:
A single random blood sample for AMH testing is sufficient to determine gonadal status. In-house methods to assess gonadal status include vaginal cytology (which requires the animal to be in oestrus), or ultrasound (which requires an experienced operator, and may miss small ovarian remnants). Serum progesterone or testosterone can be measured, but interpretation can be hampered by naturally fluctuating levels of each, particularly with stage of cycle in females and with cryptorchidism in dogs. Stimulation with gonadotrophs (hCG or GnRH) may be required to demonstrate functional gonadal tissue.
References:

INHIBIN TEST

**Species:** Equine  
**Specimen:** Serum  
**Container:** Plain  
**Collection protocol:** Standard venepuncture  
**Special handling/shipping requirements:** Standard  
**General information about the disease:** N/A  
**General information about when this test is indicated:**
Inhibin can be used to confirm suspected ovarian granulosa cell tumours in the mare. These tumours are hormonally active and can produce inhibin, testosterone and progesterone. A non-pregnant mare with inhibin concentrations greater than 0.7 ng/ml, testosterone concentrations greater than 500-100 pg/ml and progesterone concentrations less than 1 ng/ml is very likely to have a GCT. Greater than 90% of mares with granulosa cell tumours have elevated inhibin concentrations.

**Comparison with other related tests:**
Testosterone assay is no longer recommended for diagnosis of mare GCT, as the assay cannot detect these relatively low levels of circulating testosterone. Recently, serum Anti-Müllerian hormone (AMH) has been investigated for diagnosis of GCT, with promising results.

OESTRONE SULPHATE

**Species:** Equine  
**Specimen:** Serum  
**Container:** Plain (red top) tube  
**Collection protocol:** Standard venepuncture  
**Special handling/shipping requirements:** Standard  
**General information about the disease:** N/A  
**General information about when this test is indicated:**
**Pregnancy diagnosis:**
This is the most reliable means of pregnancy diagnosis in the mare from approximately 100 to 310 days. Increased concentrations of oestrone sulphate indicate a viable foetus and concentrations will drop immediately if foetal death occurs. Oestrone sulphate concentrations can decrease in late pregnancy and can give a negative result in mares in late pregnancy. A non-pregnant mare has an oestrone sulphate
concentration of < 5 ng/ml. The concentration in a pregnant mare is typically > 20 ng/ml and generally around 100 ng/ml.

**Cryptorchid (rig) identification:**

The equine testis produces ten times the quantity of oestrogen compared with testosterone and it is therefore much easier to differentiate a cryptorchid from a gelding by measuring serum oestrone sulphate rather than testosterone. This test is not recommended in horses less than 3 years of age and donkeys, which will require testosterone assay after stimulation with hCG.

**Comparison with other related tests:**

For pregnancy diagnosis, progesterone can be used from days 19-22, and PMSG from days 40-100.

---

**PREGNANT MARE SERUM GONADOTROPHIN (PMSG)**

- **Species:** Equine
- **Specimen:** Serum
- **Container:** Plain (red top) tube
- **Collection protocol:** Standard
- **Special handling/shipping requirements:** N/A
- **General information about the disease:** N/A
- **General information about when this test is indicated:**

  PMSG is detectable in mares between days 40-120 of gestation when functional endometrial cups are present. Peak concentrations in most mares occur between days 60-80. Variation in levels between mares means that a negative test result before 60 days or after 90 days from breeding does not rule out pregnancy. The test is a latex agglutination test, with results reported as positive or negative. A positive result generally indicates pregnancy but false positives do occur if there has been foetal death after the endometrial cups have formed (i.e. after day 40).

  **Comparison with other related tests:**

  Progesterone can be used for pregnancy diagnosis between days 12-22. Oestrone sulphate can be used from days 100-310.

---

**PROGESTERONE TEST**

- **Species:** All
- **Specimen:** 1 mL serum
- **Container:** Plain (red top) tube. Do not use gel serum separator tubes.
- **Collection protocol:** Standard venepuncture
- **Special handling/shipping requirements:**

  Samples should be transported to the laboratory within 12-24 hrs. Ruminant progesterone is unstable in clotted blood. Concentrations fall significantly within two hours at 17°C. In ruminants avoid the use of gel tubes and separate serum within two hours of collection.
**General information about the disease:** N/A

**General information about when this test is indicated:**

**Serum Progesterone for Timing of Mating in the Bitch**

There is variability between bitches in the time at which ovulation occurs during oestrus. Vaginal cytology, the traditional test for determining the best time to mate has its limitations as it can only place bitches into broad categories of prooestrus, oestrus, dioestrum and anoestrus. It cannot detect ovulation time per se.

In the bitch, serum progesterone is low during anoestrus and prooestrus, begins to rise in late prooestrus, increases rapidly around ovulation to reach high levels at the beginning of dioestrum. Determining serum progesterone concentrations periodically from mid prooestrus and timing mating according to results has proven very useful in bitches with reduced fertility or irregular cycles, when AI is being used and where bitches have to be taken some distance to the dog.

In bitches with a history of infertility or irregular cycles, or where the owner is not familiar with the bitch’s cycle, collect a serum sample (red top tube) at the first indication of bleeding. Alternatively, collect the first serum sample within 1-2 days of detecting >60% cornified cells on vaginal cytology. Ideally the first sample has a serum progesterone concentration of <3 ng/ml. Progesterone concentrations should then be assessed every day or every other day to determine the best time for mating. When the progesterone concentration increases to >8 ng/ml, (in most bitches) then the LH surge has occurred and the best time for mating can be determined from the concentration.

**Serum Progesterone for Estimating Day of Parturition in the bitch**

Variability in the timing of ovulation, length of oestrus and multiple breeding dates make it difficult to predict an exact due date for the litter. The duration of pregnancy has always been regarded as 63-65 days from the first mating. However, a range of 58-68 days from the first mating is more likely to be correct. There are a variety of methods available to try and get a more precise whelping date. The whelping date is likely to be 57 ± 1 days from the first day of dioestrum as determined by vaginal cytology, 63 ± 1 days from the day of ovulation and 65 ± 1 days from the LH surge as assessed by the rise in progesterone above 3 ng/ml. Progesterone concentrations fall in bitches close to parturition. Measuring progesterone in serum within 1 – 2 days of the expected parturition date can help pinpoint time of parturition.

Plasma progesterone concentrations typically fall below 2ng/mL approximately 18 – 30 hours before parturition.

**Serum Progesterone for Confirmation of Pregnancy in Animals**

**Dogs**

Serum progesterone is not a useful test in confirming pregnancy in the bitch.

Concentrations are not significantly different between pregnant and non-pregnant animals in dioestrum. Serum relaxin can be used for confirmation of pregnancy in the bitch.

**Ruminants and Horses**

Progesterone may aid in confirming pregnancy if it is high at a time it should be low if the animal was not pregnant. This situation occurs when:

- Samples are collected at a time of expected oestrus if the animal is not pregnant (e.g. day 19-22 or 40-44 post mating in cattle and horses).
- Samples are collected outside the breeding season. High concentrations at this time indicates there is a functional corpus luteum and as non-pregnant animals should be in seasonal anoestrus, the animal would most likely be pregnant. This is useful for seasonal breeders like sheep, deer, alpacas and llamas.
In most species, the serum progesterone concentration during pregnancy is not significantly different from the concentration during the mid-luteal stage of the oestrus cycle. Therefore, a single random high value is not confirmatory of pregnancy if the stage of the oestrus cycle is not known and the sample is taken during the breeding season. However, if the concentration is still high 10 days later, then the animal is most likely pregnant.

A single low serum progesterone concentration (<0.5 ng/ml) indicates the animal is not pregnant.

As well as pregnancy, other causes of persistent corpus lutea will produce high progesterone concentrations and must be considered, e.g. pyometra.

**Comparison with other related tests:** See “Pregnancy diagnosis guidelines – all species”

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**RELAXIN TEST**

**Species:** Canine

**Specimen:** 1 mL whole blood

**Container:** Lithium heparin (green top) tube, NaCitrate may also be used. EDTA anticoagulated blood and serum are not suitable.

**Collection protocol:**
Sample at 33 days after the last mating. If the mating date is unknown, and the test gives a negative or suspicious result then repeat in 7-10 days to confirm.

**Special handling/shipping requirements:**
The sample can be kept in the fridge for up to 48 hours. Plasma samples may be kept frozen for later analysis.

**General information about the disease:** N/A

**General information about when this test is indicated:**
Pregnancy in the bitch can be confirmed by measuring concentrations of the hormone relaxin. Relaxin is produced by the placenta from about 21 days post mating. By day 28 the test is positive 80% of pregnant bitches. However, depending on factors such as breed, size of bitch, litter size and time of mating relative to the LH surge it may not become positive to 33 days then remains positive for the remainder of the gestation. Pseudopregnancy does not give a positive result, as there is no placenta. As with all pregnancy tests, a positive result reflects the status at the time of testing and does not guarantee that the animal will remain pregnant. Relaxin levels decrease within 2-3 days of the loss of a viable pregnancy.

**Comparison with other related tests:** N/A

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**TESTOSTERONE**

**Species:** Canine, equine, alpaca

**Specimen:** Serum

**Container:** Plain (red top) or gel tube

**Collection protocol:** Standard venepuncture
Special handling/shipping requirements: Separate the serum and transport to the laboratory within 12-24 hrs.

General information about the disease: N/A

General information about when this test is indicated:

Testing to identify cryptorchid animals

Canine

Testosterone level in intact dogs will vary substantially throughout the day. Measurement of a single baseline testosterone can therefore sometimes give equivocal results. Results greater than 1.0 nmol/L are consistent with the presence of testicular tissue (intact or cryptorchid). General guidelines for interpreting the test are as follows:

- Castrated dog: <0.07 nmol/L
- Cryptorchid: 0.35 - 7.49 nmol/L
- Adult male with 1 or 2 scrotal testes: 3.5 - 17.4 nmol/L

An hCG stimulation test can be used to increase the chances of a meaningful testosterone result. A protocol for this test is as follows:

- Take a baseline blood sample.
- Inject GnRH at 2 ug/kg or 50 ug for larger dogs
- Collect a blood sample after 60 minutes.
- There should be a 2-3 fold increase in testosterone above baseline, and no response in a castrate.
- The test can also be performed in cats:
- Take a baseline blood sample.
- Inject 500 IU hCG IV
- Collect a blood sample after between 30 and 120 minutes.
- There should be a 2-3 fold increase in testosterone above baseline, and no response in a castrate.

Equine

Testosterone concentrations in the stallion vary with season, with a mean of 3 nmol/L in the non-breeding season rising to 12 nmol/L at the height of the breeding season. Geldings have low or undetectable concentrations (<0.19 nmol/L) of testosterone. A cryptorchid is confirmed if the serum testosterone concentration is greater than 0.30 nmol/L.

Baseline testosterone concentrations are not significantly different between stallions and unilateral cryptorchid horses. Bilaterally cryptorchid horses have a lower baseline testosterone concentration and most commercially available assays are not sensitive enough to detect these low levels. A single serum testosterone test is therefore NOT recommended for diagnosing cryptorchidism.

The equine testis produces ten times the quantity of oestrogen compared with testosterone and it is therefore much easier to differentiate a cryptorchid from a gelding by measuring serum oestriol sulphate. This test is not recommended in horses less than 3 years of age and donkeys. These younger animals require a stimulation test. A protocol for this test is as follows:

- Take a baseline blood sample.
- Inject 6000 IU hCG IB
- Collect blood between 30 minutes and 2 hours after hCG

A true cryptorchid should show an increase in testosterone from 0.3 – 4.3 nmol/L to 1 – 12.9 nmol/L. Castrated horses should remain below 0.19 nmol/L.
Alpacas and Llamas

A single testosterone measurement is often all that is required. If equivocal results are found, then an HCG stimulation test can be used. For this test, serum testosterone concentrations are measured in two blood samples collected before and 18 hours after IM administration of 5000 IU HCG. NB: Alpacas reach sexual maturity at 10-12 months of age and llamas at 20-24 months.

Comparison with other related tests:

The equine testis produces ten times the quantity of oestrogen compared with testosterone and it is therefore much easier to differentiate a cryptorchid from a gelding by measuring serum oestrone sulphate. Serum Anti-Mullerian hormone (AMH) and LH assays are now available for identification of cryptorchid dogs and cats and are replacing the use of stimulation assays. The presence of penile spines can assist in identifying intact male cats as these are testosterone-dependent.
Endocrinology – Miscellaneous

APPROACH TO DIAGNOSING DIABETES INSIPIDOUS

Approach to Diagnosing Diabetes Insipidus

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<thead>
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<tr>
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<td>Baseline Cortisol (atypical/ Addison’s)</td>
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<td>T4 +/- TSH</td>
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Species: Horse
Specimen: Serum
Container: Plain (red top) tube
Collection protocol:
6. Fast the horse/pony overnight (12 hours).
7. Give a non-glycaemic feed (e.g. chaff) containing 1g/kg bodyweight glucose or dextrose powder mixed with a little water to aid mixing/ingestion.
8. Collect a whole blood (plain tube) sample 2 hours after ingestion of the feed for measurement of insulin concentrations.
Special handling/shipping requirements: No

General information about when this test is indicated:

Resting hyperinsulinaemia is only present in approximately 30% of horses with Equine Metabolic Syndrome (EMS). The in-feed oral glucose challenge test offers a more sensitive means of identifying horses with insulin resistance, one of the hallmarks of EMS. Generally, only the post-prandial insulin concentration is interpreted. Therefore there is no need to collect a basal serum sample for insulin measurement prior to the oral glucose challenge. Measurement of triglycerides and fasting glucose concentrations may be additional tests alongside characteristic changes in the clinical picture that can be helpful in formulating a diagnosis of EMS. Note however very few horses with EMS exhibit fasting hyperglycaemia.

Comparison with other related tests: N/A

**INSULIN**

**Species:** Dog, cat, horse  
**Specimen:** Serum  
**Container:** Plain (red top) tube  
**Collection protocol:**

**Dogs and cats:**

9. Collect both a serum sample and a fluoride oxalate tube.

10. Ideally measure blood glucose concentrations in-house to confirm the dog/cat is currently hypoglycaemic. Blood glucose should be preferably < 2.8 mmol/L for a concurrent insulin measurement to have the most power in diagnosis.

11. Fasting may be necessary to induce hypoglycaemia. This should be performed in-hospital with blood glucose concentrations measured hourly during the fast until blood glucose reaches the desired level. A few small meals should be fed over 2-3 hours afterwards to help the animal recover.

12. Ideally separate the serum from the clot prior to sending as this removes any risk of haemolysis, can invalidate insulin measurement.

13. Send samples with gel ice packs to the laboratory.

**Horses - Basal Insulin:**

1. Collect a serum sample after a minimum of 6 hours fast.

2. Send sample with gel ice packs to the laboratory.

3. See ‘In feed oral glucose challenge test’ for protocol for dynamic insulin testing.

**Special handling/shipping requirements:** Yes, see above

**General information about when this test is indicated:**

**Dogs and cats:** Concurrent measurement of insulin and blood glucose concentrations is used for the diagnosis of insulinoma. Insulinoma or beta cell tumours are relatively common causes of hypoglycaemia in dogs and a rare cause of hypoglycaemia in cats.

**Horses:** Basal insulin concentrations can be used as a screening test for Equine Metabolic Syndrome (EMS) and can also have prognostic value when evaluating cases of pituitary pars intermedia dysfunction (PPID, equine Cushing's syndrome). Note however only approximately 30% of horses with EMS have fasting
hyperinsulinaemia and dynamic testing e.g. with an in-feed oral glucose challenge test, is typically recommended to capture the most cases of insulin resistance.

Feeding, particularly, pasture can cause significant increases in serum insulin concentrations. Therefore it is preferable to measure basal insulin concentrations after fasting (6 hours minimum).

**Comparison with other related tests:** N/A
Haematology

BLOOD TYPING (FELINE)

Species: Feline
Specimen: Whole blood
Container: EDTA (Lavender top)

Collection protocol: Collect as for CBC
Special handling/shipping requirements: As for CBC

General information about the test:
Cats have three blood types, A, AB and B. Cats have natural antibody against other blood groups. Type B kittens have high levels of anti A antibodies and acute transfusion reactions can occur when type B kittens are given transfused. Type B cats should only be given type B RBCs and type A RBCs should never been given to type B cats. RBCs from type B cats can be given to type A or AB cats.

General information about when this test is indicated:
Severe blood/RBC loss, haemorrhage

BLOOD TYPING (CANINE)

Species: Dogs
Specimen: Whole blood
Container: EDTA (lavender top)

Collection protocol: As for CBC
Special handling/shipping requirements: As for CBC

General information about the test:
Testing is for DEA 1.1. A universal donor should be DEA 1.1 negative. Dogs have 8 standard blood groups (DEA system: 1.1, 1.2, 3, 4, 5, 6, 7, and 8). 1.1, 1.2 and 7 are the most important antigens in transfusion medicine. Universal donors should be negative for all 3 antigens and must definitely be negative for 1.1. DEA 1.1 antibody causes an acute haemolytic reaction. DEA antibodies to 1.2 and 3 cause a more chronic immune reaction with shortened RBC life span and no systemic signs. Potential donor dogs should be clinically healthy and on no medication at time of testing.

General information about when this test is indicated: Severe blood loss, haemorrhage
COMPLETE BLOOD COUNT (CBC)

Species: Large animals
Specimen: Whole blood
Container: EDTA (lavender top)

Collection protocol:
Mix blood and anticoagulant gently and as soon as possible to prevent clotting. Make and air dry a blood smear as soon as possible to prevent artefactual changes occurring in the RBCs and WBCs. Do not refrigerate smears. Keep smears away from flies.

Special handling/shipping requirements: Keep cool and transport to laboratory as soon as possible.

General information about the test (disease):
Includes haematocrit (HCT), haemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), WBC differential, reticulocytes if anaemic, assessment of RBC morphology and any descriptions of any abnormalities, platelet assessment.

Includes measurement of fibrinogen.

Excludes reticulocyte count in horses because they do not release reticulocytes if anaemia is present.

General information about when this test is indicated: To check clinically normal animals and help with diagnosis in sick animals.

COMPLETE BLOOD COUNT (CBC)

Species: Small animals
Specimen: Whole blood
Container: EDTA (lavender top)

Collection protocol:
Mix blood and anticoagulant gently and as soon as possible to prevent clotting. Make and air dry a blood smear as soon as possible to prevent artefactual changes occurring in the RBCs and WBCs. Do not refrigerate smears. Keep smears away from flies.

Special handling/shipping requirements: Keep cool and transport to laboratory as soon as possible.

General information about the test (disease):
Includes haematocrit (HCT), haemoglobin (Hb), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC), WBC differential, reticulocytes if anaemic, assessment of RBC morphology and any descriptions of any abnormalities, platelet assessment.

General information about when this test is indicated: To check clinically normal animals and help with diagnosis in sick animals.
COOMBS TEST

Species: Dogs, cats, horses
Specimen: Whole blood
Container: EDTA (lavender top)

Collection protocol:
Mix blood and anticoagulant gently and as soon as possible to prevent clotting. Make and air dry a blood smear as soon as possible to prevent artefactual changes occurring in the RBCs and WBCs. Do not refrigerate smears. Keep smears away from flies

Special handling/shipping requirements: Keep cool and transport to laboratory as soon as possible

General information about the disease: NA

General information about when this test is indicated: Where there is anaemia and haemorrhage is ruled out as a cause of anaemia

WBC DIFFERENTIAL (BLOOD SMEAR)

Species: All species
Specimen: Glass slide with film made from EDTA blood (lavender top) or from fresh blood immediately after collection
Container: Send in a plastic slide holder, protected from breaking while on courier

Collection protocol:
Make smears preferably as soon as blood is taken, but as soon as is practicable. Degeneration of leukocytes may be seen within 15-20 mins of taking blood sample

Special handling/shipping requirements: Air dry as quickly as possible. Do not refrigerate.

Comparison with other related tests:
Some of these preparation criteria apply to general cytology smears.

BLOOD SMEAR EXAMINATION FOR FELINE MYCOPLASMA SPECIES

Species: Cats
Specimen: Whole blood
Container: EDTA (lavender top)

Collection protocol:
Mix blood and anticoagulant gently and as soon as possible to prevent clotting. Make and air dry a blood smear as soon as possible to prevent artefactual changes occurring in the RBCs and WBCs. Do not refrigerate smears. Keep smears away from flies
Special handling/shipping requirements: Make a smear as soon as possible because the organisms tend to drop off the RBCs in transit to the laboratory.

General information about the disease:
Mycoplasmas are small bacteria without cell walls that attach to the surface of RBCs. There are three species present in New Zealand namely *Mycoplasma haemofelis, Candidatus Mycoplasma haemominutum, Candidatus Mycoplasma turicensis*.

General information about when this test is indicated: When anaemia is detected or suspected in cats.

Comparison with other related tests:
A PCR test is available for confirmation of the disease if required. This requires a fresh EDTA blood sample or a sample that has not already been put through the analyser because there is a very small chance that there may be transfer of organisms from a prior infected sample.

**NEONATAL ISOERYTHROLYSIS (NI) IN FOALS**

Species: Equine  
Specimen: Whole blood  
Container: EDTA (lavender top)  
Collection protocol: As for CBC  
Special handling/shipping requirements: As for CBC  
General information about the disease:
This is a haemolytic disease of newborn foals that have suckled and ingested colostral antibodies. There are two antibodies that are most immunogenic – namely Aa and Qa. Mares that are negative for either Aa and Qa or both Aa and Qa are most at risk for causing this disease within their newborn foal, if these foals are positive.

General information about when this test is indicated:
It is required when there is a difference of blood group between the dam and foal. It is most commonly seen where mares have been sensitised by a previous pregnancy. Prediction of this disease requires an EDTA sample and serum sample from the mare and an EDTA sample from the foal for an indirect coombs test. Colostrum from the mare may also be used before the foal suckles.

**RED BLOOD CELL PARASITES**

Species: All species  
Specimen: Whole blood  
Container: EDTA (lavender top)  
Collection protocol:
Mix blood and anticoagulant gently and as soon as possible to prevent clotting. Make and air dry a blood smear as soon as possible to prevent artefactual changes occurring in the RBCs and WBCs. Do not refrigerate smears. Keep smears away from flies.
Special handling/shipping requirements:
Make a smear as soon as possible after collection of blood as some blood parasites/organisms may fall off the RBCs.

General information about the disease: Organisms present in New Zealand include
Cats: Mycoplasma haemofelis, (Formerly Haemobartonella felis), Candidatus Mycoplasma haemominutum, Candidatus Mycoplasma turicensis.

Cattle: Theileria orientalis – Ikeda, Chitose, Buffeli, Mycoplasma wenyonii, (formerly Eperythrozoon wenyonii), Candidatus Mycoplasma haemobos

Sheep: Mycoplasma ovis (formerly Eperythrozoon ovis)

Alpacas: Candidatus Mycoplasma haemolamae

Dogs: Mycoplasma haemocanis has been reported but presence was not confirmed by PCR

General information about when this test is indicated: NA

Comparison with other related tests:
See PCR section for specific tests
Histopathology

HISTOLOGY GENERAL INFORMATION

Species: All

Specimen: Fixed tissue (1:10 tissue:formalin)

Container: Plain with 10% formalin

Collection protocol:

Remember to provide as many clinical details as possible on your submission form. A picture tells a thousand words: if you can, send photos – digital photos can be emailed to your pathologist (usually firstname.lastname@gribbles.co.nz). Try to use a program that can reduce the size of your image files preferably under 1MB. Alternatively, you can provide images on a CD or upload them to a file-sharing site (e.g. Dropbox). Pathologists will view these photos with the case slides and history provided on the submission form.

Special handling/shipping requirements:

Tissues in formalin should be submitted in separate sealed plastic bags from any specimens for cytology. Formalin fumes cause artefact in the cytology specimens that reduces staining quality to a level that is often insufficient for diagnosis. Specimens should be shipped in leak-proof containers and with appropriate labelling (e.g. UN numbers).

Please be very careful not to put freshly removed tissue into narrow-necked containers. Fixed tissue becomes quite firm and warps in fixation; whilst you may have been able to squeeze it into a container, it can then conform to the shape of the container (losing its anatomic orientation) and may be impossible for us to retrieve it without cutting it or breaking the container.

The rule of thumb for tissue:fluid volume is 1:10. It is important to realise that formalin penetrates tissue slowly and might only penetrate 0.5 cm into a tissue block. This requires that the tissues placed in formalin be kept as thin as possible; having slices of only 1 cm in one dimension is optimal. If you cannot see a gross lesion, submission of a big chunk of tissue is false economy. In this situation, the centre will autolyse and it might be that only the outer, fixed part of the tissue will be trimmed anyway.

The standard all-purpose fixative is 10% buffered formalin. This is made by adding 9 volumes of water to one volume of commercial formalin (available as 40% formaldehyde) and buffering it to pH 7. Formalin that is incorrectly buffered has a deleterious effect on nuclear staining and causes yellow-brown deposits to form in the tissues (acid haematin). Neutralised (buffered) formalin (NBF) is easily made by adding 5 g of limestone chips to every 2 l of 10% solution. Otherwise, the following formula may be useful:

- Formalin (40% commercial): 100 mL
- Distilled water: 900 mL
- Na2HPO4: 6.5 g
- NaH2PO4.H2O: 4.0 g

Formalin vapour is an irritant and potentially carcinogenic. It is proven to cause dermatitis in susceptible individuals. Formalin should only be handled in well-ventilated areas and care taken at all times to avoid direct contact with the skin and mucous membranes; gloves are strongly recommended.

Formalin fixation makes most pathogens inert once complete, with the known exception of prion proteins.
BIOPSY / MASS

Species: All

Specimen: Fixed tissue (1:10 tissue:formalin)

Container: Plain with 10% formalin

Collection protocol:

Skin tumours and other specimens in which surgical margins are to be evaluated should be kept as intact as possible. While we understand that you may wish to incise through the deep or lateral margin in order to assess the adequacy of excision at the time of surgery, this has the disadvantage of causing the margin to retract during fixation, making assessment of the margins difficult and sometimes impossible.

- If masses are very large, accept that there may be some delay in processing while we wait for the specimen to fix.
- Certain sites require special approaches. For example, with splenic masses the most diagnostic site to sample is the margin between the mass and apparently normal spleen, since the centre is usually composed of blood. On the converse, the centre of lytic bone lesions should be sampled rather than the periphery, which might just be reactive bone.
- Be careful not to crush the specimen with either your fingers or forceps.
- You may use sutures or non-water-soluble inks to identify the margins. Allow the specimen to dry slightly or blot with tissue paper, gently apply ink sparingly over the entire surgical margins (avoiding any area you have sliced into) and blot the ink dry before placing the sample in formalin.
- Under no circumstances should needles be used to identify specimens or to fix specimens to cardboard to keep them straight. If you wish to orientate a specimen, use sutures to tie to cardboard if required, or just allow the specimen to dry slightly on cardboard over 30-60 seconds before putting it all in formalin.
- When submitting several lumps, place them in separate jars and label appropriately according to site; or alternatively, make sure they are clearly marked in some other way (e.g. sutures/ink) so that any specimens that have dirty margins can be identified. Otherwise if one is incompletely excised, you may not know at which site to perform further surgery.
- Do not squeeze samples into containers. Once fixed, they become firm and may be impossible to remove without cutting the specimen or the container.

Special handling/shipping requirements: All samples must be shipped in leak-proof, sealed containers with appropriate labelling (i.e. UN number). Sample containers and shipping materials can be ordered from your local Gribbles Veterinary laboratory. For more information, see Histopathology – General Information

General information about the disease: Not applicable.

General information about when this test is indicated: Not applicable.

Comparison with other related tests: See – Skin Disease Investigations - Histopathology.
FIXING AND SUBMITTING LARGE ORGANS

Species: All

Specimen: Fixed tissue (1:10 tissue:formalin)

Container: Plain with 10% formalin

Collection protocol:

Whole organs can be submitted where necessary. This is often best so that the pathologist can interpret gross findings, and/or assess the best samples for histopathology; e.g. splenic tumours, whole abnormal uteri, amputation specimens. Brains for TSE evaluation should be submitted whole, and not dissected before submission.

- If your practice is close to a Gribbles Veterinary laboratory and the sample will definitely arrive at the lab on the same day it was collected, it may be sent fresh and unfixed in double plastic bags or a pottle.
- If there may be some delay, it is best to completely fix large organs before shipping. Use some type of large wide-lidded receptacle (e.g. a bucket), to fix the sample. Change the formalin after 24 hours and leave it at least 2 days. It often helps to partially transect large organs (brain excepted) like slicing a loaf of bread; this enables the formalin to penetrate adequately. Try to make the slices 1 cm apart and not to cut completely through the organ in order that anatomical detail can still be recognised.
- If you prefer to submit partially fixed tissue, be aware that there may be a delay in reporting while we wait for the sample to fix.
- Once fixed, the pink soft texture of the centre of the cut tissue will be gone. It can then be shipped in minimal formalin. Pour most of the formalin off, then wrap the organ in several layers of plastic within sealed bags (Ziplock are good) to ensure there is no leakage. It is best to put the organ within a firm rigid container to protect it in transit.

Special handling/shipping requirements:

All samples must be shipped in leak-proof, sealed containers with appropriate labelling (i.e. UN number). Sample containers and shipping materials can be ordered from your local Gribbles Veterinary laboratory. For more information, see Histopathology – General Information

General information about the disease: Not applicable.

General information about when this test is indicated: Not applicable.

Comparison with other related tests: See – Necropsy, Histopathology – Fixing of Special Organs

FIXING OF SPECIAL Organs

Species: All

Specimen: Fixed tissue (1:10 tissue:formalin)

Container: Plain with 10% formalin

Collection protocol:

Tissues such as central nervous system and eyes require special care upon removal. Artefact induced by rough handling and poor fixation can greatly impede interpretation of histological changes in these organs.
Lesions in the brain can be localised to focal areas, and the entire intact brain should always be submitted. It is very important to preserve the mid-brain and hind-brain in cases submitted for TSE evaluation. Eyeballs need to be removed immediately after death, as autolytic change can affect the retina within 10 minutes of anoxia. Good retinal morphology is critical for diagnosis of most cases of ocular blindness. Eyeballs need to be trimmed of extraocular tissue and muscle before fixing, but leave the optic nerve in place. Intra-vitreous injection of formalin (0.25 ml in a dog or cat, 2 ml in a horse) can be used to optimise retinal histopathology; alternatively a specialist fixation protocol (e.g. use of Bouin’s or Davidson’s fixative) can be pursued.

**Special handling/shipping requirements:**

All samples must be shipped in leak-proof, sealed containers with appropriate labelling (i.e. UN number). Sample containers and shipping materials can be ordered from your local Gribbles Veterinary laboratory. For more information, see Histopathology – General Information

**General information about the disease:** Not applicable.

**General information about when this test is indicated:** Not applicable.

**Comparison with other related tests:** See – Necropsy, Histopathology – Fixing and Submitting Organs.

### HISTOPATHOLOGY – NECROPSY SPECIMENS

**Species:** All

**Specimen:** Fixed tissue (1:10 tissue:formalin)

**Container:** Plain with 10% formalin

**Collection protocol:**

Histopathology samples should be collected as soon as possible after death to avoid artefact and loss of diagnostic information due to autolysis and putrefaction. Some tissues autolyse very quickly, and in some circumstances (e.g. scouring calves with intestinal lesions) it is actually best to sacrifice a moribund animal for immediate post mortem and fixation, rather than to sample an animal that has been dead for hours.

Always include tissues with gross changes, even if you are doubtful of the significance. If the change occupies a large area of the tissue (>2 cm), a few small pieces from the edge and some from the deeper areas are recommended.

It is crucial to sample a range of organs, even if the cause of death or gross lesions seem obvious, since apparent lesions may not prove significant and it is rare to have another opportunity. The samples can be held in formalin for later analysis if required. For cases of sudden death in all species, we recommend at a minimum collecting the brain, heart, lungs, liver and kidney (known as the “Big Five”), with lymph node, gastrointestinal tract (rumen, abomasum, small intestine, large intestine), urinary bladder, skeletal muscle and spleen also often containing useful information.

Handle tissues gently, especially delicate endocrine organs (e.g. adrenals). Mucosal surfaces (e.g. intestine) should not be rubbed or washed before fixation.

Provide sections that include all the relevant architecture, e.g. for lung, include portions of large and small airways, and avoid extremities of lobes; don’t just sample areas of ventral consolidation but also a few areas from several lobes. For liver include a bile duct or two; for kidney ensure both cortex and medulla with pelvis are included.
Do not freeze tissues before or after fixing. Freezing does not absolutely preclude histopathology and sometimes frozen tissues can remain diagnostically useful, but generally speaking freezing induces artefact that can make histopathology very difficult.

Tissues fix best at warmer temperatures (up to 30°C, the warmer the temperature, the faster the fixation). On cold winter days, fixation can be aided by keeping the samples in a heated area.

**Special handling/shipping requirements:** All samples must be shipped in leak-proof, sealed containers with appropriate labelling (i.e. UN number). Sample containers and shipping materials can be ordered from your local Gribbles Veterinary laboratory. For more information, see Histopathology – General Information

**General information about the disease:** Not applicable.

**General information about when this test is indicated:** Not applicable.

**Comparison with other related tests:** See – Necropsy.

**IMMUNOHISTOCHEMISTRY**

**Specimen:** Fixed tissue processed to a paraffin block.

**Container:** Not applicable.

**Collection protocol:** Tissues are fixed after collection at biopsy or necropsy.

**Special handling/shipping requirements:** As apply generally to shipping of fixed tissue.

**General information about the disease:** Not applicable.

**General information about when this test is indicated:**

In large part, diagnostic pathology by light microscopy relies upon recognising patterns of disease at the architectural and cellular level. However, any particular disease can have a wide range of microscopic expression, and while most cases will fall into the centre of the “bell curve”, there are always one or two at the tail ends of the curve that overlap with the tail of another diagnostic “bell curve”, and consequently defy interpretation. In other words, sometimes it’s impossible to say just from light microscopy what this or that cell is; whether the lesion is lymphoma or another type of tumour; or even whether the lesion is inflammatory or neoplastic! Furthermore, there are some cases where a lesion can be recognised easily, but predicting it’s behaviour based on light microscopy is difficult (e.g. mast cell tumours in dogs).

This problem has lead to the development of a wide range of complementary diagnostic techniques including electron microscopy, karyotyping, in-situ hybridisation, PCR, flow cytometry and immunohistochemistry. In human medicine, improved diagnosis, characterisation and sub-classification of disease by multiple modalities has allowed refinement of prognosis and the best treatment for each individual patient. Medicine is becoming personalised with specific targeting of biochemical pathways promoting various diseases. In veterinary medicine we are now moving in the same direction, with better characterised diseases providing more useful prognostic information to veterinarians and the opportunity to treat with targeted therapies.

Immunohistochemistry and immunocytochemistry use immune reactions in order to identify cells or other targets (e.g. receptors or pathogens). The basic principle in most immunodiagnostics is that antibody-antigen binding between a specific antigen and a diagnostic antibody raised against it (the primary antibody), triggers a change that can be recognised in a tissue section or smear by light microscopy. In diagnostic laboratories this is usually a colour change achieved through an enzymatic reaction, with the catalysing enzyme tagged to the site by a secondary antibody that recognises the primary antibody.
Your pathologist is likely to review the stained sample alongside a control (external or internal), and then conclude the overall diagnosis and prognosis, or whether more testing is required to determine this. It should be understood that immunohistochemistry and immunocytochemistry are not always the “last word”, since like any test they can have false or uninterpretable results (e.g. over-fixation impairs antigen detection; very poorly differentiated tumours may not express some antigens; background staining can impede recognition of positive cells). Nevertheless they are very useful tools in the classification of disease by light microscopy.

The tests listed here have been validated in Gribbles Veterinary for the species listed. If you want to test another species or require an antibody or test not listed here, please contact your local Gribbles Veterinary laboratory since it may be possible, or available through one of our partner medical laboratories. Those tests may work, but have not necessarily been subjected to a validation process in our network.

If you require specific information on the clone and manufacturer of primary antibody used in a certain test, please contact your local laboratory.

**CD3**

**Species:** Dog, Cat

CD3 is part of the T-cell receptor, the critical part of T-cells responsible for recognising antigens presented by other cells. Therefore, this antibody recognises lymphocytes of T-cell lineage (including CD4+ and CD8+ cells).

This antibody is used to confirm and characterise diagnoses of T-cell lymphoma, and to help rule out lymphoma in cases of poorly differentiated “round cell” tumours. It is typically used in conjunction with a B-cell antibody (e.g. CD20 or CD79a).

This is helpful because the prognosis and treatment for different forms of lymphoma varies according to their classification. There are low-grade T- and B-cell lymphomas that may have a fair to good prognosis, medium-grade B-cell lymphomas that are often chemoresponsive, and high-grade T-cell lymphomas that may be poorly chemoresponsive and may have a poor prognosis.

**CD10**

**Species:** Dog

CD10 is an enzyme found in many tissues. Antibodies to CD10 are mainly used in humans to classify lymphoma, leukaemia and carcinomas.

In animals, it has been used to identify myoepithelial cells in normal, dysplastic and neoplastic mammary tissue. This can be helpful since histological classification and grading of mammary tumours has prognostic value. It has also been used to classify renal tumours.

**CD18**

**Species:** Dog

CD18 is part of an integrin molecule, essential for the adhesion of leukocytes to vessel walls as they migrate into tissues. Macrophages and granulocytes express 10x more CD18 than lymphocytes.

Therefore while not specific to histiocytes, this antibody helps to identify “round” or “spindle” cells in tissue sections as histiocytic, and to rule out other possibilities (e.g. lymphoma, melanoma, soft tissue sarcoma).

This sometimes requires concomitant use of other antibodies to rule out other possibilities.

This is helpful since both benign and malignant histiocytic diseases can be difficult to distinguish from reactive processes, “round cell” tumours and “spindle cell” tumours. Histiocytic sarcomas tend to metastasise more widely and to have a worse prognosis than soft tissue sarcomas.
CD20 and 79a

Species: Dog, Cat (CD20 only)

CD20 is a surface molecule found on B-lymphocytes, which plays a role in their differentiation into plasma cells.

CD79a is part of the B-cell receptor, the critical part of B-cells responsible for recognising antigens. Therefore, this antibody recognises lymphocytes of B-cell lineage (including plasma cells).

These antibodies are used to confirm and characterise diagnoses of B-cell lymphoma, and to help rule out lymphoma in cases of poorly differentiated “round cell” tumours. They are typically used in conjunction with the T-cell antibody CD3. Some plasma cell tumours are also CD79a positive.

This is helpful because the prognosis and treatment for different forms of lymphoma and plasma cell neoplasia varies according to their classification. There are low-grade T- and B-cell lymphomas that may have a fair to good prognosis, medium-grade B-cell lymphomas that are often chemoresponsive, and high-grade T-cell lymphomas that may be poorly chemoresponsive and may have a poor prognosis. Plasma cell tumours tend to be benign and have a good prognosis compared to some other round cell tumours.

CD31 and Factor VIII-related antigen

Species: Dog

CD31 (also known as PECAM-1) is an adhesive protein expressed strongly by endothelial cells, haemopoietic stem cells and more weakly on leukocytes (neutrophils, lymphocytes and plasma cells). It may also be found on megakaryocytes and platelets. It is used during transendothelial migration of leukocytes.

Factor VIII is part of clotting cascade. The Factor VIII complex is found free in the circulation, with Factor VIII-related antigen (von Willebrand factor) thought to be synthesised by endothelial cells and megakaryocytes.

These antibodies are therefore useful to identify spindle cells as endothelial, allowing the distinction of haemangiosarcomas, angiosarcomas and lymphangiosarcomas from other “spindle cell” tumours, (e.g. spindloid melanomas and soft tissue sarcomas). This is helpful since malignant vascular tumours often have a worse prognosis (e.g. early metastasis) than soft tissue sarcomas.

c-Kit / CD117

Species: Dog

c-Kit / CD117 is a tyrosine kinase receptor for stem cell factor, found on mast cells, interstitial cells of Cajal and some other cells (e.g. bone marrow stem cells, melanocytes, germ cells).

It is therefore not specific to mast cells, however mast cell tumours generally stain well with antibodies to this receptor and this test is helpful to confirm that diagnosis. Furthermore, the distribution of antibody staining in the cells may have prognostic significance in mast cell tumours.

In the intestinal tract, Gastrointestinal Stromal Tumours (arising from interstitial cells of Cajal) are differentiated from other “spindle cell” tumours (e.g. leiomyosarcoma) by this antibody. The prognosis for these tumours is not well understood; they may metastasise to local lymph nodes, mesentery and liver, but in general are thought to have a better prognosis than other malignant tumours of the lower intestinal tract.

Confirmation of a diagnosis of mast cell tumour enables the use of specific treatments such as tyrosine kinase inhibitors. These block signals stimulating cell proliferation from c-Kit and other tyrosine kinase receptors. Constitutive activation of c-Kit is seen in a percentage of canine mast cell tumours in association with mutations in the c-Kit receptor.
Cytokeratin AE1/AE3 and Cam 5.2 (Cytokeratin 8/18)

**Species:** Dog

Cytokeratins form part of the cytoskeleton in epithelial cells. They are found in different combinations in different types of epithelial cells, and therefore specific cytokeratin antibodies can identify these cell lines.

Cytokeratin AE1/AE3 is a “cocktail” of antibodies recognising most epithelial cells. Cam 5.2 (Cytokeratin 8/18) identifies simple epithelium (e.g. liver, thyroid follicular epithelium, lining cells of glands).

This is useful to differentiate carcinomas from other tumour types, which may have a different prognosis and treatment regime. Cytokeratin AE1/AE3 is often used in combination with vimentin.

**Ki67**

**Species:** Dog

Ki67 is a nuclear protein that is expressed during all stages of the cell cycle, except G0 (i.e. “resting”, non-dividing cells). Since mitotic figures are only seen in metaphase, and are sometimes hard to distinguish histologically from artefactual nuclear distortion or necrotic/apoptotic nuclear changes, antibodies to Ki67 allow more sensitive detection of proliferative activity in a population of cells.

The percentage of positive cells produces a quantitative assessment of tumour proliferation. This is useful in tumours that are difficult to prognosticate by histopathological features, such as mast cell tumours and melanomas.

**Melan A**

**Species:** Dog

Melan A is a melanocytic differentiation antigen. It can also cross-react with steroid hormone-producing cells (these do not produce Melan A however).

This antibody allows the distinction of melanomas from other “round”, “epithelioid” or “spindle cell” tumours (e.g. histiocytic sarcoma, carcinoma, soft tissue sarcoma). This is useful because the prognosis for malignant melanoma is often more guarded than for some differential diagnoses; and also because specific treatment for melanoma (the “melanoma vaccine”) may be useful in some correctly diagnosed melanoma patients.

**Neuron Specific Enolase / Synaptophysin**

**Species:** Dog

Neuron-Specific Enolase is an enzyme found in neurons and neuroendocrine cells. Synaptophysin is a component of the neuronal synaptic vesicle recycled across nerve junctions. Antibodies to synaptophysin also label neurons and neuroendocrine cells.

These antibodies are mainly used to distinguish neuroendocrine tumours from other epithelial tumours.

This test is not available in NZ.

**Vimentin**

**Species:** Dog

Vimentin is a part of the cytoskeleton in mesenchymal “spindle” cells, such as endothelial cells and fibroblasts. It can also be found in some other cells such as leukocytes, glial cells and mesoderm-derived epithelia (e.g. endometrium and ovary).
This test is used to help confirm that a tumour is of mesenchymal origin (e.g. poorly differentiated “spindle cell” tumours). It is often used with cytokeratin in order to distinguish mesenchymal tumours from undifferentiated carcinomas, and to identify tumours co-expressing vimentin and cytokeratin (e.g. mesotheliomas).

**S-100**

**Species:** Dog

The family of S-100 proteins performs a variety of intracellular and extracellular functions. They are found in a variety of cells, but in formalin fixed paraffin embedded sections, antibodies to S-100 may detect melanocytes, Schwann cells, cerebral glia, ependymal cells and certain histiocytes.

This test is mainly used to confirm a diagnosis of melanoma or peripheral nerve sheath tumour. This is useful because the prognosis for malignant melanoma is often more guarded than for other differential diagnoses; and also because specific treatment for melanoma (the “melanoma vaccine”) may be useful in some correctly diagnosed melanoma patients.

This test is not available in NZ.

**Smooth Muscle Actin, Muscle Specific Actin and Desmin**

**Species:** Dog

These Actins and Desmin are variably part of the contractile apparatus and cytoskeleton of muscle, myofibroblasts, pericytes, liver peri-sinusoidal cells and myoepithelial cells.

These antibodies can be used to determine if a tumour or cell of interest arises from any contractile cell line (Desmin, Muscle Specific Actin) or incorporates smooth muscle in particular (Smooth Muscle Actin).

They are used to help diagnose muscle tumours (such as leiomyosarcomas and rhabdomyosarcomas) and to sub-classify mammary tumours. This is clinically useful since poorly differentiated rhabdomyosarcomas can sometimes resemble “round cell” tumours such as lymphoma, spindloid muscle tumours can be indistinguishable from other “spindle cell” tumours (e.g. some forms of melanoma), and sub-classification of mammary tumours allows better prognostication.

This test is not available in NZ.

**Thyroglobulin and TTF-1**

**Species:** Dog

Thyroglobulin is produced by thyroid follicular epithelial cells, stored in colloid and complexed with iodine to produce thyroid hormones.

TTF-1 is a transcription factor regulating thyroid-specific genes, and also regulating transcription in the lung (Clara cells and Type 2 pneumocytes).

Antibodies to thyroglobulin stain follicular thyroid tumours but not C-cell (medullary cell) tumours. TTF-1 antibodies stain follicular thyroid tumours, some C-cell tumours and also stain some pulmonary carcinomas.

This is used to diagnose poorly differentiated thyroid carcinomas and to differentiate pulmonary carcinomas from other lesions, including tumours that might have metastasised to the lung.

This test is not available in NZ.
Microbiology

Microbiology really encompasses the study of disease caused by microbes, and as such may be concerned with bacteria, viruses, fungi and protozoa. As a general rule, if one is to associate disease with an isolated organism it is important to demonstrate the presence of the organism in diseased tissue, and to demonstrate changes within the tissue consistent with an infection by that organism.

This section will deal primarily deal with how to sample and submit tissue and fluid, taken from a live or dead animal, for bacterial culture. Some comments will be passed on fungal skin disease. Viral diseases will largely be dealt with under serology and specific disease entities.

Introduction

Bacteria can be found in large numbers in the external environment, and in those areas of the body that are connected to that environment e.g. cavities - GIT, vagina, upper respiratory tract; skin. Besides these areas of the body, tissues are sterile in a healthy animal, and there are considerable anatomical and physiological structures and mechanisms to keep them that way. These may be by-passed in a number of circumstances such that normal commensal bacteria and environmental saprophytes (opportunistic pathogens) gain admittance to these normally sterile areas e.g. trauma, suppression of the immune response. There are some obligate pathogens that elaborate virulence factors that allow invasion of the body in the absence of these predisposing conditions, although some bacteria may fall between these 2 extremes.

Bacterial invasion generally results in tissue destruction and inflammation resulting in disease.

Sampling

The aim of correct techniques in sampling and submission is to provide the laboratory with a sample/s that contains the same types and numbers of bacteria that were present in the sampled site of the diseased animal in life. This can be problematic in animals that are dead at the time of examination, as post mortem decomposition and tissue breakdown provide an environment where invasion of environmental, and especially gut origin bacteria are guaranteed, and will be worsened the longer the time between death and sampling, and the higher the environmental temperature.

As a rule of thumb, bacterial invasion of normally sterile tissues in dead animals may assume significance as early as 2 -4 hours after death, especially when temperature is high, and in some circumstances sampling of internal organs and tissues should not proceed if it judged to be too contaminated. This decision is easier to make if one knows the time of death, but one often does not. Thus the appearance of the tissue/carcass is used to make this judgment. If multiple animals are affected, and some are still alive, it is advisable to sacrifice one (or more animals) and freshly sample to eliminate PM autolysis and tissue invasion.

- Sampling of normally sterile sites: these are sites where in life one would expect to find no bacteria e.g. most internal organs, lower respiratory tract, body cavities. The significance of any isolate from these areas depends on aseptic sampling and submission at all times. This is especially important in a live animal to preclude introducing any organisms into previously sterile sites. Thus sampling should be as aseptic as possible, and samples obtained should always be placed in sterile containers, and forwarded as indicated below. If this is done carefully, bacteria isolated are likely to be significant

- Sampling from contaminated sites: these are those areas of the body normally in contact with the external environment, and that often have a “normal” commensal population of bacteria e.g. GIT, skin, URT. Thus a knowledge of the normal flora of these sites is required to assist the laboratory in culture method required and in interpretation of the results of culture. Often the clinician will want to know if particular organism/s are present e.g. Salmonella or Yersinia sp. from faecal cultures, so selective media can be used to eliminate or inhibit the growth of normal commensals, and encourage the growth of potentially pathogenic bacteria. The sampling procedure should still be done in a sterile a fashion as
possible, and certainly extraneous bacteria should not be introduced to confound the interpretation of culture results.

- Other samples required: the isolation of bacteria from a sample is not in itself enough to demonstrate significance/causation in the sampled disease state, except in rare instances e.g. anthrax. It is important to demonstrate that there is a response from the tissue to the potential presence of the bacteria, as this offers the clinician evidence that the isolate is not a contaminant, and is contributing to disease. There are a number of samples that can be taken to support the results of culture, and most allow the demonstration of an inflammatory response in the tissues at the site of sampling e.g. histopathology (with fixed tissue), cytology. Without these other samples it is often (nearly always) impossible to be sure of the significance of any isolate. In some contaminated sites e.g. skin/ear canal, this can also allow the clinician or pathologist to assign causation to a particular bacteria in a cultured mixed population e.g. Staphylococcus sp.

**Types of samples**

- Fluids: if culture is required all fluids should be placed into sterile containers (generally yellow top). Blood collection tubes are NOT guaranteed sterile. If the fluid is a conventional body fluid for which normal levels of protein and cells are known e.g. thoracic and abdominal fluid, joint fluid, CSF, the fluid should also be collected into an EDTA tube to prevent clotting and allow a fluid analysis.

  - Urine is a conventional body fluid. Urine is collected aseptically (cystocentesis) if culture is required, but the fluid analysis (called a urinalysis in this case) does not require EDTA preservation usually, as the cytology is done on unstained sediment. There are instances where stained cytology may be useful however e.g. suspect neoplasia, and some urine can be placed in EDTA to try and preserve cytological features.

  - Blood is also a conventional fluid, and is commonly collected into EDTA for a fluid analysis (called a CBC here). Culture of blood however is a special case where special liquid media are required to encourage any bacteria to grow, as often they can be in low numbers/or the presence of antibiotics can inhibit growth. Absolutely sterile collection is required, and culture bottle inoculation is done immediately at animal side (contact the laboratory for bottles of culture media)

- Solid tissue: from a dead animal – here it is difficult to absolutely avoid contamination, and there is always the chance of post mortem bacterial overgrowth. Thus large chunks of tissue (enough to nearly fill a 70 ml urine jar and taken from the organ system prior to opening the GIT) are the suggested sample. The size allows searing of the surface and aseptic interior sampling with “cooking” the whole sample

- Swabs: these are not an ideal sample for bacterial culture, but in some circumstances are the best that can be provided. Those for bacterial culture should always be those that are preserved by transport media; note these are NOT useful for PCR analysis however. Dry swabs are suitable for providing samples for PCR

**Packaging and Transport of samples**

Samples must be packaged and transported in such a way that you retain, as much as possible, the state of the sample at the time of sampling. Two factors are of prime importance here- time and temperature. It is ideal if the sample can arrive at the laboratory <24 hours after sampling, and be sent in a leak proof container with enough ice bricks to keep the sample chilled (not frozen) during transport. If there is a delay in transport, the sample should be kept in the cool part of the fridge, and forwarded as soon as possible.

Containers should be leak proof, clearly labeled (animal number, sample type), and not contaminated by the sample (as can occur with faeces for instance).
SAMPLING AND SUBMITTING MATERIAL

In order for microbiological examination to be useful, the submitted material must:

- Be collected and stored in as sterile a manner as possible.
- Reach the laboratory as soon as possible to allow for minimum multiplication of organisms in transit.
- Be in good condition (kept moist, kept cool) by the time it reaches the laboratory.
- Be the appropriate specimen e.g. we do not usually recommend that faecal swabs be taken, as this type of specimen reduces the chance of a diagnosis in most cases.

In order to achieve these conditions:

- Use sterile scissors and forceps for collection of small samples (mainly small animals), and clean scissors and forceps for collection of large tissue samples (mainly from necropsy cases).
- When collecting faeces don't contaminate the outside of the container.
- Collect as much of the sample as possible on swabs where appropriate and place them in sterile transport media. Dry swabs are not recommended.
- Always use sterile containers.
- Ensure the lids are threaded correctly for tight leak proof seal.
- Keep specimens as cool as possible in transit. This can be achieved by use of chilli-bin in the car, refrigerator in the clinic and coolant silica pads in the courier bag.
- Use couriers wherever possible to reduce transport time.

Transportation of microbiology samples

- Submit samples with an ice brick within 24 hours of collection.
- Refrigerate samples if they cannot be sent within 24 hours.
- The use of transport media is recommended for all swabs submitted for culture

Please see individual information sheets for specific requirements for the following:

1) Campylobacter foetus subsp. venerealis and Tritrichomonas foetus Examinations
2) Blood and Joint Culture
3) Footrot Investigation
4) Fungal Examination of the Skin
5) Leptospira
6) Milk Sampling for Culture

MILK SAMPLING FOR CULTURE

Preparation

- Wash the teats with clean running water or water containing disinfectant such as cetrimide.
- Dry the teats and udder with paper towel.
- Wipe the teat orifice with a gauze wad soaked in 70% ethanol, using a separate wad for each teat. Dry the side of the teat with a clean gauze swab, using one motion and moving away from the teat orifice.
- The teats on the side of the udder opposite to the collector should be cleaned first.
- It is essential that NO disinfectant or ethanol drip into the collection container.
Collection Method

- The collector should wear gloves or wash their hands in a solution of disinfectant. Change gloves or wash between taking samples from different animals.
- Reduce contamination by any existing milk in the streak canal by NOT collecting the first squirt of milk.
- Collect 10-20ml of milk from each quarter into a sterile screw top container.
- Samples should be collected from teats nearest the collector first.
- Ensure no milk runs off the collector’s hand into the container.
- Do not let the teat touch the container.

Containers should be clearly labelled with the animal’s identification, owner’s name and the quarter that has been sampled.

BLOOD AND JOINT CULTURE

This is indicated in animals with pyrexia of unknown origin and in disease processes where bacteraemia or septicaemia is expected (e.g. discospondylitis, endocarditis).

- Sterile blood (free of anticoagulant) is injected directly into a blood culture bottle (available from Gribbles Veterinary) – paediatric blood culture bottles only require 1-3mL blood.
- Aseptic collection of the sample is critical with the site of venepuncture being shaved and prepared as for surgical procedures. One contaminating bacterium will multiply up and produce erroneous results.
- Once the blood culture media has been inoculated DO NOT refrigerate, keep at room temperature until submitted to the laboratory. Courier to the laboratory in the usual way.
- It is recommended that joint fluid from suspected cases of septic joint disease be also inoculated into blood culture bottles as this greatly increases the sensitivity of joint cultures.

Refer to our How To Take a Blood Culture guide for further information.

CAMPYLOBACTER FOETUS SUBSP. VENEREALIS AND TRITRICHOMONAS FOETUS EXAMINATION AND CULTURE

These organisms can establish on the preputial mucosa of largely asymptomatic bulls, and be transmitted during coitus, resulting in infertility and abortion. Samples require the use of a “Tricamper” collection tool, and the sample collected can be used for culture or PCR.

NB. T. foetus culture must be done at the clinic, and culture medium can be obtained using our consumable order form.

Preputial samples

Insert the tool into the prepuce and move back and forth to scape across preputial mucosa and the surface of the penis. Block end (e.g. with finger) to prevent loss of material then remove from prepuce. Holding just off horizontal, insert tip into saline tube and remove block from end. Rinse smegma using 5 ml saline from a syringe, or cut off the black head of tricamper into saline.

Note: Tricampers can be obtained using our consumable order form.

Vaginal mucus
• Clean perineum.
• Introduce tricamper in a dorsocranial direction to avoid urethra until anterior end reaches cervix, and move gently back and forth. Block end (e.g. with finger) to prevent loss of material then remove. Holding just off horizontal, insert tip into saline tube and remove block from end. Rinse material off.

**CHLAMYDIA CULTURE**

**Species:** Avian, feline

**Specimen:** Conjunctival scrapings (cat or bird), cloacal scrapings, whole fresh spleen or liver

**Container:** Chlamydia culture media

**Collection protocol:** Firm swabbing of the affected tissue

**Special handling/shipping requirements:** Ship chilled, double bagged in leak proof container as this is a zoonotic organism.

**General information about the disease:** Chlamydia are intra-epithelial organisms so epithelial cells need to be collected in the specimen. This requires fairly vigorous scraping of the mucosa.

**General information about when this test is indicated:** Although cats can carry chlamydia in their conjunctiva, birds carrying chlamydia usually have conjunctivitis. It is therefore recommended that conjunctival scrapes from birds only be done on those showing clinical conjunctivitis.

**Comparison with other related tests:** This is a referral test so turnaround time can be prolonged. See Chlamydia ELISA and PCR testing as these tests are other options.

**FUNGAL EXAMINATION OF THE SKIN**

In cases where lesions are present, pluck as many hairs as practicable from the peripheral areas for mycology examination.

In suspect infections that are inapparent to the naked eye, and negative to Wood's lamp illumination, brush the animal with an unused toothbrush. Collect the hairs and scurf into a container (e.g. by having newspaper under the animal during brushing). Submit the container with samples and the entire toothbrush to the laboratory for fungal examination.

DO NOT use paraffin oil to aid in the collection of these types of specimens.

**Please note that absence of positive KOH test on hairs and scale does NOT rule out ringworm. Culture is required and this can take up to three weeks.**

**LEPTOSPIROSIS DIAGNOSIS**

**Species:** Cattle, pigs, sheep, horses, goats, deer, dogs

**Specimen:** Fresh urine, body cavity fluid or fresh post mortem tissue (e.g. kidney, liver).

**Container:** Sterile yellow top pot (no additives needed).

**Special handling/shipping requirements:** Store and transport refrigerated. Submit within a week of collection.
General information about the disease

Leptospirosis is one of the world’s most widespread zoonotic diseases. Native, feral and domestic animals may serve as reservoirs, with rats and other rodents recognised as the most important maintenance hosts. Leptospirosis affects a range of domestic animals including cattle, pigs, dogs, sheep, horses, goats and deer. Classically the serovar linkages are cattle with serovar Hardjo and pigs with serovars Pomona and Tarrasovi. There is serological evidence for the infection of sheep, particularly with serovar Hardjo, and sporadic evidence that sheep occasionally experience disease. Infection of horses may also occur, although most cases are subclinical. Infection of dogs with serovar Copenhageni occasionally occurs in Australia.

The clinical signs of infection can range from the inapparent to severe and be influenced by factors such as species, inoculation dose, immune status and age of the animal. Some of the clinical signs associated with acute disease include high fever, jaundice, haemoglobinuria, pulmonary congestion and death. The clinical signs most associated with chronic infections tend to be infertility and reproductive failure. Agalactia can be associated with clinical signs of the disease in dairy cattle. In pigs at slaughter, visible kidney lesions ('white spotting') are often used as an indication that a group of pigs carries leptospirosis infection. However, white spotting may be of limited value in identifying individual pigs infected with *Leptospira*.

There have been 24 *Leptospira* serovars identified in Australia. Human infections result from contact with contaminated soil, vegetation or water, or with the body fluids of infected animals. The average annual incidence of leptospirosis in humans in Australia is 1.0 per 100,000 of population over the last 10 years, and in New Zealand is approximately 2.4 per 100,000 per year. The incidence of leptospirosis in the animal population in Australia and New Zealand is unknown.

General information about when to use PCR versus serology: serology indicates whether an animal has been infected in the past. PCR determines if the agent is still present thereby informing the clinician that an active infection is in progress. The *Leptospira* real time (or quantitative) PCR has been designed to detect pathogenic leptospira isolates. The PCR is based on the method developed by Smythe *et al* (2002).

References:

**Necropsy**

**POST MORTEM GUIDELINES**

Some of our laboratories offer a necropsy service. PLEASE RING and discuss the case and find out if your local lab offers this service before sending in any carcasses. The likely cost can also be discussed at this point. Small birds, fish or reptiles may be submitted whole in formalin, but ideally at least open the body cavities and skull to allow penetration of formalin. Again contact your local laboratory to discuss first.

For cases with multiple mortalities it may be worthwhile examining multiple animals.

Submission of necropsy samples will be generally charged per slide. The first slide is the most costly, where as additional slides are relatively inexpensive. So try to send as many relevant tissues as possible.

IF YOU NEED TO DO THE NECROPSY/ POST MORTEM EXAMINATION YOURSELF

Before you start:

This protocol includes brief guidelines for performing post mortem examinations in the clinic or on farm. Remember to consider your personal safety and that of your assistants and the laboratory staff with any post mortem examination and submission.

If you euthanasing the animal for necropsy, consider taking a range of blood samples prior to euthanasia.

Concentrate on the organs relevant to the clinical signs, but look at everything. This way you familiarise yourself with size, orientation and colour of normal organs.

Write notes. Try your best to describe any changes using as many descriptors as possible- colour, size (actual measurements rather than comparing to inanimate objects), distribution, number, shape, consistency, smell, weight etc. Aim to ‘paint a picture’ of the lesion in the mind of the reader. TAKE PHOTOS if you’re unsure if something is significant- you can always ask one of us later.

Take sections of tissues no thicker than 1cm for histopathology (in any one dimension- it doesn’t have to be a cube). Also consider taking fresh samples for culture or virology, ideally 1-2cm cube of the affected tissue into a sterile pot.

Add abundant formalin (1:10 tissue:formalin is recommended). For larger tissues e.g. brain, you can allow them to fix for several days (ideally 5 days for brain) and then send the tissue in only a small amount of formalin, ideally wrapped in formalin soaked gauze.

When submitting samples from any case, always ensure the outside of containers are clean and dry, no glassware or needles are submitted, and there is appropriate labelling.

Make it clear on the submission form (after notifying the relevant government authorities) if a notifiable or potentially zoonotic disease is considered among the differentials.

**Initial Examination**

Lay the animal on its side. Small animals and horses are generally placed right side down. Ruminants may be done on either side, but some prefer left side down due to placement of the rumen.

Check superficially for wounds, blood in the mouth, eyes sunken, previous surgery, external genitals, condition (fat, thin), skin (alopecia, smell, numerous fleas, thickened or thinned etc).
Opening the Carcass

1. Reflect both the front and back limb then skin the top half of the carcass. If you don’t have a sharp PM knife, then a scalpel should suffice.

2. After skinning, open the abdominal cavity just behind the ribs, along the midline and lay back like a flap.

3. Check the diaphragm is intact then pierce it to check for inrush of air (there should be negative pressure unless there has been pneumothorax or other significant pulmonary lesions).

4. Remove the rib cage with rib cutters or foot shears.

5. Check that all organs appear to be in their correct sites and are of normal size. Check for evidence of previous spay surgery in females.

Removing the Pluck:

1. Ideally, open the mouth and check for lesions, then slit open the intermandibular space to pull the tongue out. Free the tongue from the mandible and cut the hyoid apparatus in the neck. Remove the trachea with oesophagus by holding the tongue and cutting around the soft tissues of the neck.

2. With continued gentle traction on the tongue and oesophagus cut the connections in the chest holding the lungs and heart in place. Cut the oesophagus next to the diaphragm, as well as the aorta and vena cava, to allow removal of the entire pluck.

3. Look for the thyroids.

Oesophagus and Lung Investigation:

First slice along the length of the oesophagus with your scissors. Then slice open the larynx and trachea, looking for froth. Follow the bronchi down into the lung as far as your scissors allow (? lungworm). Then check each lung lobe by feeling for softness, any masses etc. Using a new scalpel blade, cut into each lobe and check for edema and congestion, as well as any abscesses or other masses detected. Check the thoracic lymph nodes by assessing their size and slicing into them (normal ones can be hard to find).

Heart Investigation:

Start by examining the size of the atria and ventricles compared to each other. With scissors, slit open the vena cava and open the right atrium, then cut through the atrioventricular valve and enter the right ventricle. Examine the valves and endocardium. Open the pulmonary artery and examine the valve. Open the left atrium and left ventricle (a slice down the middle of them both is easiest) and examine the AV valve. Then cut through the AV valve to open up the aorta and examine the aortic valve. For histopathology ideally take a section which includes the right and left ventricle as well as intraventricular septum. Depending on the size of the animal, you could submit the whole heart if you have concerns.

Abdominal Investigation:

1. Feel for formed faeces in rectum then cut the rectum, and remove the entire intestinal tract and stomach, as well as the stump of the oesophagus.

2. Open the stomach or abomasum and forestomachs to check for feed quantity, type and assess the mucosa- any erosions or ulcers.

3. Open the small intestine at least at the level of duodenum, jejunum, cecum and colon. If the primary signs are GIT, open the entire tract. It is recommended to take several representative samples throughout the intestines (duodenum, jejunum, ileum, colon) before opening the organ. Keep the tubular sections for formalin small enough that the formalin will penetrate through e.g. 1-2cm long.
For production animals in particular, collect fresh faecal or small intestinal material for possible parasitology or culture.

4. Spleen; check size, any lumps, then slice open in a few areas.

5. Mesenteric lymph nodes; slice open a few, check the size is normal.

6. Liver; quickly check for evenness of colour, any reticular pattern and whether the gall bladder is full. Make serial slices throughout the organ.

7. Kidney; does the capsule peel off easily? Size normal? Any discolouration? Find and follow the ureters- are they even in size?

8. Genital Tract and Bladder; quickly check for abnormalities and slice open the bladder to check the mucosa. Consider collecting urine with a needle and syringe before opening the bladder.

**Bone Marrow:**

If you suspect a problem here, crack open the proximal femur and collect some marrow into a cassette (if available, or an individual small pot of formalin).

**CNS Investigation:**

If the necropsy has so far been inconclusive or the history was consistent with neurological disease, sampling of the CNS is recommended.

- Remove the brain and place in a large amount of fixative. We generally recommend fixing the brain whole, but if you think there may be bacterial infection then consider taking one or two small samples for culture.

- If there was ataxia or paralysis, open the vertebral canal and collect the spinal cord.

- If there was blindness remove the eyes and place them in fixative whole.

- Note: For ruminants in particular, if a metabolic condition is suspected, you can collect ocular fluid for analysis of some biochemical analytes i.e. magnesium, calcium, urea, nitrate/nitrite (For further information see Edwards, G & Foster A. 2009. Use of ocular fluids to aid postmortem diagnosis in cattle and sheep. In Practice 31:22-25).

**Musculoskeletal system:**

Open several joints and examine the joint fluid for any thickening or discolouration. Take note of the appearance of muscle that has been exposed during the necropsy procedure e.g. diaphragm.

**Suspect toxicity or poisoning**

Collect fresh- stomach contents, liver, kidney, skeletal muscle and fat (in separate sterile pots).
### SAMPLES TO COLLECT DURING NECROPSY

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Number of samples (minimum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tongue</td>
<td>1</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>1</td>
</tr>
<tr>
<td>Trachea</td>
<td>1</td>
</tr>
<tr>
<td>Thyroid glands</td>
<td>2 (whole or section both)</td>
</tr>
<tr>
<td>Lung</td>
<td>4 (different lobes)</td>
</tr>
<tr>
<td>Heart</td>
<td>1-2 (include all 3 walls)</td>
</tr>
<tr>
<td>Thymus (of present)</td>
<td>1</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>2 (both whole)</td>
</tr>
<tr>
<td>Kidney</td>
<td>2 (section from each)</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>1</td>
</tr>
<tr>
<td>Liver</td>
<td>2 (from separate lobes)</td>
</tr>
<tr>
<td>Spleen</td>
<td>1</td>
</tr>
<tr>
<td>Stomach</td>
<td>1</td>
</tr>
<tr>
<td>Duodenum</td>
<td>1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>1</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1</td>
</tr>
<tr>
<td>Ileum</td>
<td>1</td>
</tr>
<tr>
<td>Colon</td>
<td>1</td>
</tr>
<tr>
<td>Mesenteric lymph node</td>
<td>1</td>
</tr>
<tr>
<td>Sciatic nerve</td>
<td>1</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>2 (fore and hind-limb)</td>
</tr>
<tr>
<td>Brain</td>
<td>Whole</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>If relevant</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>1 (from femur)</td>
</tr>
<tr>
<td>Reproductive organs</td>
<td>1 of each (whole ovary)</td>
</tr>
</tbody>
</table>

Note: Ensure all fixed tissue is placed in formalin (ratio tissue:formalin must be at least 1:10).

Fresh tissue recommendations - if suspect poisoning/toxicity, vitamin/mineral problem (place in separate yellow pottle): fat, stomach contents, liver, kidney, skeletal muscle.
FINFISH EXAMINATION

Examination of fish is best undertaken in fish that are alive as fish tissues undergo rapid post mortem changes making examination of fish that have been dead >2h of no / minimal diagnostic value.

NOTE: FISH CAN ONLY BE SUBMITTED TO LABORATORIES WHERE NECROPSIES ARE UNDERTAKEN. IT IS ESSENTIAL TO CONTACT YOUR CLOSEST LABORATORY TO ASCERTAIN IF NECROPSIES ARE UNDERTAKEN AT THAT LABORATORY. PLEASE CONTACT THE LABORATORY TO ARRANGE A SUITABLE TIME.

In general fish are submitted to determine:

1. Cause of death/disease; or,
2. For export certification.

To determine cause of mortalities/disease

- Submit 4-6 fish, live, in water.
- Provided that the fish will reach the laboratory within at most 2h after death, one can submit 4-6 fish packed ‘on ice’, NOT FROZEN.
- supply a pertinent background history
- If water quality testing has been undertaken e.g. oxygen level, ph, these findings should be included in the background history.
- Alternatively supply a water sample. We recommend at least 250-500 mL in a plastic pot/bottle. This will be forwarded to a referral laboratory for water quality testing.

The fish will be necropsied and samples collected for histopathology and bacterial culture and forwarded for other tests as deemed necessary by the pathologist on duty.

For export certification

- Hatchling or fingerling fish can be submitted, usually 25 - 50 in 10% buffered formalin.
- Histopathology is undertaken and, if, requested culture.
- For the latter, need fingerlings (30-60mm), smaller fish are too small for gill and kidney sampling for culture.
- Supply a list of the diseases for which the importing country requires exclusion testing.

KEEP IN MIND THAT WE REQUIRE AT LEAST 5 WORKING DAYS TO PROCESS AND EXAMINE THE FISH PRIOR TO ISSUING A REPORT.

PLEASE NOTE:

We only certify for absence of histopathological lesions consistent for the diseases specified. For definitive exclusion testing we advise the submitter to contact the relevant government laboratory to determine what samples should be collected.
AVIAN POST MORTEM EXAMINATION

Basic Outline

- Weigh, external appearance
- Wet
- Reflect legs
- Reflect skin
- Pectoral muscles
- Remove or reflect keel bone and pectorals. Airsacs
- Reflect liver, spleen, alimentary tract (GIT). Examine liver, spleen.
- Remove beak- turbinates. Infrac orbital sinuses.
- Oral cavity, choanal cleft
- Oesophagus and crop
- Larynx and trachea
- Heart
- Lungs
- Reproductive tract
- Kidneys, adrenal glands
- Sciatic nerve, brachial plexus
- Spine, head, brain
- Joints, tendons, section bone, snap long bones
- GIT- proventriculus, gizzard, SI (Meckels diverticulum), pancreas, caecae (lymph tissue)
- Lymphoid system- thymus, Bursa of Fabricius

Good reference books:

Poultry Diseases, 4th or 5th edn, Jordan, F.

A colour atlas of diseases of the domestic foal and turkey, Ranadall, C.J., 1985
Mineral testing

BETA-HYROXYBUTYRATE (BHOB)

Species: Cattle, sheep
Specimens: Serum or plasma
Optimum number of animals to test for mob/herd/flock: 8-10

BHOB, a stable ketone, is commonly used as an indicator of an energy deficit (subclinical and clinical ketosis) and is generally most useful in the transition period from just before calving to mid lactation (just prior to mating) in cattle and in late pregnancy in sheep. It is not a useful test in non-lactating or non-pregnant cattle or sheep and it may be falsely elevated in cows ingesting poor quality silage high in butyrate. In sheep pregnancy toxaemia usually occurs 4-6 weeks before lambing.

Reference interval (cattle): Optimum concentration for milking cows <1.0 mmol/L
Subclinical ketosis (reduced milk production) >1.2 mmol/L
Clinical ketosis >2.0 mmol/L

Reference interval (Sheep): Normal < 1.0 mmol/L however twin and triplet bearing ewes can have slightly increased BHOB concentrations yet appear clinically normal.

COBALT (B12)

Species: Cattle, sheep, goats, deer, alpaca
Specimens: Serum or liver
Optimum number of animals to test for mob/herd/flock deficiency: 8 (serum) and 4 (liver).

General information about the test:
Cobalt is measured indirectly in animals by measuring vitamin B12 a cobalt containing vitamin. Order of susceptibility to cobalt deficiency (high to low) is lambs>adult sheep>calves> kids >fawns>adult goats> cattle>deer.

Serum B12 concentrations can be elevated in lambs by > 6 hours of yarding or a concurrent liver disease (eg. facial eczema). In cattle vitamin B12 analogues produced by some diets can produce sometimes markedly variable and higher than expected results in a group of cows grazing together. This does not appear to occur in sheep.

The liver is the storage organ for vitamin B12.

Reference interval (serum B12): Cattle - Adequate > 150 pmol/L
Sheep - Adequate > 500 pmol/L
Alpaca - Adequate > 70 pmol/L

Reference interval (liver B12): Cattle - Adequate > 220 pmols/kg
Sheep - Adequate > 375 pmols/kg

There are no reference ranges available for deer or goats.
COPPER

Species: Cattle, sheep, deer, goats, alpaca

Specimens: Serum, whole blood (EDTA or Heparin) or liver.

Number of animals to test for mob/herd/flock deficiency: 8 (serum, whole blood) and 4 (liver).

General information about the test:

Copper is stored in the liver so measurement of plasma, ferroxidase or serum copper do not give an estimation of the liver reserves until they are very low < 4.5 umol/L (serum or plasma) or ferroxidase <7 U/L. However if more than two serum coppers are low in a group then the liver coppers of all animals in the group will also be low.

Ferroxidase is a copper containing enzyme which deteriorates over time so using old or haemolysed serum samples can affect results. However it does correlate with serum copper if fresh samples are used.

Although plasma copper also measures the copper which would have been retained in the clot if serum copper had been measured there appears to be no clinical advantage in using plasma copper over serum copper as the reference ranges have been adjusted and both are equally poor indicators of liver stores but can give an indication if copper deficiency is the cause of the current problem.

Serum (live animal) and kidney (dead animal) are the samples to test for copper toxicity. Serum copper >40 umol/L and a kidney copper >150 umol/Kg indicate toxicity. Liver coppers >4000 umol/Kg in liver biopsies or liver taken at slaughter plants from normal cattle indicate the potential for copper toxicity.

Reference interval (serum Cu):
- Cattle - Adequate >8 umol/L
- Sheep - Adequate >8 umol/L
- Deer - Adequate >8 umol/L
- Goat - Adequate >11 umol/L
- Alpaca - Adequate >5 umol/L

Reference interval (plasma Cu):
- Cattle - Adequate >9 umol/L

Reference interval (ferroxidase):
- Cattle - Adequate >14 IU/L

Reference interval (liver Cu):
- Cattle - Adequate >95 umol/kg
- Sheep - Adequate >65 umol/kg
- Deer - Adequate >100 umol/kg

IODINE

Species: Cattle, sheep

Specimens: Serum, plasma or urine (sheep only).

Optimum number of animals to test for mob/herd/flock deficiency: 3-5 (serum, plasma) or 10 pooled urines (sheep only).
General information about the test:

Inorganic iodine is a very stable compound. Inorganic iodine measures the iodine intake of the animal over the previous 2-3 days and the effect of oral or parenteral treatment with iodine supplements. It does not measure the reserves of iodine which are only in the thyroid gland.

Serum thyroxine is not a useful test to detect iodine deficiency in farm animals.

Reference Interval (serum, plasma, urine):

Cattle - Adequate > 40 umol/L
Sheep - Adequate > 40 umol/L (large goitres (60 grams) have been found in neonatal lambs produced by ewes with serum iodine <10 umol/L)
Horse - Serum iodine measured in low numbers of normal horse suggest that their normal range may be much lower compared with other grazing animals i.e. 10-30 umol/L so serum thyroxine may perhaps be a more useful test in this species.
There are no reference ranges available for deer, alpaca or goats.

MAGNESIUM

Species: Cattle and sheep
Specimens: Serum or eye fluid (in recently dead cows or sheep)
Optimum number of animals to test for mob/ herd/ flock: 8-10

General information about the test:

Magnesium is a stable element. Magnesium deficiency occurs most frequently in dairy and beef cattle in the late winter and spring period. This deficiency can be due to a combination of low magnesium and high potassium in spring pasture, the application of slurry to pasture and nutritional stress. A variety of clinical signs may be observed in a deficient herd from peracute (found dead) to chronic (unthrifty cows with an udder oedema). An increased incidence of milk fever and reduced milk yield can indicate subclinical hypomagnesaemia in a herd. Wet cold days and the pasture is wet and lush may precipitate attacks as cows will have lower intakes of pasture. Clinical hypomagnesaemia also occurs in sheep—the causes are similar to those seen in cattle.

Reference Interval:

Cattle - Supplied with results
Sheep - Adequate 0.74 -1.15 mmol/L
Eye Fluid - Adequate > 0.60 mmol/L

NON-ESTERIFIED FATTY ACIDS (NEFA)

Species: Cattle
Specimens: Serum or plasma
Optimum number of animals to test for mob/ herd/ flock: 8-10
Collection protocol: Samples need to be submitted chilled and tested within 2 days of collection.

General information about the test:

NEFA’s is useful for detecting cows in negative energy balance (when body fat is being mobilised) 2-14 days before calving and perhaps colostrum cows. Avoid testing cows that are on the point of calving. Outside
these times it has limited use. Diurnal variation in serum concentrations of NEFA can occur and their lowest concentration is reached 4-5 hours after feeding so blood for testing should collected just before feeding to obtain peak concentrations.

Reference interval (Cattle): Optimum concentration for milking cows <1.0 mmol/L, although some studies have found that normal values for cows in positive energy balance can be as low as < 0.2 mmol/L

PHOSPHORUS

Species: Cattle

Specimens: serum or (soil/ pasture)

Optimum number of animals to test for mob/herd/flock deficiency: 8-10

General information about the test:

Serum phosphorus can be elevated by haemolysis, aging of the sample, azotaemia, enteric disease, milk fever and anorexia. Phosphorus deficiency is commonly seen in dairy cattle a month either side of calving and the usual signs are anaemia and haemoglobinuria. This syndrome is called post parturient haemoglobinuria (PPH). Deficiencies can occur under drought conditions and where pasture phosphates are low due to recent conversions from sheep farms where the requirements for phosphates are much lower.

Reference interval: Cattle - Adequate > 1.4 mmol/L

Note: In PPH, anaemic cattle commonly have serum phosphorus levels < 0.8 mmol/L

SELENIUM

Species: Cattle, sheep, horse, alpaca, deer, goats

Specimens: Serum, whole blood (EDTA or Heparin) or liver.

Number of animals to test for mob/herd/flock deficiency: 5 (liver, serum or whole blood)

Sample collection (liver):

Necropsy sampling - take entire caudate lobe. Refrigerate or freeze;

Biopsy sampling - >50 mg of liver – remove any blood clots at the time of collection and refrigerate. Special collection tubes are available at no charge on request.

General information about the test:

Selenium is not stored for any length of time in tissue-depletes rapidly after dosing. Serum selenium is very stable as is whole blood selenium. Liver or whole blood can be used to diagnose selenium toxicity: >30,000 nmol/Kg (or/L) indicates toxicity. However recently dosed animals (<24-48 hours) can have liver concentrations of selenium up to 30,000 nmol /Kg.

Serum selenium measures current intake and approximates liver concentration.

Whole blood selenium and Glutathione peroxidase (GSHPx) correlate after steady state reached 3 months after dosing. GSHPx is less stable than whole blood selenium so should be measured within 24-48 hours after collection if kept at room temperature or within a week if stored at 4 degrees C. GSHPx can be used to assess selenium status of stock if they have been grazing the same soil type and no selenium supplementation for three months.
As with serum selenium, liver selenium measures current intake as selenium is not stored in any tissue.

**Ref Interval (serum Se):**
- Cattle: Adequate >140 nmol/L
- Sheep and deer – no reference ranges at present

**Ref Interval (whole blood Se):**
- Cattle: Adequate >250 nmol/L
- Sheep: Adequate >250 nmol/L
- Horse: Adequate >1600 nmol/L
- Alpaca: Adequate >350 nmol/L
- Deer and goats - no reference range at present

**Ref Interval (GSHPx):**
- Cattle: Adequate >2 kU/L
- Sheep: Adequate >3 kU/L
- Deer, horse, alpaca and goats - no reference range at present

**Reference Interval (liver Se):**
- Cattle: Deficient < 600 nmol/kg
- Sheep: Deficient < 250 nmol/kg
- Deer: Outbreaks of myopathy (WMD) have been recorded in young fawns with a liver selenium of < 600 nmol/kg.

Alpaca, horse and goats - no reference range at present

**SODIUM**

**Species:** Cattle

**Specimens:** Urine or (pasture/diet).

**Optimum number of animals to test for mob/herd/flock deficiency:** 8-10

In lactating dairy cattle sodium requirements are high. Deficiency signs are reported to include poor conception rates, irregular oestrus cycles and poor milk yield. Because of homeostatic mechanisms the measurement of serum sodium gives erroneous results. The measurement of the potassium (mmol/L):sodium (mmol/L) ratio in urine appears to overcome these problems.

**Reference Interval:**
A median K:Na ratio of <15 suggests a sodium deficiency in a mob of cattle.

**Reference:**

**ZINC**

**Species:** Cattle, sheep

**Specimens:** Serum or liver (serum preferred).

**Optimum number of animals to test for mob/herd/flock deficiency:** 6-10 (serum) and 4 (liver).

**General information about the test:**
There are no significant mobilisable stores of zinc in the body. High levels of calcium, soil and sulphur in the diet can reduce zinc availability to the animal. Do not test sick animals or cows within two weeks of calving as initial values can double within 24 hours.

**Reference interval (serum):**  
- Cattle and sheep - Adequate 9-18 umol/L  
- Toxic >27 umol/L  

**Reference interval (liver):**  
- Cattle and sheep - Adequate 460-1150 umol/kg  
- Toxic - 1760-10200 umol/kg
Neonatal diarrhoea

Neonatal diarrhoea is often a multifactorial problem involving colostrum, nutrition, husbandry, climate and infectious agents. The age of the animal is of utmost importance. In many instances, the infectious agents suspected can be ruled out based on this knowledge.

SAMPLE COLLECTION

Many of the causative infectious agents are transiently present, or produce villous atrophy that is obscured by autolysis; therefore, it is important to keep in mind that intestinal mucosa show histological signs of autolysis within 15 minutes of death, hindering diagnosis. *If fresh samples cannot be obtained, think carefully before requesting histopathology interpretation.* For histology, take multiple 1 cm long segments and immerse in abundant formalin. Minimal handling of the fresh tissue is advised as the mucosa is very fragile and susceptible to manipulation. Alternately, cut the wall of the intestine; at least 1 centimetre along its length, to allow proper fixation.

We suggest you to collect the following range of samples, depending on the age of the animals affected. You can nominate your own tests or let us select the appropriate ones for you.

1) Faeces or colonic content
2) Fixed small and large intestine from fresh intestine (freshly dead animal).
3) Serum
4) Dried milk powder feed

CALF DIARRHOEA

The following table provides a guide for matching ages of scouring calves with the most appropriate tests. 
*Note: SI (Small intestine); LI (Large intestine).*

<table>
<thead>
<tr>
<th>Calf age</th>
<th>Samples</th>
<th>Aetiology</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 7 days</td>
<td>Faeces</td>
<td>E. coli (k99), Salmonella, Cryptosporidium, Rotavirus, Coronavirus, Failure of colostral transfer, Poor quality milk powder</td>
</tr>
<tr>
<td></td>
<td>Fixed SI/LI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sera</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk powder</td>
<td></td>
</tr>
<tr>
<td>1-4 weeks</td>
<td>Faeces</td>
<td>Salmonella, Cryptosporidium, Rotavirus, Failure of colostral transfer, Poor quality milk powder</td>
</tr>
<tr>
<td></td>
<td>Fixed SI/LI</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sera (if &lt; 4 days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Milk powder</td>
<td></td>
</tr>
<tr>
<td>4-8 weeks</td>
<td>Faeces</td>
<td>Salmonella, Coccidia</td>
</tr>
<tr>
<td></td>
<td>Fixed SI/LI</td>
<td></td>
</tr>
</tbody>
</table>
CALF SCOUR PANELS

Gribbles laboratories offer a variety of calf diarrhoea test packages *(only for faecal samples)*. Common packages are as follows. *Note: Individual tests for the listed agents can be requested*

<table>
<thead>
<tr>
<th>&lt; 1 week panel</th>
<th>1-4 weeks panel</th>
<th>4-8 weeks panel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli (K99)</td>
<td>Salmonella</td>
<td>Salmonella</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Rotavirus</td>
<td>Coccidia</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>Cryptosporidium</td>
<td></td>
</tr>
<tr>
<td>Rotavirus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronavirus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Specific information for some agents

1. *Escherichia coli* – Apart from histology and culture, *Enterotoxigenic E. coli* in calves can be diagnosed by antigen *k99 detection with ELISA*. This antigen can also cause diarrhoea in piglets and lambs. *K88 antigen detection is also available* and this antigen is specific for piglets.

   **Gribbles laboratories offer culture for the diagnosis and confirmation of k88 infection and a faecal immunoassay for the diagnosis of k99 infection.**

   **Strain typing is not offered by Gribbles – so cases negative for k88 and k99 are not necessarily negative for E.coli infection.**

   Besides fresh intestine for culture and formalin fixed intestine for histopathology, formalin-fixed tissues of liver and lung may help in diagnosing enteropathogenic E.coli cases.

   Please be advised that even with both culture and histopathology, a small percentage of E.coli cases can still be missed.

2. *Yersinia sp.* is not included in the calf scour panels; therefore, consider requesting it in addition to the calf scour panels above; in calves that are getting close to weaning.

3. *Cryptosporidium*: Pathogenic infections with Cryptosporidium sp. are uncommon in calves > 3 weeks old; therefore, we routinely do not recommend testing for *Cryptosporidium sp. in calves > 3 weeks.*

Other tests

- **GGT** – Levels of GGT are directly proportional to the quantity of colostrum absorbed.

  We recommend taking 5-10 sera samples from affected and in-contact calves. The half-life of GGT in serum is relatively short and so *this test can only be interpreted in calves less than 5-7 days of age.*

- **Milk powder testing** - Curd testing gives an assessment of the powder’s *casein content/quality.*

  Powders that give poor quality may contribute to cases of diarrhoea. *The curd test is only a valid test if it is known that the predominant protein in the milk powder is casein*, as noncasein proteins can be can be successful in raising healthy calves, even they do not tend to form good curds.

  Other analyses include milk fat, carbohydrate and protein levels alone, or assessment or presence and quality of other sugars such as glucose and sucrose, individually or in combination as “total sugars”. Total carbohydrates, including oligo- or polysaccharides can also be assayed. *(This is a referred test, so please indicate the depth of the analyses required when submitting the sample).*
Testing for fat, protein and lactose allows an assessment of feed quality. Although it does not define the digestibility of type of protein sources, it can indicate if high lactose is present and how much of the energy is supplied by the fat fraction.

**GENERAL INFORMATION ABOUT NEONATAL DIARRHOEA IN OTHER SPECIES**

**Neonatal swine**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Age</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli, Coronavirus (Transmissible gastroenteritis), Rotavirus, Isospora suis</td>
<td>&lt; 3 weeks of age</td>
<td>E. coli: No gross lesions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Isospora: &gt;5-6 days</td>
</tr>
<tr>
<td>Clostridium perfringens type C and Clostridium difficile</td>
<td>&lt; 3 weeks of age</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** *Bacteroides fragilis, Salmonella and Klebsiella sp.* have rarely been implicated as a cause of diarrhoea in neonatal pigs. *Strongyloides ransomi* may infect young piglets causing diarrhoea.

**Small ruminants**

- **Lambs:** Causes identified in neonatal lambs are *E.coli, Rotavirus and Cryptosporidium*, overfeeding and very occasionally milk replacer contaminated with bacteria at manufacture. Outbreaks of diarrhoea in colostrum deprived orphan lambs held in sheds and fed milk supplements are not unknown.

  *Salmonellosis* may occur rarely in young lambs. *Bacteroides fragilis*, producing enterotoxin, has been implicated as a cause of diarrhoea in neonatal lambs.

- **Goats:** Causes of undifferentiated diarrhoea are poorly defined, but appears to be minor under usual conditions of husbandry. A similar spectrum of agents that affects lambs may be expected and sought.

  *Clostridium perfringens type B in lambs and kids under 8-10 days* can be recognized as severe haemorrhagic enteritis, occasionally with necrotic ulcers.

  *Coccidiosis* due to *Eimeria sp.* in lambs and kids may occur in animals as young as 3 weeks of age.

  *Strongyloides* may also be associated with diarrhoea in ruminants only a few weeks old.

**Foals**

The most common causes are foal heat diarrhoea, *Strongyloides westeri, Salmonella, E. coli, Rhodococcus equi (1-4 months), Cryptosporidium and Rotavirus*. *Enterotoxigenic E coli and Coronavirus are not proven to cause diarrhoea in foals. Actinobacillus equuli* may cause severe diarrhoea and haemorrhagic enteritis, with lesions of bacteraemia in other organs. Fibrinonecrotic enteritis in foals less than 1 week of age may be due to *Clostridium perfringens type B or C*, or *Clostridium difficile. Rhodococcus equi* may cause chronic diarrhoea and wasting in foals. *Clostridium piliforme* (Tyzzer’s disease) which is restricted to foals under 6 weeks of age may be associated with diarrhoea; however, the liver lesions predominate.

**Fawns**

Outbreaks of diarrhoea and death in unweaned fawns < 2 years of age on pasture have been caused by *Cryptosporidium*. Diarrhoea outbreaks have also been recorded in slightly older fawns exposed to wallows heavily contaminated with faecal bacteria.

E. COLI K99 ANTIGEN ELISA

Species: Ovine, porcine, bovine
Specimen: Faeces
Container: Sterile container
Collection protocol: Collect a 1-2g faecal sample
Special handling/shipping requirements: Standard

General information about the disease:
Enterotoxigenic E. coli (ETEC) is one of the major causes of diarrhoea in neonatal pigs, calves and lambs. K99 refers to one of the specific pilus adhesin antigens in ETEC diseases. Bacteria possessing K99 adhesins adhere mainly to the jejunum and ileum mucosa.

General information about when this test is indicated:
Diarrhoea in young animals in the first week of life. The test determines the presence or absence of antigen in faeces

Comparison with other related tests:
Although pigs can be affected by K99 E. coli, the main cause of ETEC neonatal diarrhoea in piglets is E. coli with K88 adhesins.
Parasitology

COCCIDIA FAECAL OOCYST COUNTS

**Species:** Bovine, Canine, Feline, Avian, Porcine  
**Specimen:** Faeces  
**Container:** Pottle  
**Collection protocol:** Directly from rectum or freshly passed sample from clean surface  
**Special handling/shipping requirements:** Samples may be refrigerated prior to transport

**General information about the disease:** Coccidia of domestic animals are relatively host and organ specific. Those associated with enteric infection belong to the *Eimeria* and *Isospora* genera. Enteric coccidiosis is typically a disease of intensively managed, young animals. The economic cost of coccidiosis in the food animal species is considerable, manifested in mortality, morbidity, subclinical disease, and the cost of management. They are mainly related to malabsorption induced by villus atrophy, anaemia, hypoproteininaemia, and dehydration due to exudative enteritis and colitis.

**General information about when this test is indicated:** Investigation of enteric disease, ill thrift, and production loss in production, companion, and captive wildlife species and to aid prevention and treatment decisions. Oocysts are reported using a semi-quantitative system (- to ++++).

**Comparison with other related tests:** Coccidia oocyst counts are included in the calf scour 4-8 week panel and the dog and cat diarrhoea panel

CRYPTOSPORIDIUM ANTIGEN ELISA

**Species:** Cattle, sheep, goats, dogs, cats  
**Specimen:** Faeces (minimum 0.5g)  
**Container:** Pottle  
**Collection protocol:** Directly from rectum or freshly passed sample from clean surface  
**Special handling/shipping requirements:** Samples may be refrigerated for up to 7 days prior to testing. Freezing is possible for longer term storage but test sensitivity is lost.

**General information about the disease:**

*Cryptosporidium* is a small apicomonad protozoan, found on the surface of epithelium in the gastrointestinal, biliary, and respiratory tracts of mammals, birds, reptiles, and fish. The disease in mammals is generally enteric, while respiratory infection is more significant in birds. *Cryptosporidium parvum* is parasitic in ruminants and *C. canis* and *C. felis* occur in dogs and cats respectively. All are potentially zoonotic and may be associated with contamination of water sources and food products. Cryptosporidiosis particularly occurs in neonates and the immunocompromised. It results in intestinal villus atrophy with lesions most significant in the distal small intestine. It is an important component of undifferentiated neonatal diarrhoea in calves and frequently occurs concurrently with other agents.
General information about when this test is indicated: Diarrhoea investigations, particularly in calves and other young animals.

Comparison with other related tests:
Cryptosporidium antigen ELISA is included in the calf scour <1 week panel, the calf scour 1-4 week panel, the cryptosporidium-giardia combined ELISA test, the small animal diarrhoea panel, and the small animal mini parasitology panel. Cryptosporidia may also be detected by acid fast staining of faeces.

CRYPTOSPORIDIUM OOCYST DETECTION

Species: Cattle, sheep, goats, dogs, cats
Specimen: Faeces (minimum 0.5g)
Container: Pottle
Collection protocol: Directly from rectum or freshly passed sample from clean surface
Special handling/shipping requirements: Samples may be refrigerated if there is any delay in sending to the laboratory (e.g. over weekend)

General information about the disease: Cryptosporidium is a small apicomplexan protist, found on the surface of epithelium in the gastrointestinal, biliary, and respiratory tracts of mammals, birds, reptiles, and fish. The disease in mammals is generally enteric, while respiratory infection is more significant in birds. Cryptosporidium parvum is parasitic in ruminants and C. canis and C. felis occur in dogs and cats respectively. All are potentially zoonotic and may be associated with contamination of water sources and food products. Cryptosporidiosis particularly occurs in neonates and the immunocompromised. It results in intestinal villus atrophy with lesions most significant in the distal small intestine. It is an important component of undifferentiated neonatal diarrhoea in calves and frequently occurs concurrently with other agents.

General information about when this test is indicated: Diarrhoea investigations, particularly in calves and other young animals. Cryptosporidia oocyst numbers are scored using a semi-quantitative system (- to ++++) similar to that used for coccidia oocysts.

Comparison with other related tests: Cryptosporidia may also be detected using an antigen ELISA on faeces.

CYATHOSTOME LARVAE DETECTION

Species: Horses
Specimen: Faeces (5g)
Container: Pottle
Collection protocol: Directly from rectum or freshly passed sample from clean surface
Special handling/shipping requirements: Sample may be refrigerated if transport is delayed

General information about the disease: Cyathostomin (small strongyle) larvae migrate deep into the mucosa or submucosa of the equine large bowel (mainly cecum and ventral colon) to molt and develop. Third or fourth stage larvae may undergo hypobiosis persisting in nodules in the bowel wall. Emergence of the larvae (sporadically or synchronously)
causes significant mucosal damage and eosinophilia, resulting in diarrhoea and protein-loss. Horses >1-year-old are affected, typically in late winter, spring, and summer when larvae emerge.

**General information about when this test is indicated:** Investigation of diarrhoea and ill thrift in horses and evaluation of drench efficacy and parasite control programs

**FAECAL EGG COUNTS – COMPOSITE**

**Species:** Cattle, sheep

**Specimen:** 2-4g faeces per animal

**Container:** Plastic potte (the use of egg cartons is not recommended due to drying out and cross-contamination of samples during transport)

**Collection protocol:** Collect samples directly from rectum of animal (not from the ground unless freshly passed onto clean yard). Sample from 10-15 animals per group. Equal volumes of faeces may be pre-bulked or sent individually.

**Special handling/shipping requirements:** Submit fresh or store in a refrigerator* until transport to a laboratory. Do not freeze.

*It is important that faecal samples for larval cultures are not refrigerated at all. In cases where both FECs and larval cultures are required on the same set of samples it is recommended that a sub-sample be removed from each individual sample and pooled. The individual samples for FEC can be refrigerated while the pooled sample should be clearly identified as being for larval culture and kept at room temperature.

**General information about the disease:** Grazing ruminants in Australia and New Zealand are rarely free of worm infection, though effects on stock health and productivity vary widely. Clinical effects of enteric parasitism include ill thrift, diarrhoea, anaemia, and death in severe cases. The degree of damage is influenced by the numbers and identities of the parasites present, host age, immunity, general health, and nutrition.

**General information about when the test is indicated:** Composite FECs are used to give an overview of the gastrointestinal nematode parasite status of a flock or herd. Indications include monitoring the effectiveness of control programmes, assisting in drench decision-making, and helping to identify the causes of scouring and wasting. They can also be used as a common pre-treatment control group for faecal egg count reduction tests.

**Specific comments relating to disease in Australia and NZ:** None

**Comparison with other related tests:** FECs provide little information on the identity of the worm genera represented. This can be overcome by the use of faecal larval cultures in conjunction with FEC. FEC and larval culture are both necessary for the calculation of faecal egg count reductions to determine drench resistance. Total worm counts on abomasal and small intestinal contents can provide more accurate information on parasite burden than FECs. Serum pepsinogen may be used as an estimate of abomasal ostertagiasis in young cattle.

**FAECAL EGG COUNTS – INDIVIDUAL**

**Species:** Cattle, sheep, horses, camelids, deer, dogs, cats, birds

**Specimen:** 2-4g faeces per animal
**Container:** Plastic pottle (the use of egg cartons is not recommended due to drying out and cross-contamination of samples during transport)

**Collection protocol:** Collect samples directly from rectum of animal (not from the ground unless freshly passed onto clean yard)

**Special handling/shipping requirements:**
Submit fresh or store in a refrigerator* until transport to a laboratory. Do not freeze.

*It is important that faecal samples for larval cultures are not refrigerated at all. In cases where both FECs and larval cultures are required on the same set of samples it is recommended that a sub-sample be removed from each individual sample and pooled. The individual samples for FEC can be refrigerated while the pooled sample should be clearly identified as being for larval culture and kept at room temperature.

**General information about the disease:**
Grazing ruminants in Australia and New Zealand are rarely free of worm infection, though effects on stock health and productivity vary widely. Clinical effects of enteric parasitism include ill thrift, diarrhoea, anaemia, and death in severe cases. The degree of damage is influenced by the numbers and identities of the parasites present, host age, immunity, general health, and nutrition.

**General information about when the test is indicated:**
FECs are used to monitor the effectiveness of worm control programmes, help in the differential diagnosis of cases of scouring and ill thrift, aid drench decision-making, and investigate suspected drench resistance.

Any statement regarding significance of FECs will only be a guide. Interpretation must take into account the age, class, origin, state of nutrition, environment, stage of season, and concurrent disease state of infected animals.

**SHEEP**
FECs provide a relatively robust estimate of worm burdens in sheep and goats, especially those in the first year of life. An exception applies in the case of Nematodirus infections, where limited reliance should be placed on egg counts for diagnostic purposes. A FEC of 500 eggs per gram is generally considered high enough to require treatment in order to limit pasture contamination and subclinical disease. Treatment may be advisable at lower counts depending on the circumstances.

**CATTLE**
FEC in cattle are considered more variable and of less diagnostic value than those in small ruminants due to the stereotypic suppression of Ostertagia ovulation as host immunity develops. However, Ostertagia infections rarely occur in isolation and Cooperia and Trichostrongylus egg production may not be affected by host immunity to the same degree. Consequently, in mixed infections, FECs may still provide useful guidelines regarding herd parasite status, especially in cattle less than 18 months old. FECs in older cattle are frequently unreliable unless a breakdown in host immunity reveals high FECs. In cattle, a FEC of 150-250 is generally considered high enough to require treatment. Clinically affected cattle with diarrhoea often have >1,000 epg.

**HORSES**
FECs are only an approximate guide to worm burden. Clinical history and knowledge of seasonal pattern of parasites in specific geographical regions will assist in interpretation of FECs. The following FECs may indicate clinical disease:

Up to 15 months of age – 100 epg

2-year-old – 200 epg
2-6-year-old – 400 epg
>6-year-old – 600 epg

**Comparison with other related tests:** FECs provide little information on the identity of the worm genera represented. This can be overcome by the use of faecal larval cultures in conjunction with FEC. FEC and larval culture are both necessary for the calculation of faecal egg count reductions to determine drench resistance. Total worm counts on abomasal and small intestinal contents can provide more accurate information on parasite burden than FECs. Serum pepsinogen may be used as an estimate of abomasal ostertagiasis in young cattle. FEC is part of the small animal diarrhoea panel (FEC, coccidia, *Campylobacter*, *Salmonella*, *Giardia*, and *Cryptosporidium*).

### FASCIOLA HEPATICA (LIVER FLUKE) ANTIBODY (MILK)

**Species:** Bovine  
**Specimen:** 50 mL milk  
**Container:** sterile pottle  
**Collection protocol:**
Collect from a well stirred vat one hour after milking finishes. Alternatively, arrange collection from the milk processing company. Contact your local Gribbles laboratory for details  
**Special handling/shipping requirements:** Ship chilled  
**General information about the disease:** Liver fluke establish in the bile ducts of ruminants and interfere with liver function.  
**General information about when this test is indicated:**
This test is offered on bulk tank milk (BTM). This test is a simple way of assessing whether a particular herd is infected and to what extent. As with the serum antibody test, infection by immature flukes is detected and results are reported as graded positives.  
**Comparison with other related tests:** Serum test possible in non-lactating animals

### FASCIOLA HEPATICA (LIVER FLUKE) ANTIBODY (SERUM)

**Species:** Bovine, ovine  
**Specimen:** 10 mL serum  
**Container:** Plain (red top) or gel tube  
**Collection protocol:** Venepuncture  
**Special handling/shipping requirements:** Standard  
**General information about the disease:**
Liver fluke establish in the bile ducts of ruminant and interfere with liver function.  
**General information about when this test is indicated:**
This test can be used to detect liver fluke infection in an individual animal or in a group. Up to ten sera can be pooled. Results are reported as graded positives and correlate with the level of current and/or recent infection.
infection. The test has the advantage of detecting infection with immature flukes – animals are seropositive by six weeks post infection.

**Comparison with other related tests:** Milk test possible in lactating animals

**FLUKE (TREMATODE) EGG COUNTS - INDIVIDUAL**

**Species:** Cattle, sheep  
**Specimen:** Faeces (minimum 3g)  
**Container:** Plastic pottle  
**Collection protocol:** Directly from rectum or freshly passed sample from clean surface  
**Special handling/shipping requirements:** Samples may be refrigerated if there is any delay in sending for analysis (e.g. over weekend)  
**General information about the disease:**  
Fascioliasis in grazing ruminants in Australasia is caused by the trematode *Fasciola hepatica*. Infection occurs in wet, swampy areas and irrigated pastures. The fluke requires an intermediate host in the form of snails in the *Lymnea* genus. Development of eggs through to the infective metacercarial stage occurs mainly in spring and summer and the adult flukes live in the bile ducts. Disease may be acute or chronic, but mostly presents as ill thrift, loss of production, and anaemia in autumn and winter.

**General information about when this test is indicated:** Investigation of production loss, ill thrift, and anaemia in grazing ruminants in fluke risk areas.

**Any specific comments relating to the disease in NZ:** Fluke risk areas include the North Island of New Zealand, and the north and west of the South Island.

**Comparison with other related tests:** In addition to fluke egg counts, a liver fluke ELISA is available to test individual serum samples, pooled serum samples, and bulk milk tank samples, and may be more useful for screening groups of animals.

**GIARDIA ELISA**

**Species:** Feline, canine  
**Specimen:** Faeces  
**Container:** Sterile pottle  
**Collection protocol:** Collect faecal sample either per rectum or once passed  
**Special handling/shipping requirements:** Standard  
**General information about the disease:**  
The combined cryptosporidia and Giardia antigen ELISA identifies both these enteric pathogens. The ELISA detects cryptosporidia and the generic GA 65 antigen released by the Giardia trophozoite when it encysts, rather than relying on the presence of the trophozoite or cyst.

**General information about when this test is indicated:** In animals with non-responsive chronic diarrhoea

**Comparison with other related tests:** A dual test involving cryptosporidia as well.
PARASITE LARVAL CULTURE AND RECOVERY

**Species:** Cattle, sheep

**Specimen:** Faeces (3-5g)

**Container:** Pottle

**Collection protocol:** Directly from rectum or freshly passed sample from clean surface

**Special handling/shipping requirements:**
Submit samples fresh. It is important that faecal samples for larval cultures are not refrigerated. In cases where both FECs and larval cultures are required on the same set of samples it is recommended that a sub-sample be removed from each individual sample and pooled. The individual samples for FEC can be refrigerated while the pooled sample should be clearly identified as being for larval culture and kept at room temperature.

**General information about the disease:**
Grazing ruminants in Australia and New Zealand are rarely free of worm infection, though effects on stock health and productivity vary widely. Clinical effects of enteric parasitism include ill thrift, diarrhoea, anaemia, and death in severe cases. The degree of damage is influenced by the numbers and identities of the parasites present, host age, immunity, general health, and nutrition.

**General information about when this test is indicated:**
Apart from those of *Nematodirus, Strongyloides,* and *Trichuris,* the similarities in size and appearance of most strongyle eggs found in domestic livestock makes differentiation largely impossible. Their third stage (infective) larvae are sufficiently distinct to allow differentiation to at least the generic levels. Faecal culture allows eggs to hatch and develop to the third larval stage. It may be necessary to identify which species are present because some are more pathogenic than others and for drench resistance testing purposes.

**Comparison with other related tests:**
Larval culture may be combined with composite or individual faecal egg counts in faecal egg count reduction testing for the purposes of estimating drench efficacy against parasites.

LUNGWORM LARVAE DETECTION

**Species:** Cattle, deer, horses

**Specimen:** Faeces (minimum 5g)

**Container:** Pottle

**Collection protocol:** Directly from rectum or freshly passed sample from clean surface

**Special handling/shipping requirements:** Samples may be refrigerated if there is any delay in sending for analysis (e.g. over weekend)

**General information about the disease:**
*Dictyocaulus viviparus* is a common and important cause of respiratory disease in cattle in cool seasonal climates. Calves in their first grazing season are most at risk, though clinical disease may occasionally occur in adult cattle that have had insufficient exposure to develop immunity. Clinical signs range from a soft husky cough to fatal dyspnoea. Adult nematodes inhabit the large bronchi. Eggs are embryonated when laid and hatch rapidly. First stage larvae are coughed up, swallowed, and expelled in the faeces. *Dictyocaulus*
arnfieldi is mainly a lungworm of donkeys but is an occasional cause of chronic coughing in horses. Dictyocaulus eckerti causes lungworm disease in deer.

**General information about when this test is indicated:** Investigation of respiratory disease in farmed cattle and deer, horses and donkeys.

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**OSTERTAGIA MILK ELISA**

**Species:** Bovine  
**Specimen:** Milk  
**Container:** Sterile pottle  
**Collection protocol:**  
Collect from the vat or arrange collection via the dairy factory. A form is available from Gribbles Veterinary to authorise milk collection from the factory  
**Special handling/shipping requirements:** Refrigerate until testing or store frozen  
**General information about the disease:**  
Ostertagia larvae infect the abomasal of adult cattle, can lead to abomasal damage, reducing nutrient absorption, and leading to decreases in milk production.  
**General information about when this test is indicated:**  
Collect milk samples near the end of lactation. The milk ELISA test demonstrates the ostertagia parasite burden in the herd. The numerical result generated is the ratio of the sample to the positive control and the values range from 0.1 to about 1.5. Higher levels indicate higher worm burdens. In overseas herds, bulk milk ratios greater than 0.5 have been associated with a depression of milk production and linked with a response to anthelmintic treatment.  
**Comparison with other related tests:** Serum pepsinogen measurement is another test to gauge if there has been abomasal damage. For general parasite screening of adult cattle faecal egg counts are another option.

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**TAENIA OVIS ELISA**

**Species:** Canine  
**Specimen:** Serum  
**Container:** Plain (red top) or gel tube  
**Collection protocol:** Venepuncture  
**Special handling/shipping requirements:** Standard  
**General information about the disease:**  
Taenia ovis is the tapeworm parasite responsible for ovine cysticercosis (sheep measles). Taenia ovis is the subject of a national eradication campaign in New Zealand. A serological ELISA test was developed in New Zealand to supersede the need to purge and test dogs.  
**General information about when this test is indicated:**
If a problem with ovine cysticerosis has been detected in an area, dogs can be bled to detect infected individuals.

**Comparison with other related tests:** N/A

**WORM COUNTS**

**Species:** Cattle, sheep, deer, goats

**Specimen:** Tied off abomasum (for abomasal worm count); tied off proximal 10 meters of small intestine (for SI worm count); tied off cecum and colon to 25cm below spiral colon (for LI worm count).

**Container:** Secure, leak-proof container (e.g. plastic bucket with lid)

**Collection protocol:** Harvest organs at necropsy, tying off proximally and distally to prevent loss of contents.

**Special handling/shipping requirements:** Submit samples fresh in securely sealed container (e.g. bucket with sealed lid).

**General information about the disease:**

Grazing ruminants in Australia and New Zealand are rarely free of worm infection, though effects on stock health and productivity vary widely. Clinical effects of enteric parasitism include ill thrift, diarrhoea, anaemia, and death in severe cases. The degree of damage is influenced by the numbers and identities of the parasites present, host age, immunity, general health, and nutrition.

**General information about when this test is indicated:**

Post-mortem worm counts are used to determine the numbers and identities of gastrointestinal worm burdens in grazing ruminants. The procedure provides a more direct quantification of worm burden than FEC but is more costly and time consuming. Because the level and composition of worm infection may vary considerably between individuals, worm counts need to be performed on several animals in order to obtain meaningful information on the parasite status of the herd or flock as a whole.

In cattle and probably deer, worm counts should ideally include digestion or prolonged saline/water soaking of the abomasal mucosa, particularly in animals >1-year-old. This helps to detect early 4th stage larvae of *Ostertagia* and helps in the diagnosis of type II ostertagiosis, as well as allowing improved quantification of *Trichostrongylus axei*, which may remain adhered to the mucosa after washing.

**REFERENCE(S):**


Carter MJ. Detection of Cryptosporidium oocysts in faecal samples. *New Zealand Journal of Medical Laboratory Techniques* 38:122-3, 1984


McKenna PB. How do you mean? The case for composite faecal egg counts in testing for drench resistance. *New Zealand Veterinary Journal* 55:100-1, 2007

McKenna PB. The diagnostic value and interpretation of faecal egg counts in sheep. *New Zealand Veterinary Journal* 29:139-32, 1981

McKenna PB. The effect of cold storage on the subsequent recovery of infective third-stage larvae from sheep faeces. *Veterinary Parasitology* 80:167-72, 1998


PCR testing

16S BACTERIAL RIBOSOMAL PCR TESTING

Species: All

Specimen: This test is generally referred in-house from initial samples such as histological material, fluid or cultured material

Container: N/A

Collection protocol: N/A

Special handling/shipping requirements: N/A

General information about the disease:

The bacterial 16S rRNA polymerase chain reaction test (PCR) is a conventional PCR which identifies the presence of bacterial species by DNA amplification and agarose gel electrophoresis. The PCR targets the 16S ribosomal RNA region allowing for speciation by sequencing the amplified product. The result is analysed by comparing it to known sequences on the GenBank database held at NCBI in the USA. In most cases, that analysis is able to identify the bacterial species present.

Note that in some cases where bacteria are detected by PCR, sequencing may fail due to the presence of a mixed bacterial population. Also, the sequence analysis is limited to what is recorded on the GenBank database. Due to this an exact species can’t always be clearly identified. In these cases, multiple species may be reported.

Polymerase chain reaction (PCR) detects the presence of infectious agents by identifying the genomic material of the agent being investigated. Unlike serology, which indicates whether an animal has been infected either recently or in the past, PCR determines if the agent is still present thereby informing the clinician that an active infection is in progress. It is often more sensitive and specific than other available tests including culture (in particular for viruses) and is often more rapid than culture.

General information about when this test is indicated:

Sample material where bacterial infection has been identified and is likely to be a pure population.

Comparison with other related tests:

Gram stain can identify many bacteria in histological section but does not allow speciation. Not all bacteria will stain with Gram. Culture may sometimes yield bacterial colonies that cannot be identified by conventional means. PCR will identify dead bacteria whereas culture will not. Coccidia faecal oocyst counts

BOVINE HERPESVIRUS PCR (INFECTIONOUS BOVINE RHINOTRACHEITIS VIRUS, IBR)

Species: Bovine

Specimen: Dry swab

Container: Sterile container

Collection protocol:
For respiratory cases nasopharyngeal, nasal and conjunctival swabs are recommended. For cases with suspect genital tract infection, a vulval/vaginal swab, a penile mucosal swab or exudate from these sites are preferred. Semen for bulls is also accepted.

**Special handling/shipping requirements:**

Dry swab samples are stable at room temperature and can be sent unrefrigerated. If collecting in the field in hot conditions, it is recommended that swabs be stored out of the heat preferable in a cooler until they can be transferred in doors to a controlled environment.

**General information about the disease:**

Bovine Herpesvirus 1 (BHV1) is an infectious agent that generally is found in the respiratory or genital tracts. It is the causative agent of a number of serious diseases in cattle and if not recognised early can cause significant problems in a herd including death in extreme cases.

There are three subtypes recognised worldwide: BHV1.1, BHV1.2a and BHV1.2b, although in Australia, only subtype 2b has been reported (Hungerford 1990). All subtypes have been recognised to cause infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV) or infectious balanoposthitis (IBP), but the virus has been found associated with several other clinical conditions. BHV strains belonging to subtypes 1.1 and 1.2a, which are not relevant to Australia, are more virulent and severe than those belonging to subtype 1.2b. BHV1.1 and 1.2a viruses that cause severe respiratory disease and several other syndromes, including abortion.

Infectious bovine rhinotracheitis is a highly infectious disease. The virus naturally infects cattle, water buffaloes, goats, pigs and deer. The disease is characterised by nasal discharge, rhinitis, tracheitis, conjunctivitis and fever. In general, symptoms are short lived but can be prolonged in cases where other infections are present. Infected cattle can be excrete virus for some time after recovering from the clinical manifestations and therefore risking other members of the herd. This is particularly relevant to cattle kept in close quarters such as in feed lots.

The BHV PCR will detect all type 1 strains of BHV and other closely related strains. The PCR is based on that developed by Wang et al. (2007; 2008) and accredited by the OIE.

**General information about when this test is indicated:**

Suspicion of bovine herpesvirus infection; screening for disease in new introductions and breeding animals.

**References:**

BVD PCR

Species: Bovine
Specimen: 10 mL serum, 50 mL milk
Container: Red top or gel tube, sterile 50 mL pottle

Collection protocol:
Serum - venepuncture
Milk – collect from a well stirred vat one hour after milking finishes. Alternatively, arrange collection from the milk processing company. Contact your local Gribbles laboratory for details

Special handling/shipping requirements:
Standard, keep chilled

General information about the disease:
Bovine viral diarrhoea virus ("pestivirus") is one of the most significant viral diseases in cattle. Clinically, there are three forms of the disease:

- A persistently infected (PI) form which may/may not have clinical signs
- An acute transient form characterised by fever and diarrhoea and short term immunosuppression. These animals will mount an immune response and clear the virus in 10-14 days.
- Mucosal disease (MD) only occurring in PI animals. PI animals are infected by a noncytopathogenic strain of the virus. A subsequent spontaneous mutation of the virus to a cytopathogenic strain within the PI animal results in MD, characterised by seromucoid nasal secretions, severe erosive lesions in the oral and intestinal mucosa, diarrhoea and death.

General information about when this test is indicated:
Screening bulk milk samples from lactating cows is a convenient and swift way of determining the virus status of large numbers. PCR milk testing can detect virus from one infected animal in a herd of 5000.

Serum testing by PCR is performed on pools of 20 animals as the most cost-effective pool size to work with. If virus is detected BVD antigen ELISA is used to individually test each sera and identify the viraemic animal.

Virus screening of all keeper calves is also recommended.

Comparison with other related tests:
PCR virus testing is used in conjunction with BVD antigen ELISA, and antibody ELISA in various forms to determine the BVD status of herds and sub-groups of animals.

Further information:
Collection of bulk tank milk
### BVDv testing summary:

BVDV tests to use in calves from conception to 10 months of age

<table>
<thead>
<tr>
<th>Age</th>
<th>PCR</th>
<th>Antigen ELISA</th>
<th>Antibody ELISA</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception-40 days</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>N/A</td>
</tr>
<tr>
<td>40-120 days gestation</td>
<td>✓</td>
<td></td>
<td>x</td>
<td>Foetal fluid</td>
</tr>
<tr>
<td>150 days – birth</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>Foetal fluid</td>
</tr>
<tr>
<td>Birth-35 days</td>
<td>✓</td>
<td></td>
<td>x</td>
<td>Serum</td>
</tr>
<tr>
<td>35 days -10 months</td>
<td>✓</td>
<td>✓</td>
<td>x</td>
<td>Serum/skin</td>
</tr>
<tr>
<td>10 months and older</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serum/skin</td>
</tr>
</tbody>
</table>

Individual tests for BVDV depending on disease or physiological state

<table>
<thead>
<tr>
<th>Disease or physiological state</th>
<th>PCR</th>
<th>Antigen ELISA</th>
<th>Antibody ELISA</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient infection (TI)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serum/skin</td>
</tr>
<tr>
<td>Conceptus loss</td>
<td>x</td>
<td>x</td>
<td>✓</td>
<td>Serum</td>
</tr>
<tr>
<td>Pregnant (Trojan)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>N/A</td>
</tr>
<tr>
<td>Persistent infection (PI)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serum/skin</td>
</tr>
<tr>
<td>Mucosal disease</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serum/skin</td>
</tr>
</tbody>
</table>

Group test options

<table>
<thead>
<tr>
<th>Physiological state</th>
<th>PCR</th>
<th>Antigen ELISA</th>
<th>Antibody ELISA</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milking</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>Bulk milk</td>
</tr>
<tr>
<td>Non milking</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>Pooled serum</td>
</tr>
</tbody>
</table>

x = No, ✓ = Yes

### EQUINE HERPES VIRUS-1 PCR

**Species:** Equine

**Specimen:** Swabs from nasopharynx, fresh foetal lung, thymus spleen and liver

**Container:** Sterile tube for swabs; Sterile pottle for fresh tissues

**Collection protocol:**

If EHV-1 abortion is suspected:

Collect fresh samples from the aborted foetus - entire foetus ideally or thymus, lung, spleen and liver. Place tissues in a sterile container and transport to Gribbles Veterinary Pathology within 12 hours of collection. It is best to transport these samples in an insulated (polystyrene) container with ice bricks. These tissues will be tested using a PCR protocol unless otherwise stated.
Special handling/shipping requirements: Transport to laboratory chilled within 12 hrs of collection

General information about the disease:

There are 9 equine herpes viruses, 5 of which cause disease in horses. The two most important horse herpes viruses are EHV-1 (which causes abortion, neonatal death, respiratory disease and neurological disease) and EHV-4 (which mainly causes respiratory disease). Other equine herpes viruses include EHV-3 (equine coital exanthema) and the gammaherpesviruses EHV-2 and EHV-5. All of these equine herpes viruses can be detected by virus isolation in cell culture or by PCR tests. Antibody to these herpes viruses can be detected by ELISA (for EHV-1 and EHV-4) or by virus neutralisation tests for all equine herpes viruses.

General information about when this test is indicated: n/a

Comparison with other related tests:

- If active EHV-1 infection is suspected (acute and convalescent serum samples), e.g. If EHV-1 abortion is suspected, but the foetus is not available for testing:
  Submit serum samples for antibody detection by ELISA
  a) Collect initial blood sample (red top tube) for serum and transport to the laboratory within 12 hours of collection. Sample to be held at Gribbles Veterinary and tested with convalescent sample once received.
  b) A second sample should be taken from the horse at between 2 weeks (no less) and four weeks (no longer) after the initial sample.
  c) All samples should be transported to Gribbles Veterinary within 12 hours of collection.
  d) After the second sample has been received by Gribbles Veterinary, both samples will be referred together for paired serological analysis by ELISA testing.

- EHV1-4 screening test (horse moving onto a new property)
  Collect blood sample (plain tube) for serum and transport to the laboratory within 12 hours of collection. The serum will be referred for ELISA testing.

FELINE IMMUNODEFICIENCY VIRUS (FIV) PCR

Species: Feline

Specimen: 0.5-1 mL of EDTA anticoagulated blood.

Container: EDTA or Lithium Heparin tube

Collection protocol: Venepuncture

Special handling/shipping requirements: Standard

General information about the disease:

Feline immunodeficiency virus (FIV) is a lentivirus in the family Retroviridae. It contains RNA and its life cycle involves the integration of its RNA into the DNA of the genome of the host using the enzyme reverse transcriptase. This proviral DNA is then replicated as the cell divides. The proviral DNA is then translated back into viral RNA, and viruses are released from the host cell, the virus receiving its envelope from the host cell membrane. It shows many similar features to HIV, but is unrelated. A number of different subtypes or clades of FIV have been identified by sequencing the gene involved with the viral envelope. Isolates have been divided into five phylogenetic subtypes designated A, B, C, D and E. New Zealand has been found to
have subtype C as the predominant subtype, fewer numbers of subtype A, and a novel, as yet unknown subtree. There is also a putative A/C recombinant strain.

Infected cats carry the virus for life and should be considered infectious at all times. Transmission is predominantly through bite wounds and infected cats are persistently viraemic. Viral replication occurs primarily in CD4+ (T helper) lymphocytes and macrophages, resulting in eventual disruption of cell mediated immunity. Although FIV itself can lead to fatal disease, its main complication is immunodeficiency of the carrier cat making it susceptible to other infections. It is important to know the FIV status of a cat so that these secondary infections, which may be of little consequence in a healthy cat, can be diagnosed and treated before they become serious.

**Stages of Infection**

1. The acute phase: This stage lasts several weeks. Signs include fever, diarrhoea, gingivitis, jaundice, uveitis, conjunctivitis, generalized lymphadenopathy and neutropenia. The severity depends on age. Young kittens have a more florid lymphadenopathy during the acute phase and there is increased severity in adolescents, while geriatric cats show minimal signs but progress more rapidly to the next stages of disease.

2. Asymptomatic carrier: This stage lasts from months to years with no obvious signs, and the cats appear apparently healthy. This stage may last up to 5 years but cats infected at >10 years of age progress through this stage faster than younger cats.

3. Persistent generalised lymphadenopathy and AIDS related complex: Lasts for 6 months to several years. It is characterized by vague, non-specific signs of illness, weight loss, enlarged lymph nodes, stomatitis, anorexia, anaemia, leucopenia, neurological signs and apathy. This is the stage at which the majority of cats are presented to veterinarians.

4. Terminal AIDS-like phase: Lasts less than a year. Cats are emaciated. There are opportunistic infections, lymphoid depletion and miscellaneous disorders including neurologic, renal, immunologic and neoplastic disease.

PCR tests are needed to prove infection is present, if the vaccinial status is not known. The demonstration of the FIV proviral DNA sequence in the host genome is consistent with FIV infection. A subclinical phase of several months to years is common in FIV. Infected cats can succumb to various opportunistic infections, however an FIV positive cat may live for several years without any signs of illness.

All kittens born to infected queens will have maternal antibody present, although only one third will be infected with FIV. Maternally derived antibodies may persist for up to 3 months. Then it may be a further two months before infected kittens seroconvert. PCR testing will not be affected by the presence of maternal antibodies, and a positive PCR test in a cat of less than 6 months would therefore indicate FIV infection.

**General information about when this test is indicated:**

- Lymphadenopathy.
- Persistent unexplained pyrexia.
- Chronic infections (oral, respiratory, ocular, skin, gastrointestinal).
- Neoplastic disease, particularly lymphoma.
- Neurological disease (behavioural change, peripheral lymphadenopathy).
- Introduction of adult cats into multi-cat households.

The real time PCR test detects the presence of the viral genome (antigen), thereby confirming the FIV status of the cat. PCR diagnosis can be made at about 4-5 weeks post infection at which time there should be sufficient virus within the blood stream to make a definite diagnosis (PCR detects integrated virus genome in white cells). Unlike with antibody tests, the presence of vaccinial or maternal antibodies will not affect the PCR result thus a positive PCR test in a cat of less than 6 months would therefore indicate FIV infection.
Comparison with other related tests:
Until recently, diagnosis of FIV has been based on serological tests to identify antibodies to FIV. Infected cats are persistently viraemic and the presence of antibodies in animals over 6 months of age was therefore diagnostic for FIV. The recent release of a FIV vaccine has complicated the diagnosis of FIV. The vaccine elicits a humoral response, thus seroconverting the vaccinated cat. The real time PCR test overcomes this obstacle by detecting the presence of the viral genome (provirus) incorporated into the cat’s lymphocyte genome. This does not occur with vaccination and the detection of FIV genetic material in the lymphocyte genome is therefore specific for FIV infection.

FELINE LEUKAEMIA VIRUS (FELV) PCR

Species: Feline

Specimen: 0.5-1 mL of EDTA anticoagulated blood, bone marrow aspirate (up to 0.5 mL collected into an EDTA tube)

Container: EDTA, lithium heparin or sodium citrate

Collection protocol: Standard venepuncture or bone marrow biopsy

Special handling/shipping requirements:
Blood samples are stable at room temperature and can be sent un-refrigerated. If collecting in warm conditions, it is recommended that samples be stored out of the heat, preferably in an insulated container, until they can be transferred indoors to a controlled environment.

General information about the disease:
Feline Leukaemia Virus (FeLV) is a retrovirus; a single stranded RNA virus belonging to the same viral family as the more common Feline Immunodeficiency Virus (FIV). It is found worldwide but incidence can be quite varied. Although many cats will overcome infection with FeLV, experiencing a transient viraemia or seroconverting with no detectable viraemia, some become persistently infected. Most persistently viraemic cats will develop a range of conditions including anaemia and/or lymphoma, cancers, intermittent immunosuppression and reproductive problems, and die within 3 years. In particular, cats may become susceptible to secondary infections if immunosuppressed. The cat’s age at the time of infection is a major determinant of clinical outcome. A cat found to be persistently viraemic should be isolated from other cats to reduce the risk of passing on the virus.

FeLV invades and replicates in some cells of the cat’s immune system and blood-forming cells. During viral replication, the nucleic acid of FeLV inserts itself into the genome of the infected cells it has invaded. The result can be death of the cell or the viral insert being carried by the cell and passed on to the next generation during cell division. The change in the cells genetic code can also potentially result in cellular changes that can lead to neoplastic disease (cancer). The development of cancer or other conditions may not occur for months or years after the initial infection.

A cat that has overcome viraemia will remain latently infected. Once a cat becomes latently infected, it remains so for life. FeLV may be reactivated on rare occasions from these cats when immunosuppressed or under chronic stress and such cats should still then be considered potential sources of infection.

FeLV is passed from cat to cat via saliva. Unlike FIV that is transmitted via biting, the transfer of FeLV is usually between friendly cats. This can occur during grooming or by sharing food bowls. Occasionally mothers can pass the infection to their kittens either in the womb or via milk. Kittens are particularly susceptible to contracting persistent infections, whereas most adult cats are able to eliminate the virus. Once
a cat becomes persistently infected, it remains so for life. Cats known to be persistently infected should be isolated from other cats to reduce the risk of passing on the virus.

Polymerase chain reaction (PCR) detects the presence of infectious agents by identifying the genomic material of the agent being investigated. Unlike serology, which indicates whether an animal has been infected in the past, PCR determines if the agent is still present thereby informing the clinician that an active infection is in progress. It is often more sensitive and specific than other available tests including culture (especially for viruses) and is usually more rapid to achieve a result.

**General information about when this test is indicated:**

This test is a quantitative PCR test for the detection of FeLV proviral DNA. The FeLV PCR will detect the majority of strains of the virus.

**Comparison with other related tests:**

The FeLV PCR will detect the majority of strains of the virus. The PCR is based on the method used by most diagnostic laboratories and is considered to be both reliable and sensitive for the diagnosis of viral infection. Serology (to detect antibody) is also available but is considered less sensitive and specific; positive serology indicates the animal has been exposed to FeLV, whereas positive PCR indicates genuine persistent infection is present.

**References**


**FELINE CALICIVIRUS PCR**

**Species:** Feline

**Specimen:** Conjunctival and/or a oropharyngeal swab

**Container:** Pottle or sterile tube (no transport media)

**Collection protocol:**

- Moisten a clean, dry swab well with tears/exudate
- Firmly and vigorously swab both of the conjunctival sacs (a local anaesthetic may be used). For FCV oropharyngeal and conjunctival swabs are recommended but nasal and throat swabs are also acceptable.
- Swabs from clinical lesions in the nasal and pharyngeal areas and tissue fragments or biopsies may also be useful.
• Place the swab in a sterile container and keep at 4°C until submission.

Special handling/shipping requirements:
Dry swab samples should be sent in a chiller box with an ice block. Do not place swabs in any transport media as this may affect the sensitivity of the assay. If storing for a period before sending, samples must be stored at 4°C. All samples should be received at the laboratory within 3 days of collection as sensitivity may be impacted by prolonged storage.

General information about the disease:
Feline calicivirus is widespread in the feline population. The virus is shed in oral, nasal and conjunctival secretions. Cats can continue shedding the virus for more than 30 days (sometimes for years) after recovery. Viral RNA may be detected by qPCR in samples from these “carrier” cats but may not be the cause of the current clinical disease. In addition, error-prone replication of the viral RNA generates a high degree of variability in FCV genomes and results in the evolution of many different strains. Although it is difficult to develop a sensitive qPCR assay to detect all the strain variants being generated, the current assay is able to detect the majority of those circulating in the population. The test is most reliable in cases with clinical disease. Recent vaccination should have no effect on the results of the PCR test.

General information about when this test is indicated: Diagnosis of feline calicivirus infection in cats. Can be used in cats showing clinical signs but also recovered cats to check for the presence of virus.

Comparison with other related tests: A single conjunctival or oropharyngeal swab can be submitted and used to test for feline calicivirus, herpesvirus and chlamydophila.

FELINE CHLAMYDOPHILA PCR

Species: Feline
Specimen: Dry swab
Container: Pottle or swab carrier (no transport media)

Collection protocol:
• Moisten a clean, dry swab well with tears/exudate
• Firmly and vigorously swab both of the conjunctival sacs (a local anaesthetic may be used). As the organism is intracellular, it is important that as much cellular material, in the form of conjunctival exudates, be collected to increase the likelihood of detection.
• Place the swab in a sterile container and keep at 4°C until submission.

Special handling/shipping requirements:
Dry swab samples should be sent in a chiller box with an ice block. Do not place swabs in any transport media as this may affect the sensitivity of the assay. If storing for a period before sending, samples must be stored at 4°C. All samples should be received at the laboratory within 3 days of collection as sensitivity may be impacted by prolonged storage.

General information about the disease:
The PCR test is a sensitive test for detecting the presence of the upper respiratory pathogen *Chlamydophila felis*. The test is most reliable in cases with clinical disease. Patients receiving antibiotic treatment for Chlamydophila can be expected to have negative test results after 2-3 days of treatment. Recent vaccination should have no effect on the results of the PCR test.
General information about when this test is indicated: Diagnosis of *Chlamyphila felis*, infection in cats.

Comparison with other related tests: N/A

**FELINE HERPESVIRUS-1 PCR**

**Species:** Feline

**Specimen:** Conjunctival and/or oropharyngeal swab

**Container:** Pottle or sterile tube (no transport media)

**Collection protocol:**
- Moisten a clean, dry swab well with tears/exudate
- Firmly and vigorously swab both of the conjunctival sacs (a local anaesthetic may be used). For FeHV-1 oropharyngeal and conjunctival swabs are recommended but nasal and throat swabs are also acceptable.
- Swabs from clinical lesions in the nasal and pharyngeal areas and tissue fragments or biopsies may also be useful.
- Place the swab in a sterile container and keep at 4°C until submission.

**Special handling/shipping requirements:**
Dry swab samples should be sent in a chiller box with an ice block. Do not place swabs in any transport media as this may affect the sensitivity of the assay. If storing for a period before sending, samples must be stored at 4°C. All samples should be received at the laboratory within 3 days of collection as sensitivity may be impacted by prolonged storage.

**General information about the disease:**

The feline upper respiratory tract disease complex includes those illnesses typified by rhinosinusitis, conjunctivitis, lacrimation, salivation, and oral ulcerations. Feline herpes virus (FHV) is widespread in the cat population. The main source of infection is virus present in ocular, nasal and oral secretions of infected cats. Latent chronic infection is the typical outcome of an acute FHV infection, and intermittent virus reactivation (following stress or corticosteroid treatment) gives rise to viral shedding, despite vaccination. Some clinically normal cats may shed virus and thus qPCR results need to be interpreted with the clinical history. Therefore when FHV DNA is detected by qPCR it may indicate the primary cause of disease, virus reactivation secondary to a primary disease, or virus reactivation unrelated to the cause of the current clinical disease.

The test is most reliable in cases with clinical disease. Negative test results are expected in patients with latent herpes infections as the virus is found in the trigeminal ganglion during this period. A negative test does not therefore exclude feline herpesvirus infection. Recent vaccination should have no effect on the results of the PCR test.

**General information about when this test is indicated:**

Diagnosis of feline herpesvirus infection in cats. Can be used in cats showing clinical signs but also recovered cats to check for the presence of virus.

**Comparison with other related tests:** Can be used in cats showing clinical signs but also recovered cats to check for the presence of virus.
FELINE UPPER RESPIRATORY TRACT PATHOGENS PCR

Species: Feline
Specimen: Dry swab
Container: Bottle or swab carrier (no transport media)

Collection protocol:

- Moisten a clean, dry swab well with tears/exudate
- Firmly and vigorously swab both of the conjunctival sacs (a local anaesthetic may be used).
- Swabs from clinical lesions in the nasal and pharyngeal areas and tissue fragments or biopsies may also be useful.
- Place the swab in a sterile container and keep at 4°C until submission.

Special handling/shipping requirements:

Dry swab samples should be sent in a chiller box with an ice block. Do not place swabs in any transport media as this may affect the sensitivity of the assay. If storing for a period before sending, samples must be stored at 4°C. All samples should be received at the laboratory within 3 days of collection as sensitivity may be impacted by prolonged storage.

General information about the disease:

The feline upper respiratory tract disease complex, also known as feline respiratory disease (FRD) syndrome, is caused by a range of infectious agents and includes those illnesses typified by rhinosinusitis, conjunctivitis, lacrimation, salivation and oral ulcerations. The common infectious causes include Feline Herpesvirus type 1 (FeHV-1), Feline Calicivirus (FCV), *Chlamydomphila felis* (formerly *Chlamydia psittaci* var. *felis*), *Bordetella bronchiseptica* and *Mycoplasma* spp. infections. FeHV-1 and FCV are the most common viral causes of sneezing and nasal discharge in the cat. Calicivirus infection is most commonly associated with oral ulceration while corneal ulceration is more likely in FeHV-1 infections. FeHV-1 is commonly a persistent infection with long periods of latency and has been associated with chronic stomatitis, facial dermatitis, and endogenous uveitis. All these infectious agents are more prevalent in multiple-cat households and catteries and concurrent infections are common.

All these agents are spread through close animal contact, often through the ocular or nasal discharges associated with infection. Aerosols due to sneezing can also be a significant source of infection. Most importantly, these infections are more prevalent in multi-cat households or in catteries and concurrent infections with more than one agent found often.

The PCR test is a sensitive test for detecting the presence of the upper respiratory pathogens *Chlamydomphila felis*, Feline Herpesvirus and Feline Calicivirus. The test is most reliable in cases with clinical disease. Negative test results are expected in patients with latent herpes infections as the virus is found in the trigeminal ganglion during this period. A negative test does not therefore exclude feline herpesvirus infection. Patients receiving antibiotic treatment for *Chlamydomphila* can be expected to have negative test results after 2-3 days of treatment. Recent vaccination should have no effect on the results of the PCR test.

Polymerase chain reaction (PCR) detects the presence of infectious agents by identifying the genomic material of the agent being investigated. Unlike serology, which indicates whether an animal has been infected either recently or in the past, PCR determines if the agent is still present thereby informing the clinician that an active infection is in progress. It is often more sensitive and specific than other available tests including culture (in particular for viruses) and is often more rapid than culture.

The FRD PCR is performed in two separate assays the first detecting FeHV-1 and C. felis and a second to identify FCV. All assays include appropriate PCR and sample controls to identify problem samples or failures in processing.
**General information about when this test is indicated:** Specific diagnosis of upper respiratory tract infection in cats.

**Comparison with other related tests:** N/A

**References**


Helps et al. (2005), *Factors associated with upper respiratory tract disease caused by feline herpesvirus, feline calicivirus, Chlamydo phila felis and Bordetella bronchiseptica in cats: experience from 218 European catteries.* Vet. Record 156: 669-673.

**HAIRY SHAKER DISEASE/ BORDER DISEASE VIRUS (BDV/HSD) PCR**

**Species:** Ovine

**Specimen:** Serum, or heart blood from a dead foetus

**Container:** Plain (red top) or gel tube

**Collection protocol:** Venepuncture

**Special handling/shipping requirements:** Standard

**General information about the disease:**

Hairy shaker disease (HSD) is caused by a Pestivirus. Border disease (BD) viruses survive for only a short time in the environment. Spread is usually by the oral/nasal route thus the rate of virus transmission is increased under conditions of intensive husbandry. As with BVDV the persistently infected (PI) sheep is a major component of disease transmission. Healthy non-pregnant sheep exposed to the virus may have mild symptoms with a slight fever, a short lived viraemia and develop a long-lived immunity. The virus appears to be able to cross the placenta with ease.

When previously naïve ewes are first exposed during pregnancy effects similar to those in BVDV infection are seen and vary depending on the age of the foetus at the time of infection. It appears that prior to 16 days gestation the zygote is refractory to infection, and after 90 days gestation the ovine fetal immune system is capable of eliminating the infecting virus. However infection between 16-90 days of gestation causes: early embryonic death, abortions and stillbirths, birth of lambs with malformations, dead or alive (often dying soon after if alive), birth of small, weak lambs, some often 'hairy' and 'shaking'. Combinations of all of the above can be seen on one property.

The 'hairy' fleece on lambs is the most obvious clinical sign but if lambs survive past a few months of age this may disappear. It can be useful to identify 'hairy' lambs at tailing/docking. As not all persistently infected lambs (PI's) are 'hairy' it is important to check flock members by blood testing. The 'shaky' syndrome (sometimes with severe locomotor disturbances and an inability to stand) is due to a deficiency of myelin at all levels of the CNS and cerebellar hypoplasia. Surviving lambs are persistently infected and excrete virus in their urine, faeces, saliva and blood. They have poor growth rates and are often susceptible to many other
diseases. Infected ewe lambs that survive to sexual maturity and breed will always produce a PI lamb. Infected males that reach sexual maturity will have poor quality, highly infective semen and reduced fertility. These PI lambs should be culled or identified as a non-replacement at the time of tailing/docking. As with cattle, PI animals can develop 'mucosal disease' type infections and often die within a few weeks.

**General information about when this test is indicated:**

No antibody is produced by PI animals. A border disease virus specific and sensitive PCR test is available that will detect the Australasian strains of BDV.

**Comparison with other related tests:**

The cattle BVDV PCR used at Gribbles Veterinary also detects Border Disease Virus.

### LEPTOSPIRA PCR

**Species:** Ovine, cervine, bovine, caprine, llamoid, canine, equine, porcine

**Specimen:** Whole blood, urine

**Container:** EDTA tube, sterile pottle

**Collection protocol:** Venepuncture, mid-stream urine or cystocentesis

**Special handling/shipping requirements:**

Double bagged in a leak proof container as this is a zoonotic disease and can be spread to humans through breaks in the skin

**General information about the disease:**

Leptospirosis is a zoonosis caused by one of the many pathogenic serotypes of the genus *Leptospira*, a spirochete that is transmitted by direct contact of abraded skin or mucous membranes with urine or tissues of an infected animal or, more commonly, by indirect contact with mud or water contaminated by urine of infected animals. Rodents are the most common carrier of Leptospira. Most mammals are considered as carriers of the bacteria but the most commonly encountered domestic animal carriers are pigs and cattle.

*Leptospira* species are a diverse group of organisms, many of which are non-pathogenic. There are numerous species and serovars throughout the world. Leptospira serovars are divided into serogroups depending on their serological responses. The main *pathogenic* serovars isolated from animals belong to the *Leptospira* species *borgpetersenii* and *interrogans*. Rats, mice, hedgehogs, pigs, dogs, possums and cattle have all been reported as carriers of leptospirosis. Pigs are maintenance hosts for Tarassovi and Pomona, while cattle are maintenance hosts for Hardjo. Serovars Copenhageni and Ballum have been isolated from herds of healthy calves, which showed no clinical signs attributable to leptospirosis. Sheep have been shown to maintain serovar Pomona with significant economic losses on affected properties. Farmed deer are maintenance hosts for serovars Hardjo, Pomona and Copenhageni. Pathogenic leptospires such as Copenhageni, Tarassovi, Pomona and Hardjo can "accidentally" infect dogs and may cause severe disease.

The bacteria are spread through the urine of infected animals due to a chronic infection of the renal tubules. The clinical signs of infection can be influenced by factors such as inoculation dose, immune status and age of the animal. Severity ranges from the inapparent to severe. Some of the clinical signs associated with acute disease include high fever, jaundice, haemoglobinuria, pulmonary congestion and death. The clinical signs most associated with chronic infections tend to be infertility and reproductive failure. Agalactia can be associated with clinical signs of the disease, particularly in dairy cattle.
General information about when this test is indicated:

PCR allows rapid specific and sensitive diagnosis of clinical leptospirosis. The assay can detect DNA from as few as 1-10 organisms per ml of urine sample. Using PCR, it is possible to quantify the amount of template and therefore the number of target organisms. The PCR test also paves the way for screening of sub-clinical shedders of the organism in situations where there is a significant health and safety risk, e.g. dairy sheds, piggeries, sheep farms and abattoirs. Currently PCR is unable to identify the infecting serovar.

Comparison with other related tests:

PCR has a very high positive and negative predictive value but only determines if pathogenic leptospires are present or not. Confirmation of the serotype requires blood sampling of surviving and/or contact animals. Confirmatory tests include serologic testing such as MAT to detect antibody production to leptospira. Very high antibody titres are suggestive of infection, but paired serum titres produce more reliable prognostic information. Direct detection of the bacterium may be done by culture of urine or blood culture, identification of leptospiral DNA, fluorescent antibody staining of urine, or urine dark-field microscopy. Culture of *Leptospira* spp. can be difficult and time-consuming. Early in the course of infection, PCR tests on the blood and slightly later the urine will be positive but at this time antibody titres will be negative. Antibody takes a week to ten days to start to develop and then longer for high diagnostic titres to occur. Where late in the course of the disease, urine can be PCR negative while the animal will be antibody positive. The time taken for development of antibody titres varies depending on innate factors within the individual animal, infective dose, infective organisms, etc. To detect animals shedding *Leptospires* submit urine samples. An EDTA blood sample from an acutely infected animal may identify Leptospiraemic animals. Body fluids from aborted foetuses can be submitted where leptospirosis is suspected.

**MALIGNANT CATARRHAL FEVER (MCF) PCR**

**Species:** Bovine, cervine, swamp buffalo

**Specimen:** Whole blood

**Container:** EDTA tube

**Collection protocol:** Venepuncture

**Special handling/shipping requirements:** Standard

**General information about the disease:**

Malignant catarrhal fever (MCF) is a systemic viral disease caused by sheep-associated ovine herpes virus-2 (OHV-2) affecting cattle, deer and swamp buffalo. Cases can present with head and eye, peracute and mild forms of disease and there is usually a markedly elevated rectal temperature. The head and eye form of disease includes central nervous disease, ocular, nasal or oral lesions. There may also be diarrhoea, weight loss or respiratory disease.

**General information about when this test is indicated:**

Lesions in the eyes are the most likely reason veterinary attention will be sought for cattle suffering from MCF. Affected animals are also likely to be pyrexic with markedly elevated rectal temperatures as high as 42ºC. Many animals will have oral or nasal lesions as well but some animals will present with only one set of symptoms, such as neurological signs or ill thrift. Cases of; oral lesions only, lymph node swelling only, and skin lesions only, are also possible so it is important to keep MCF in your differential list for many conditions, and consider further testing if other diagnoses are ruled out.
Comparison with other related tests:
Differentials diagnoses for other diseases e.g. oranasal disease due to IBR can be excluded with IBR PCR and gastrointestinal disease e.g. BVDv, Yersinia, by PCR, or bacterial culture

Further information

MYCOBACTERIAL NON TB (GENERIC) PCR TESTING

Species: All

Specimen: Fresh tissue, formalin-fixed tissue from processed histology blocks; swab

Container: Pottle (for fresh tissue or swabs), 10% neutral buffered formalin (for histological material)

Collection protocol: Standard

Special handling/shipping requirements: Standard

General information about the disease:
Mycobacteria can infect a wide range of domestic and non-domestic animal species and cause a range of disease patterns. These typically include granulomatous diseases affecting any organ system but often seen in the subcutaneous tissues, gut and viscera. This test will detect all mycobacterial species.

General information about when this test is indicated: Granulomatous inflammatory disease.

Comparison with other related tests:
Mycobacteria can also be detected by acid-fast staining of swabbed material (such as wound exudate) and histological staining of affected tissues. PCR has the advantage of detecting mycobacteria when numbers are too low for visualisation by staining, and amplified genetic material can be sequenced and compared to known databases to determine the species of mycobacteria involved.

NEOSPORA PCR

Species: Bovine, ovine

Specimen: Fresh (or formalin fixed) brain and/or stomach contents from aborted fetus

Container: Sterile container

Collection protocol: Necropsy

Special handling/shipping requirements: Standard

General information about the disease:
Neospora caninum, an apicomplexan protozoan parasite infection, affects mostly cattle where it may result in abortions. These events may take the form of sporadic or low-level endemic occurrences of abortion, or be of epidemic, “storm-like” proportions. These abortion storms, in particular, can affect large proportions of the at-risk (i.e. in-calf) cow population and cause large economic losses.

Some work suggests sheep may be affected too, but the likely sero-prevalance is low.
General information about when this test is indicated:

The PCR is most suitable for abortion investigations to detect *Neospora caninum* DNA in the brain or stomach contents of an aborted fetus. This test has not been used to see if the organism is present (transiently) in the blood of animals in the early stages of infection.

A “detected” result indicates animals infected with *Neospora caninum* but will not necessarily distinguish acute or chronic subclinical encysted infection. A “not detected” result indicates that *Neospora caninum* DNA was undetectable in the sample and in most cases this means that clinical signs are unrelated to infection with *Neospora*. However, the organism may be present at levels below the detection limit of the assay or not present in the sample submitted and examined.

Comparison with other related tests:

Use the PCR result in combination with IFAT and the clinical signs for investigating the Neospora status of individual animals. The IFAT is the most appropriate assay for individual abortion diagnoses in the dam, as titres are elevated around the time of abortion and then quickly decline within a matter of weeks. In the dam, an IFAT titre of ≥1/600 is indicative of an association between the abortion and Neospora infection. For investigation of reproductive disease in groups use the Neospora ELISA.

**MYCOPLASMA HEMOFELIS PCR**

*Species:* Cats  
*Specimen:* 1 mL whole blood  
*Container:* EDTA (purple top) tube  
*Collection protocol:* Venepuncture  
*Special handling/shipping requirements:* Standard  

General information about the disease:

*M. haemofelis* is a blood parasite of cats that can cause severe regenerative anaemia. This parasite was formerly known as *Haemobartonella felis* but is now classified as a mycoplasma. Although infected cats may not show signs of clinical disease, in association with other agents (or immunosuppression) *M. haemofelis* can cause significant disease including potentially fatal anaemia. Common symptoms are intermittent fever, lack of appetite, depression, lethargy and pallor. Symptoms can be more severe when associated with other conditions such as Feline Leukaemia Virus infection.

A related organism of lesser pathogenicity, Candidatus *M. haemominutum*, is also detected and reported separately in this assay. The pathogenicity of Candidatus *M. haemominutum* is not fully understood. However, it is recommended that cats positive to either organism and showing clinical signs, be treated. These mycoplasmas should be considered as potential complications in cats that have been shown to be positive for feline immunodeficiency virus or feline leukaemia virus.

The detection of Mycoplasma in a blood sample should not necessarily be interpreted as that organism being the primary cause of the disease. Other causes of anaemia should be excluded including blood loss into the gut, effusions, neoplasia and chronic viral infections.

The frequency of *M. haemofelis* infection in a normal cat population, including Candidatus *Mycoplasma haemominutum*, has been reported to be as high as 40%. The published figures do however vary greatly as a result of the lack of sensitivity of the conventional blood smear method and differences in methodologies of the PCR assays published to date.
General information about when this test is indicated:

- Unexplained anaemia.
- Lethargy or depression.
- Intermittent fever, pale mucous membranes, jaundice and splenomegaly

Comparison with other related tests:

Traditionally, *M. haemofelis* has been detected by staining freshly prepared blood smears with Wright-Giemsa stain and examining for the presence of parasites on the erythrocytes. This often gives an equivocal result, as the parasites tend to fall off the erythrocytes soon after the blood is taken making an accurate diagnosis difficult. PCR testing is significantly more sensitive and specific than examination of a blood smear.

References:


**STREPTOCOCCUS EQUI SUBSPECIES EQUI PCR**

**Species:** equine

**Specimen:**

Dry swab from nasal, throat or nasopharangeal area, or nasopharangeal lavages or aspirates. Guttural pouches for detection of persistent infections.

**Container:** Place dry swab in a sterile pottle

**Collection protocol:** swab, lavage or aspirate the affected area

**Special handling/shipping requirements:** Samples should be held and transported chilled - do not freeze

**General information about the disease:**

Strangles is a contagious bacterial disease of horses caused by *Streptococcus equi* subsp. *equi*. Clinical manifestations include purulent nasal discharge, fever, anorexia and swollen submandibular and retropharyngeal lymph nodes which frequently form abscesses. Diagnosis has traditionally involved culture of nasal swabs, washes or pus aspirated from abscesses. While this is considered the ‘gold standard’
method, detection and confirmation of *S. equi* subsp. *equi* can take several days and identification may be complicated by the presence of other group C β-haemolytic streptococci such as *Streptococcus equi* subsp. *zooepidemicus* and *Streptococcus dysgalactiae* subsp. *equisimilis*.

**General information about when this test is indicated:**

This PCR is a sensitive and rapid test compared to culture; a positive result by PCR will indicate the presence of the *S. equi* subsp. *equi* DNA in the sample (even if the bacteria are dead). Thus the PCR can still detect *S. equi* subsp. *equi* when antibiotic treatment has already commenced and this is particularly important if the culture result is negative. If a PCR positive result is returned, the recommendation would be to complete the antibiotic treatment and then re-test about 2 weeks post treatment to confirm clearance of the bacteria. Healthy horses may be tested for the absence of infection, the organism may still be present in the guttural pouch.

**Comparison with other related tests:**

Although the primary purpose of this assay is to detect *S. equi* subsp. *equi*, the multiplex PCR will also be able to determine the presence of *S.equi* subsp. *zooepidemicus* DNA in the sample and this will be reported. The *S. equi* subsp. *equi* PCR is not approved for export testing.

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**THEILERIA ORIENTALIS IKEDA PCR**

**Species:** Bovine  
**Specimen:** Whole blood  
**Container:** EDTA tube  
**Collection protocol:** Venepuncture  
**Special handling/shipping requirements:** Standard  
**General information about the disease:**

This is a tick borne protozoan parasite infection of cattle red blood cells resulting in anaemia.

**General information about when this test is indicated:**

In anaemic cattle; to confirm the cause of anaemia is *T orientalis* Ikeda related, and to rule out other causes of anaemia. In healthy cattle; to screen for the presence/absence of *T orientalis* Ikeda.

In the South Island a government subsidy exists for PCR testing of anaemic cattle.

**Comparison with other related tests:**

Diagnosis can be made by examination of red blood cells on smears where the parasite can be visualised. A PCR for *Theileria orientalis* chitose and *Theileria orientalis* buffeli are available as separate tests.
**TRITRICHOMONAS FETUS PCR - CATTLE**

**Species:** Bovine

**Specimen:**
Preputial scraping/washing and vaginal mucus. These samples can be collected with a scraping device called a ‘Tricamper’ (contact Gribbles Veterinary for supplies)

**Container:** ‘Tricamper’ end clipped off into a sterile pottle or plain tube

**Collection protocol:** Use the ‘Tricamper’ to scrape the preputial lining or the vagina

**Special handling/shipping requirements:** Ship chilled

**General information about the disease:**

*Trichomonas fetus* is a flagellate, pyriform protozoan parasite that can cause infertility in cattle.

*Trichomonas fetus* resides in the preputial cavity of bulls, with some concentration in the fornix and around the glans penis, with little or no clinical signs. Chronically infected bulls show no gross lesions. For bulls older than 3–4 years, spontaneous recovery rarely occurs, resulting in a permanent source of infection in herds. In bulls under 3–4 years old, infection may be transient.

In the infected cow, the initial lesion is a vaginitis and animals may exhibit irregular oestrous cycles. Cows usually clear their infection and generally become immune, at least for that breeding season. If infected cows become pregnant the organism may invade the cervix and uterus with various outcomes including placentitis leading to early abortion (1–16 weeks), uterine discharge, and pyometra. Not all infections result in abortion and a normal calf is born.

Transmission of infection occurs by coitus, by artificial insemination, or by gynaecological examination of cows. Bulls are the main reservoir of the disease as they tend to be long-term carriers, whereas most cows clear the infection spontaneously. For these reasons samples from bulls are usually preferred for diagnosing and controlling the disease in herds.

**General information about when this test is indicated:**

PCR provides a very sensitive and specific method for detection of *Trichomonas fetus* in clinical samples. The organisms do not need to be viable and it will detect very lower numbers. Currently this method is not approved for export testing.

This is a disease of low prevalence in New Zealand

**Comparison with other related tests:**

The traditional method for diagnosis is culture and microscopic examination but the sensitivity of this method is relatively poor given as it depends on a relatively uncontaminated sample being collected in a manner that maximises the number of organisms present and for the sample to reach the lab in a viable state for culture.

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**TRITRICHOMONAS FETUS PCR - FELINE**

**Species:** Feline

**Specimen:** A small sample (~50 grams - a lima bean-sized sample) of litter-free faeces or an In-PouchTM culture (Biomed Diagnostics).

**Container:** Pottle for faeces, In-Pouch system for cultures

**Collection protocol:** Passed faeces or collected per rectum
**Special handling/shipping requirements:** Standard

**General information about the disease:**

*Tritrichomonas fetus* is a protozoan parasite that has been reported in cats since 1956. Infection with this parasite causes chronic diarrhoea accompanied by large bowel inflammation and faecal incontinence. The faeces may be haemorrhagic and/or mucoid and may be accompanied by flatulence and tenesmus. Little is known as to how infection occurs. It is not known if the bacteria and other flora of the intestinal tract contribute to the ability of the *T. fetus* to establish and maintain infection in the intestine. It is possible that there are breed susceptibilities to infection. There appears to be no differences in infection between sexes.

Concurrent infection such as immunosuppression with retroviral (FIV, FeLV) infections may predispose to infection. Most infections resolve spontaneously, but this can take years and relapses can occur. Treatment of symptomatic cats is usually recommended due to the long carrier status and potential to infect other cats during this period. Treatment with Ronidazole, 30mg/kg, dietary and environmental management are recommended. Metronidazole is not effective and tinidazole has only partial efficacy.

**General information about when this test is indicated:**

Infection with *T. fetus* should be considered in cases where there is chronic diarrhoea, and other examinations for bacteria, nematodes, giardia and cryptosporidium are negative. Diagnosis in the past was based upon the observation of the live organism in a direct smear or cultured sample. The PCR test to detect *T. fetus* in cat faecal samples has the advantage of not requiring viable organisms, so transport temperatures are not critical. The PCR is very specific and has a higher sensitivity than microscopy or culture. PCR can detect viable and non-viable organisms, so a positive PCR in a cat without diarrhoea may not indicate the need for therapy.

As antibiotics can temporarily reduce faecal shedding, they should be withheld for around seven days prior to testing.

**Comparison with other related tests:**

Inoculation of the In-PouchTM culture system from Biomed Diagnostics is another option for culture and identification of the organism. PCR can also be used to test the inoculant of the In-Pouch system for *T fetus.*
Serology

Production animals:

**BOVINE RESPIRATORY SYNCYTIAL VIRUS ELISA**

Species: Bovine  
Specimen: Serum  
Container: Red top or gel tube  
Collection protocol: Venepuncture  
Special handling/shipping requirements: Standard  

**General information about the disease:**

Bovine respiratory syncytial virus (BRSV) virus can cause severe primary pneumonia when cattle are first infected. There is also a subsequent mild to subclinical respiratory infections. BRSV is an important component of the bovine respiratory complex as there are often multiple pathogens involved.

**General information about when this test is indicated:**

To determine if pneumonia has a BRSV infection as part of the aetiology

**Comparison with other related tests:**

Virus isolation is also a possible diagnostic step to confirm the presence of virus. Histopathology of lung from affected cattle is another possible means of diagnosis as characteristic syncytial cells form in the lung.

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**BOVINE HERPESVIRUS-1 ELISA (BOHV-1; IBR/IPV)**

Species: Bovine  
Specimen: Serum  
Container: Red top or gel tube  
Collection protocol: Venepuncture  
Special handling/shipping requirements: Standard  

**General information about the disease:**

Bovine herpesvirus-1 causes two diseases in cattle: infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis/ balanoposthitis (IPV/IBV). The clinical signs of IBR are characterised by fever and involvement of the upper respiratory tract, including conjunctivitis, rhinitis and tracheitis. Secondary bacterial infections may lead to pneumonia, especially in intensively managed livestock, such as beef cattle in feedlots. The venereal forms of the disease result in pustular lesions in the prepuce and penile epithelium of the bull and vulva and vagina of the cow. These lesions can impair reproduction. Abortigenic strains of BoHV-1 have not been found in Australasia.
The virus is spread both within and between herds mainly by horizontal transmission such as direct and indirect contact (fomites) and aerosol droplets, or from infected bulls by coitus and in infected semen either by artificial or natural insemination. Infection with BoHV-1 results in a lifelong latent infection that may be re-activated following stress or corticosteroid treatment resulting in virus excretion. A proportion of infected bulls will chronically excrete virus in their semen.

**General information about when this test is indicated:**

To determine if there has been previous infection with bovine herpes virus and a serological response. The ELISA test generates a positive or negative result to determine if infection has occurred or not. To assess the size of the antibody response, virus neutralisation titres (VNT) on paired convalescent sera are required as an additional but more expensive test.

ELISA can also be used to determine freedom from infection

**Comparison with other related tests:**

PCR can be used to detect if bovine herpes virus is present in discharges.

### BVD ANTIBODY ELISA

**Species:** Bovine

**Specimen:** 10 mL serum, 50 mL milk

**Container:** Red top or gel tube, sterile 50 mL pottle

**Collection protocol:** Serum – venepuncture; Milk – collect from a well stirred vat one hour after milking finishes. Alternatively, arrange collection from the milk processing company. Contact your local Gribbles laboratory for details

**Special handling/shipping requirements:** Standard, keep chilled

**General information about the disease:**

Bovine viral diarrhoea virus (“pestivirus”) is one of the most significant viral diseases in cattle. Clinically, there are three forms of the disease:

- A persistently infected (PI) form which may/may not have clinical signs
- An acute transient form characterised by fever and diarrhoea and short term immunosuppression. These animals will mount an immune response and clear the virus in 10-14 days.
- Mucosal disease (MD) only occurring in PI animals. PI animals are infected by a noncytopathogenic strain of the virus. A subsequent spontaneous mutation of the virus to a cytopathogenic strain within the PI animal results in MD, characterised by seromucoid nasal secretions, severe erosive lesions in the oral and intestinal mucosa, diarrhoea and death.

**General information about when this test is indicated:**

- Limited to cattle older than 10-months-of-age once all colostral immunity has waned or pre-suckling calves.
- Once infected, cattle will be viraemic for 10-14 days. About 2-4 weeks after infection is cleared, antibodies to BVDV will be produced and can be detected.
- Detecting BVDV antibody in the fetus indicates there has been viraemia of both the dam and fetus after 150 days gestation, when the fetus is immuno-competent and antibody has been formed. However antibody positive fetuses have not necessarily died because of BVDV infection. Nevertheless, antibody is a significant finding as it shows there was virus circulating in the herd.
• Pooled antibody testing is possible in milk from lactating animals or 15 pooled sera from non-lactating animals.

• Pooled results are reported as a numerical S/P value. This is the sample value divided by the positive control. The S/P value can be compared with epidemiologically derived values to predict the likelihood of virus infection in the group.

• Individual ELISA values are reported as positive or negative.

• For surveillance, annual testing is recommended of either pooled serum samples from 15 yearling animals and/or a bulk milk antibody test on the lactating animals.

Comparison with other related tests: Used in conjunction with PCR and antigen ELISA testing

BVDV testing summary:

BVDV tests to use in calves from conception to 10 months of age

<table>
<thead>
<tr>
<th>Age</th>
<th>PCR</th>
<th>Antigen ELISA</th>
<th>Antibody ELISA</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conception-40 days</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>N/A</td>
</tr>
<tr>
<td>40-120 days gestation</td>
<td>✓</td>
<td>x</td>
<td>x</td>
<td>Foetal fluid</td>
</tr>
<tr>
<td>150 days – birth</td>
<td>✓</td>
<td>x</td>
<td>✓</td>
<td>Foetal fluid</td>
</tr>
<tr>
<td>Birth-35 days</td>
<td>✓</td>
<td>x</td>
<td>x</td>
<td>Serum</td>
</tr>
<tr>
<td>35 days - 10 months</td>
<td>✓</td>
<td>✓</td>
<td>x</td>
<td>Serum/skin</td>
</tr>
<tr>
<td>10 months and older</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serum/skin</td>
</tr>
</tbody>
</table>

Individual tests for BVDV depending on disease or physiological state

<table>
<thead>
<tr>
<th>Disease or physiological state</th>
<th>PCR</th>
<th>Antigen ELISA</th>
<th>Antibody ELISA</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transient infection (TI)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serum/skin</td>
</tr>
<tr>
<td>Conceptus loss</td>
<td>x</td>
<td>x</td>
<td>✓</td>
<td>Serum</td>
</tr>
<tr>
<td>Pregnant (Trojan)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>N/A</td>
</tr>
<tr>
<td>Persistent infection (PI)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serum/skin</td>
</tr>
<tr>
<td>Mucosal disease</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Serum/skin</td>
</tr>
</tbody>
</table>

Group test options

<table>
<thead>
<tr>
<th>Physiological state</th>
<th>PCR</th>
<th>Antigen ELISA</th>
<th>Antibody ELISA</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milking</td>
<td>✓</td>
<td>x</td>
<td>✓</td>
<td>Bulk milk</td>
</tr>
<tr>
<td>Non milking</td>
<td>✓</td>
<td>x</td>
<td>✓</td>
<td>Pooled serum</td>
</tr>
</tbody>
</table>

x = No, ✓ = Yes
BVD ANTIBODY VIRUS NEUTRALISATION TITRE (VNT)

Species: Bovine
Specimen: 10 mL serum
Container: Red top or gel tube
Collection protocol: Venepuncture
Special handling/shipping requirements: Standard

General information about the disease:
Bovine viral diarrhoea virus (“pestivirus”) is one of the most significant viral diseases in cattle. A range of tests are available to investigate disease in various ages and classes of cattle.

General information about when this test is indicated:
If cattle are suspected as having been infected with BVD virus, a VNT can be used on paired convalescent sera. This is a more expensive test than other BVD testing options and not used as frequently now the BVD antibody ELISA is a quantitative test.

Comparison with other related tests: Can be used in conjunction with BVD antibody ELISA, PCR and antigen ELISA testing.

BVD ANTIGEN ELISA

Species: Bovine
Specimen: 10 mL serum or skin sample (3mm diameter minimum)
Container: Red top or gel tube, sterile pottle
Collection protocol: Serum – venepuncture; Skin – punch biopsy from ear
Special handling/shipping requirements: Ship chilled

General information about the disease:
Bovine viral diarrhoea virus (“pestivirus”) is one of the most significant viral diseases in cattle. Clinically, there are three forms of the disease:

- A persistently infected (PI) form which may/may not have clinical signs
- An acute transient form characterised by fever and diarrhoea and short term immunosuppression. These animals will mount an immune response and clear the virus in 10-14 days.
- Mucosal disease (MD) only occurring in PI animals. PI animals are infected by a noncytopathogenic strain of the virus. A subsequent spontaneous mutation of the virus to a cytopathogenic strain within the PI animal results in MD, characterised by seromucoid nasal secretions, severe erosive lesions in the oral and intestinal mucosa, diarrhoea and death.

General information about when this test is indicated:
BVD antigen ELISA is used to detect BVD virus in individual animals. On the ELISA test, a numerical optical density value, called S-N (sample value minus negative control value) is created. Transiently infected (TI) animals have S-N values of < 1.2 and PIs have S-N values > 2. Reporting shows weak positive (S-N <1.2), positive (S-N 1.2-2), and high positive (S-N >2) results against individual animal results. A weak positive result can be taken to indicate an animal is TI while a high positive result indicates the animal is PI.
positive result could be TI or PI so retesting is indicated. Values are proportionately lower in skin samples. Pooling is not possible with antigen ELISA testing.

Virus screening of all keeper calves is also recommended.

**Comparison with other related tests:** Used in conjunction with PCR and BVD antibody ELISA testing.

**BRUCELLA OVIS SEROLOGY**

**Species:** Ovine  
**Specimen:** 10mL serum, 1mL semen  
**Container:** Red top or gel tube, sterile pottle  
**Collection protocol:** Venepuncture, semen collection  
**Special handling/shipping requirements:** Ship chilled  

**General information about the disease:**

*Brucella ovis* is a bacterial infection of sheep producing clinical or subclinical disease characterised by genital lesions in rams, and placentitis in ewes.

**General information about when this test is indicated:**

If epididymal lesions are palpated in sheep an investigation of the *B. ovis* status of the ram flock should be undertaken. Annual palpation and serum sampling of *B. ovis* free accredited flocks is a requirement of the *B. ovis* scheme.

The cold complement fixation test (CFT) is most commonly used screening test and a serum sample is required. Doubling dilutions from 1:4 to 1:128 are tested. If positive CFT results occur management of *B. ovis* infection is required: see below

**B. ovis** infection management

For further clarification of *B. ovis* disease status, gel diffusion (GD) tests on the positive CFT tests are one possibility. This test has a high specificity and is the gold standard *B. ovis* test. A positive GD test confirms *B. ovis* infection. For additional evidence of infection, semen culture of serologically positive rams could be an additional step.

*B. ovis* ELISA tests on all the sera negative to the CFT. This test has high sensitivity, as it will identify potentially infected rams earlier than either the CFT or GD.

Rams positive to the ELISA could be removed and culled at this point. If farmers insist on a re-test ensure these rams are isolated and tested again 30 days later.

Separate the rams into smaller isolated sub-groups to more easily manage the disease if additional infected rams are discovered.

A month later bleed the rams again. Request the ELISA test for earlier detection of positive reactors.

If all are negative this would rank as the first clear test. Wait 60 days and bleed again, requesting the CFT. If all negative at the 2nd test the flock is now accredited.

Shortcuts and earlier bleeding may lead to misdiagnosing infected animals and poses risks.

**Comparison with other related tests:**

For further confirmation of infection, culture of semen samples for *B. ovis* is possible. Collect a fresh sample of semen into a sterile container by electroejaculation and submit chilled to the laboratory.
CAPRINE ARTHRITIS AND ENCEPHALITIS (CAE) ELISA

Species: Caprine
Specimen: Serum
Container: Red top or gel tube
Collection protocol: Venepuncture
Special handling/shipping requirements: Standard

General information about the disease:
Caprine arthritis and encephalitis (CAE) is caused by a lentivirus and most prevalent in dairy goats. Arthritis is the most common clinical sign in adult goats while encephalomyelitis can present in young kids.

General information about when this test is indicated:
Identification of seropositive i.e. previously infected goats can allow decisions for management of infected animals or culling options. Testing groups of animals to confirm absence of infection is also useful.

Comparison with other related tests:
Characteristic pathology is found in infected animals so post mortem and histopathology could be used to confirm infection.

ENZOOTIC BOVINE LEUCOSIS (EBL) ELISA

Species: Bovine
Specimen: 10mL serum
Container: Red top or gel tube
Collection protocol: Venepuncture
Special handling/shipping requirements: Standard

General information about the disease:
A rare viral disease of cattle capable of inducing neoplasia. The ELISA tests for the presence of antibody to the virus. Either individual or pooled samples (n=10) can be tested. This disease is virtually eradicated in the national dairy herd but the status of the beef herd is unknown.

General information about when this test is indicated:
Health screening for sale or stock movement. Also used in cases of lymphoid neoplasia to exclude viral involvement.

Comparison with other related tests:
A suite of tests to confirm the disease status of any suspicious or positive results are held by government contracted laboratories.
JOHNE’S DISEASE AGAR GEL IMMUNODIFFUSION (AGID)

**Species:** Ovine, cervine, bovine, llama, caprine

**Specimen:** Serum

**Container:** Plain (red top) or gel tube

**Collection protocol:** Venepuncture

**Special handling/shipping requirements:** Standard

**General information about the disease:**

Johne’s disease is an enteric infection of grazing ruminants caused by *Mycobacterium paratuberculosis*. This test has a reported specificity of >99% indicating that an animal with a positive result almost certainly has Johne’s infection. The sensitivity of the test depends on the stage of the disease. In preclinical cases that are faecal culture positive, the sensitivity is approximately 60%. However, with clinically affected animals, the sensitivity improves to approximately 87%.

**General information about when this test is indicated:** ELISA is the first test of choice. AGID is used for specific requirements of some countries and for ancillary testing

**Comparison with other related tests:** ELISA and CFT are other serological tests and faecal Ziehl Nielsen stains are also possible to check for acid fast organisms

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JOHNE’S COMPLEMENT FIXATION TEST (CFT)

**Species:** Ovine, cervine, bovine, llama, caprine

**Specimen:** Serum

**Container:** Plain (red top) or gel tube

**Collection protocol:** Venepuncture

**Special handling/shipping requirements:** Standard

**General information about the disease:**

Johne’s disease is an enteric infection of grazing ruminants and llamas caused by *Mycobacterium paratuberculosis*. This test has a reported specificity of >99% indicating that an animal with a positive result almost certainly has Johne’s infection. The sensitivity of the test depends on the stage of the disease. In preclinical cases that are faecal culture positive, the sensitivity is approximately 60%. However, with clinically affected animals, the sensitivity improves to approximately 87%.

**General information about when this test is indicated:** ELISA is the first test of choice. CFT is used for specific requirements of some countries and for ancillary testing

**Comparison with other related tests:** ELISA and AGID are other serological tests and faecal Ziehl Nielsen stains are also possible to check for acid fast organisms

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JOHNE’S DISEASE ELISA

**Species:** Ovine, cervine, bovine, llama, caprine

**Specimen:** Serum

**Container:** Plain (red top) or gel tube
Collection protocol: Venepuncture

Special handling/shipping requirements: Standard

General information about the disease:

Johne’s disease is an enteric infection of grazing ruminants and llamoids caused by *Mycobacterium paratuberculosis*. This test has a reported specificity of >99% indicating that an animal with a positive result almost certainly has Johne’s infection. The sensitivity of the test depends on the stage of the disease. In preclinical cases that are faecal culture positive, the sensitivity is approximately 60%. However, with clinically affected animals, the sensitivity improves to approximately 87%.

General information about when this test is indicated: When ill thrift is present in animals

Comparison with other related tests: CFT and AGID are other serological tests and faecal Ziehl Nielsen stains are also possible to check for acid fast organisms

LEPTOSPIRA MICROSCOPIC AGGLUTINATION TEST (MAT)

Species: Ovine, cervine, bovine, caprine, llamoid, canine, equine, porcine

Specimen: Serum

Container: Plain (red top) or gel tube

Collection protocol: Venepuncture

Special handling/shipping requirements: Standard

General information about the disease:

Leptospirosis is a zoonosis caused by one of the many pathogenic serotypes of the genus *Leptospira*, a spirochete that is transmitted by direct contact of abraded skin or mucous membranes with urine or tissues of an infected animal or, more commonly, by indirect contact with mud or water contaminated by urine of infected animals. The MAT is a serum test and encompasses a wide variety of serovars. High titres usually indicate recent infection, while low titres suggest chronic infection or residual antibody in a recovered or vaccinated animal.

Low titres may be interpreted in a number of ways:

- Very recent infection (clinical signs required to be present) and titre still rising
- Chronic infection (may be subclinical, especially L. hardjo)
- Convalescent titre
- Maternal antibody in calves
- Cross reaction to other serovars
- Vaccination titres

In dogs L. copenhageni is a cause of liver and renal failure. If sera test negative in highly likely cases, it may be due to acuteness of disease and should not necessarily be taken as a true negative. Convalescent sera is recommended in this situation.

General information about when this test is indicated: To confirm if clinical disease is due to leptospira infection soon after infection and convalescent

Comparison with other related tests: PCR can be used to detect the presence of leptospira in blood or urine
NEOSPORA ELISA

Species: Bovine, ovine

Specimen: Serum

Container: Plain (red top) or gel tube, NB: only undertaken on 10 or more samples

Collection protocol: Venepuncture

Special handling/shipping requirements: Standard

General information about the disease:

*Neospora caninum*, an apicomplexan protozoan parasite infection, affects mostly cattle where it may result in abortions. These events may take the form of sporadic or low-level endemic occurrences of abortion, or be of epidemic, “storm-like” proportions. These abortion storms, in particular, can affect large proportions of the at-risk (i.e. in-calf) cow population and cause large economic losses.

Some work suggests sheep may be affected too, but the likely seroprevalence is low. The ELISA can be used to investigate Neospora exposure in sheep flocks.

General information about when this test is indicated:

The ELISA is most suitable for herd or flock investigations, and (as titres persist longer) for the detection of chronically infected animals. To demonstrate an association between abortions/reproductive failure and *N caninum* infection, a blood sample should be taken from 10 empty cows (or ewes), and a control group of 10 pregnant cows (ewes). All should be tested by ELISA for Neospora antibodies. To establish if there is a correlation between the Neospora results and pregnancy status calculate the relative risk \( (a / (a + b)) / (c / (c + d)) \); where \( a \) is ELISA positive and aborted, \( b \) is ELISA positive but not aborted, \( c \) is ELISA negative and aborted and \( d \) is ELISA negative but not aborted. A relative risk greater than 1 indicates an association. Also use the ELISA if you want to determine if cattle have been infected or not.

Comparison with other related tests:

For investigating the Neospora status of individual animals, especially related to abortion use the Neospora IFAT.

The ELISA test is reported as a positive or negative result. The ELISA and IFAT tests correlate well and a positive ELISA means that the IFAT titre is >1:600. Neospora serology is preferred in epidemiological investigations or where no foetal tissue is available. For the initial abortion investigation on a farm, histopathology of a range of tissues is recommended.

NEOSPORA IMMUNOFLUORESCENT ANTIBODY TEST (IFAT)

Species: bovine, canine

Specimen: Serum

Container: Plain or gel tube

Collection protocol: Venepuncture

Special handling/shipping requirements: Standard

General information about the disease:

*Neospora caninum*, an apicomplexan protozoan parasite infection, affects mostly cattle where it may result in abortions. These events may take the form of sporadic or low-level endemic occurrences of abortion, or be of
epidemic, “storm-like” proportions. These abortion storms, in particular, can affect large proportions of the at-risk (i.e. in-calf) cow population and cause large economic losses.

Dogs may suffer from a variety of clinical manifestations of *N. caninum* infection (ranging from dermatitis to pneumonia), but as in cattle, abortions, stillbirths and neonatal deaths are frequent occurrences, while an ascending paralysis of the hind legs is almost pathognomonic in younger affected dogs, less than six months of age.

**General information about when this test is indicated:** A high IFAT titre (>1:1000) in a cow that has aborted in the last 2-3 weeks is very strong evidence that Neospora was the cause of the abortion. IFAT titres fall quickly from several thousand to a few hundred over a couple of months following abortion. A low IFAT titre (<1:200) in a cow that has aborted in the previous 2-3 weeks rules out Neospora as the cause of abortion.

An elevated IFAT titre in a dog (> 1:800) with clinical signs of disease would confirm infection. Clinically unaffected dogs can have titres of 1:200 indicating previous exposure and seroconversion.

**Comparison with other related tests:** The IFAT is the most appropriate assay for individual abortion diagnoses, as titres are elevated around the time of abortion and then quickly decline within a matter of weeks. In the dam, an IFAT titre of ≥1/600 is indicative of an association between the abortion and Neospora infection. For investigation of reproductive disease in groups use the Neospora ELISA.

### PARAINFLUENZA 3 ELISA

**Species:** Bovine

**Specimen:** Serum

**Container:** Plain (red top) or gel tube

**Collection protocol:** Venepuncture

**Special handling/shipping requirements:** Standard

**General information about the disease:** Parainfluenza 3 (PI-3) virus causes mild to subclinical respiratory infections but can serve as an initiator to the development of secondary bacterial pneumonia.

**General information about when this test is indicated:** To determine if PI 3 infection is an aetiological factor when investigating pneumonia

**Comparison with other related tests:** Virus isolation is also a possible diagnostic step to confirm the presence of virus

### PORCINE PARVOVIRUS ELISA

**Species:** Porcine

**Specimen:** Serum

**Container:** Plain or gel tube

**Collection protocol:** Venepuncture

**Special handling/shipping requirements:** Standard

**General information about the disease:**
Porcine parvovirus is a virus disease of pigs associated with reproductive problems, including abortion, small litters, still births, neonatal deaths and weak piglets. Disease occurs when sero-negative dams are infected in the first half of gestation and the virus crosses the placenta killing the feti.

**General information about when this test is indicated:**

Disease could be suspected if there is a history of small litters (less than five piglets), irregular returns to service, fetal mummifications and abortion. Collect serum samples from aborted sows and/or neonatal pigs. Detection of antibodies in piglets means in utero infection as antibodies do not cross the placenta. Interpret positive antibody results in sows with care as infection is widespread.

**Comparison with other related tests:** Necropsy of aborted piglets and histopathology of fetal tissues can be used to investigate other causes of abortion.

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**Companion animals**

**ANTINUCLEAR ANTIBODY TEST (ANA)**

**Species:** Canine, feline  
**Specimen:** 5 mL serum  
**Container:** Red top or gel tube  
**Collection protocol:** Venepuncture  
**Special handling/shipping requirements:** Standard  
**General information about the disease:** This serum test detects the presence or absence of antibody to various nuclear antigens.

**General information about when this test is indicated:** It is mostly indicated in cases of suspected systemic lupus erythematosus, where loss of immune autoregulation results in the production of antibodies against a range of membrane and soluble antigens. The most characteristic of these are the antinuclear antibodies directed against double-stranded DNA, RNA, nucleoprotein and histone-related antigens. The test is mostly applicable to dogs and a negative result should not rule SLE out. A cat ANA test is available. ANA can be non-specific, present in a number of disease states, induced by some therapeutic drugs, as well as occasionally being present in normal individuals.

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**CANINE DISTEMPER VIRUS VACCINAL ANTIBODY ASSESSMENT**

**Species:** Canine  
**Specimen:** Serum  
**Container:** Red top or gel tube  
**Collection protocol:** Venepuncture  
**Special handling/shipping requirements:** Standard  
**General information about the disease:**

Canine distemper is a rare viral infection of viral disease of dogs. Vaccination is effective at preventing infection and vaccination programs have been developed to thoroughly protect dogs.
General information about when this test is indicated:
This test is used to assess if vaccinal immunity directed against canine distemper is present and sufficient. A positive test indicates a serum neutralisation titre of 1:16 or greater. Dogs with a negative test are not protected from infection but they may be protected from clinical disease since anamnestic antibody or cell mediated immunity may be protective.

Comparison with other related tests:
If clinical disease is suspected, characteristic lesions are observed by histopathology.

CANINE PARVOVIRUS (CPV-2) VACCINAL ANTIBODY ASSESSMENT

Species: Canine
Specimen: Serum
Container: Red top or gel tube
Collection protocol: Venepuncture
Special handling/shipping requirements: Standard

General information about the disease:
Parvovirus infection is a serious, highly infectious viral disease of dogs. Vaccination is effective at preventing infection and vaccination programs have been developed to thoroughly protect dogs.

General information about when this test is indicated:
This test is used to assess if vaccinal immunity directed against canine parvovirus is present and sufficient. A positive test indicates a haemagglutination inhibition titre of 1:80 or greater. Dogs with a negative test are not protected from infection but they may be protected from clinical disease since anamnestic antibody or cell mediated immunity may be protective.

Comparison with other related tests:
A faecal parvovirus ELISA is available to test for the presence of virus if clinical disease is suspected. Characteristic lesions are seen in the intestinal tract in fatal cases of infection.

FELINE CORONA VIRUS (FIP) ELISA

Species: Feline
Specimen: Serum
Collection protocol: Red top or gel tube
Special handling/shipping requirements: Standard

General information about the disease:
FIP is a coronavirus infection of cats of any age but most prevalent among cats < 3 years (particularly 4-16 months). Mortality is high once clinical signs appear. FIP virus arises through specific mutations in a common, non-pathogenic, enteric feline coronavirus ubiquitous in cats. Most kittens are exposed to coronavirus by nine weeks of age but only a small proportion of cats develop FIP related to genetic susceptibility, age of first infection, and stressors at the same time as infection.
General information about when this test is indicated:
The ELISA detects both enteric coronavirus and FIP virus antibody titres so the result needs to be interpreted in conjunction with clinical signs and other related tests as detailed below. A combination of clinical signs and test results needs to be considered to confirm a diagnosis.

Comparison with other related tests:
Blood tests: common abnormalities include chronic non-regenerative anaemia, neutrophilia and lymphopaenia, elevated globulin and decreased albumin.
Effusions: fluid in the abdomen or pleura are suggestive of FIP and are protein rich exudates with moderate cellularity, often viscous and yellow-tinged
Intracocular and neurological signs: uveitis, meningitis and/or meningomyelitis are highly suggestive of FIP in young cats
Histopathology: characteristic pathological lesions are seen on histopathology of affected tissues (by biopsy or necropsy collection)

FELINE LEUKAEMIA VIRUS (FELV) ANTIGEN ELISA
Species: Feline
Specimen: Serum or plasma
Container: Red top or gel tube, EDTA or heparin tube
Collection protocol: Venepuncture
Special handling/shipping requirements: Standard

General information about the disease:
Feline Leukaemia Virus (FeLV) is a gammaretrovirus that infects cats and other small felids. Although many cats will overcome infection with FeLV, experiencing a transient viraemia or seroconverting with no detectable viraemia, some become persistently viraemic. Most persistently viraemic cats will develop a range of disease conditions, the most common being anaemia and/or lymphoma and immunosuppression, and die within 3 years. The cat's age at the time of infection is a major determinant of clinical outcome. A cat found to be persistently viraemic should be isolated from other cats to reduce the risk of passing on the virus.
A cat that has overcome viraemia will remain latently infected. Once a cat becomes latently infected, it remains so for life. FeLV may be reactivated on rare occasions from these cats when immunosuppressed or under chronic stress and such cats should still then be considered potential sources of infection.

General information about when this test is indicated:
Antigen ELISA and immunochromatography are the most commonly used tests to diagnose FeLV infection and are valid screening tests with good sensitivity and specificity. However as the prevalence of disease is low the positive predictive value (PPV) of these tests is poor (50%) increasing the likelihood of false positive results. A positive test result in a cat with clinical signs of FeLV infection or with FeLV-consistent haematological changes is probably a true positive.

Comparison with other related tests:
A healthy cat testing FeLV positive should always be retested preferably using the qPCR for detection of provirus DNA to confirm the result. Cats that positive on the FeLV test with no clinical signs may clear the
viraemia over 2-16 weeks or longer and should be kept separated and retested. If the still positive after 1 year it is likely it will remain viraemic for life.

FIV ANTIBODY ELISA

**Species:** Feline

**Specimen:** Serum, plasma

**Container:** Red top or gel tube for serum, EDTA or heparin tube for plasma

**Collection protocol:** Venepuncture

**Special handling/shipping requirements:** Standard

**General information about the disease:**

Feline immunodeficiency virus (FIV) is a lentivirus in the family Retroviridae. It contains RNA and its life cycle involves the integration of its RNA into the DNA of the genome of the host using the enzyme reverse transcriptase. This proviral DNA is then replicated as the cell divides. The proviral DNA is then translated back into viral RNA, and viruses are released from the host cell, the virus receiving its envelope from the host cell membrane. It shows many similar features to HIV, but is unrelated. A number of different subtypes or clades of FIV have been identified by sequencing the gene involved with the viral envelope. Isolates have been divided into five phylogenetic subtypes designated A, B, C, D and E. New Zealand has been found to have subtype C as the predominant subtype, fewer numbers of subtype A, and a novel, as yet unknown subtype. There is also a putative A/C recombinant strain. Transmission is mostly associated with biting/fighting, hence free roaming male cats are more at risk than others. All kittens born to infected queens will have maternal antibody present, although only one third will be infected with FIV. Maternally derived antibodies may persist for up to 3 months. Then it may be a further two months before infected kittens seroconvert.

**Stages of Infection**

1. The acute phase: This stage lasts several weeks. May see fever, diarrhoea, gingivitis, jaundice, uveitis, conjunctivitis, generalized lymphadenopathy and neutropenia. The severity depends on age. Young kittens have a more florid lymphadenopathy during the acute phase and there is increased severity in adolescents, while geriatric cats show minimal signs but progress more rapidly to the next stages of disease.

2. Asymptomatic carrier: This stage lasts from months to years with no obvious signs, and the cats appear apparently healthy. This stage may last up to 5 years but cats infected at >10 years of age progress through this stage faster than younger cats.

3. Persistent generalised lymphadenopathy and AIDS related complex: Lasts for 6 months to several years. It is characterized by vague, non-specific signs of illness, weight loss, enlarged lymph nodes, stomatitis, anorexia, anaemia, leucopenia, neurological signs and apathy. This is the stage at which the majority of cats are presented to veterinarians.

4. Terminal AIDS-like phase: Lasts less than a year. Cats are emaciated. There are opportunistic infections, lymphoid depletion and miscellaneous disorders including neurologic, renal, immunologic and neoplastic disease.

**General information about when this test is indicated:**

Antibody to FIV appears at about 2-4 weeks post infection. The majority of commercial tests test for presence of antibody against membrane and core proteins (ELISA and immunodiffusion test kits). In New Zealand the majority of FIV positive results are true positive results because of the high prevalence and the
excellent sensitivity and specificity of the tests available. However false negative results may occur early in the disease before there is a sufficient antibody response. They may also occur late in the disease when the cat is severely immunosuppressed. False positive results may occur in cats that have been vaccinated with the FIV vaccine. Currently vaccinated cats cannot be differentiated from naturally infected cats by routine serological means such as the ELISA test kits, because both classes of cats will have antibody present. Antibody may also be present in kittens up to 12 weeks of age that are born to cats that have been either infected or vaccinated and have transferred maternal antibody to their kittens.

**Comparison with other related tests:** FIV PCR testing is another option for disease status investigation

**Further information:** FIV testing information sheet

### RHEUMATOID FACTOR

- **Species:** Dogs
- **Specimen:** Serum
- **Container:** Plain (red top) or gel tube
- **Collection protocol:** Venepuncture
- **Special handling/shipping requirements:** Standard

**General information about the disease:**
Affected dogs have episodes of anorexia, depression and fever with generalised or shifting lameness associated with swelling around the joints. Rheumatoid factors are IgM and IgG antibodies. They react with an antigen, which is altered endogenous IgG protein to form immune complexes in the joints. It is not clear why the IgG protein changes to become recognised as foreign by the immune system. Many of the dogs affected are of the small and toy breeds, with a significant number being Shetland sheepdogs.

**General information about when this test is indicated:** In dogs with clinical signs suggestive of immune mediated arthritis

**Comparison with other related tests:** It is important to rule out bacterial arthritis, bacterial endocarditis and systemic lupus erythematosus before considering rheumatoid arthritis

### TOXOPLASMA GONDII LATEX AGGLUTINATION TEST

- **Species:** Ovine, caprine, feline, canine
- **Specimen:** Serum, fetal fluids
- **Container:** Plain (red top) or gel tube
- **Collection protocol:** Venepuncture
- **Special handling/shipping requirements:** Standard

**General information about the disease:**
Toxoplasmosis, caused by *Toxoplasma gondii* infection, is one of the most common infectious causes of ovine abortion and positive ewe sera indicates exposure to the organism, not necessarily proving toxoplasmosis as the cause of abortion. Characteristic histological changes can be seen in foeti infected with toxoplasma and this is the adjunct method of diagnosis in abortion cases. Toxoplasma titres in fluids of aborted fetuses also assist in the confirmation of infection.
The serological confirmation of clinical toxoplasmosis in cats and dogs requires the demonstration of a rising titre in acute and convalescent sera as toxoplasma titres are very common in normal dog and cat sera. It is recommended for paired serology that the samples be taken 10-14 days apart.

**General information about when this test is indicated:** Cases of abortion. The test is useful in foetal fluids and maternal sera to indicate exposure to Toxoplasma. A serological titre is generated by the test.

**Comparison with other related tests:** Histopathology of fetal tissues and placenta help clarify if toxoplasma infection is the cause of abortion.
Skin Disease Investigation

GENERAL INFORMATION

Species: All.

Collection protocol:

Skin disease can be frustrating in clinical practice, but it should be remembered that the skin is one of the few organs that is easily available for examination. By careful systematic observation, the diagnoses of many dermatoses can be determined, or at least a differential list established. Specialist dermatologists will often diagnose and treat animals on the basis of the history, signalment, physical examination and a few ancillary tests (e.g. cytology).

It is important that all of the skin and the external mucous membranes are examined. Recognising the morphology of skin lesions is essential in diagnosing skin problems. Often primary lesions (e.g. vesicles) are obscured by secondary ones (e.g. crusts). Changes due to medication and self-induced trauma are also common secondary problems encountered in examination.

It is helpful for the pathologist if skin lesions and their distribution are accurately described on the accession form. It's also very helpful to indicate on the drawing from which anatomical area the biopsies were taken. The signalment, previous history and previous treatments of the case are critical in diagnosing skin disease, and we can help you much more armed with that information. Your clinical differential diagnoses are also important. Do not worry about introducing “bias” into our process; we cannot see lesions that are not there, and rather than influencing us in the wrong direction, it is much more likely that your clinical information will allow us to provide specific and accurate interpretations.

Digital pictures are very useful as another means of submitting extra information to us. Please contact Gribbles for the email address of a pathologist to send them to. Lastly, do not hesitate to call and ask a pathologist what they think before you undertake a test.

Comparison with other related tests: See - Skin Disease Investigation – Examination for Parasites, Skin Disease Investigation – Examination for Dermatophytes (Ringworm), Skin Disease Investigation – Examination for Bacteria, Skin Disease Investigation – Histopathology, Skin Disease Investigation – Immunologic Tests, Skin Disease Investigation – Endocrine Tests, Skin Disease Investigation – PCR Tests.

ENDOCRINE TESTS

Species: All.

Specimen: See specific test.

Container: See specific test.

Collection protocol:

Thyroid hormone plays an important role in controlling metabolism of the skin. Hypothyroidism should be ruled out as the underlying cause of pyoderma. See – Thyroid function in Dogs.

Skin diseases due to cortisol abnormalities are also important – See Hyperadrenocorticism (Cushing’s’ Disease) in Dogs and Cats, Pituitary Pars Intermedia Dysfunction or Hyperadrenocorticism in Horses.

Dermatoses due to sex hormone abnormalities are uncommon, and should be considered only where overall patient assessment including the results of general blood and dermatological testing support the diagnosis. Hyperoestrogenism and hyperandrogenism are recognised, often in dogs with testicular tumours. These are difficult to diagnose by blood sex hormone concentrations since those fluctuate during the day, but high
concentrations of oestradiol or testosterone can be found. Oestradiol testing may need to be performed at a referral laboratory.  

**General information about when this test is indicated:** Dermatoses in older animals, particularly with non-inflammatory alopecia.

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**EXAMINATION FOR BACTERIA**

**Species:** All.

**Specimen:** Pustules or Vesicle contents, or scale on microscope slide or swab; fresh (unfixed) skin biopsy.

**Container:** Microscope slide holder, swab or plain container.

**Collection protocol:**

The normal skin is remarkably resistant to bacterial infection and even colonisation. When damaged, however, the environment becomes much more conducive to bacterial growth.

- Pustules, papules and vesicles can contain acantholytic cells, bacteria and the predominant granulocyte population. The presence of intracellular bacteria confirms their role in inflammation.
- Unruptured pustules/papules/vesicles need to be used. These lesions should not be prepared or sterilised before sampling.
- The sample is collected via needle puncture and/or aspiration with transfer to a sterile swab for culture, then smearing for cytology.
- Secondary bacterial infection is common in eroded, ulcerated or crusted types of lesions, and culturing these is seldom helpful.
- Culture of fresh skin biopsies may be indicated for plaques, nodules, fistulous tracts, deep lesions or cellulitis. (These sites should be sterilised before surgery). It is helpful to also aspirate these for cytology at the same time.
- Sticky tape preparations of surface material are difficult to work with and not recommended.

The majority of primary bacterial skin infections are caused by coagulase positive *Staphylococcus*. Occasionally *Proteus, Escherichia coli* or *Pseudomonas* are involved, usually as secondary invaders. In cats, bacterial infections are less common and usually involve *Rhodococcus, Pasteurella* or β-haemolytic *Streptococcus*.

Rare infections are caused by anaerobes or higher bacteria including *Nocardia, Actinomyces* and mycobacteria (e.g. feline leprosy). Unstained direct smears and fresh skin biopsies can be submitted for cytology and culture if one of these infections is suspected. Mycobacteria in particular can be very slow-growing and therefore these infections are usually initially diagnosed by cytology or histopathology. Follow-up testing to identify the species of mycobacteria is available by PCR.

**General information about when this test is indicated:** Dermatoses with prominent pustules, vesicles, exudate, cellulitis, nodules or fistulous tracts.

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**EXAMINATION FOR DERMATOPHYTES (RINGWORM)**

**Species:** All mammals.

**Specimen:** Hairs, skin exudate on microscope slide, skin scrapings or nails.

**Container:** Microscope slide holder, envelope or plain container.

**Collection protocol:**
Wood's lamp is an ultraviolet light instrument, often with an incorporated magnifying glass. When exposed to the UV light, hairs invaded by *Microsporum canis* or *Microsporum equinum* can give a yellow-green fluorescence (30-80% of isolates). This is due to tryptophan metabolites produced by the organisms. Fluorescence is not present in the scale or crusts, or in cultures of *M. canis*. There are other, less common dermatophytes that may also fluoresce. Medication can interfere with the test, for example, iodine washes can destroy the fluorescence and some bacteria and chemicals can give false positive results. Lack of fluorescence is inconclusive, and it should also be noted that most dermatophytes of horses do not fluoresce.

Hair is the specimen most commonly collected for the isolation of fungi. Using forceps, select hairs that fluoresce under the Wood's lamp.

Alternatively the toothbrush method is effective in selecting specimens.

- Use a new toothbrush and gently comb the hair onto paper.
- Submit the brushings and toothbrush in an envelope or non-air tight sealed container for fungal isolation.
- Choice of container is important to prevent moisture build up, which allows bacterial overgrowth.
- The toothbrush comb technique is recommended in cats that are suspected carriers of *M. canis*.

Besides fungal isolation, which can take some time, KOH digest of hair is a rapid diagnostic screening technique for dermatophytes, which can aid in light microscope diagnosis. If negative, dermatophytosis still cannot be ruled out.

Skin samples can be submitted for fungal isolation.

- Clean the skin gently of extraneous debris with gauze soaked in 70% alcohol.
- Scrape from the periphery of the lesion (ideally a new one) and adjacent skin.
- **Do not** use a paraffin-coated scalpel to collect samples for microbiology.

Samples of nail and paw pad can also be cultured for dermatophytes. Since these are often heavily contaminated with micro-organisms, they should be cleaned with 70% alcohol before sampling. Samples should be selected from the concave side of the claw, or from within the claw.

Skin impression smears can be quite useful in the diagnosis of yeast infections. Simply press the slide directly onto the lesion and allow to air dry. Sticky tape preparations are difficult to work with and not recommended.

**General information about when this test is indicated:** Dermatoses with prominent alopecia, follicular lesions, crusting or scaling.

**EXAMINATION FOR PARASITES**

**Species:** All.

**Specimen:** Microscope slide.

**Container:** Slide holder.

**Collection protocol:**

Skin scrapings are an often under-utilised diagnostic tool and can be very useful if correctly done (do not forget about those cases of localised demodicosis!)

Use a paraffin-coated scalpel and gently scrape the area whilst squeezing the skin to help extrude *Demodex* mites from hair follicles. (Hairs may need to be plucked to find mites in some cases of demodicosis). *Sarcoptes* live on the skin surface, but while very hard to find on skin scrapings, the finding of even one mite is significant. *Cheyletiella* mites, *Chorioptes* mites and lice should be found easily in coat brushings or Sellotape preparations of the coat. *Otodectes* may be found by scrapings or Sellotape preparations.
Don’t forget faecal egg count as part of the work up in suspected parasitic dermatitis cases. Hookworm penetration of the skin can cause inflammation and the only way to confirm this is by testing faeces for eggs.

**General information about when this test is indicated:** Dermatoses with prominent alopecia, pyoderma, follicular disease, crusting or scaling.

**HISTOPATHOLOGY**

**Species:** All.

**Specimen:** Fixed tissue (1:10 tissue:formalin).

**Container:** Plain.

**Collection protocol:**

Skin biopsies are often an effective diagnostic tool. The results should be able to give you an idea of what the disease process is (e.g. inflammatory vs. neoplastic), and sometimes what agent is causing it. Even if a specific cause is not identified, by knowing if the lesion is follicular or deep dermal, neutrophil-rich or granulomatous, you can effectively reduce your differential list and focus on the most likely aetiologies. Some diseases can only be diagnosed by skin biopsy, however it is important to also recognise that biopsies are not always indicated.

- Biopsies often show non-specific changes in syndromes of allergic skin disease. Carefully consider the history, clinical signs and results of other tests before proceeding to biopsy.
- Select primary lesions to biopsy (e.g. papules, pustules, vesicles, nodules) before secondary lesions (e.g. lichenification, excoriatiion).
- If you are sampling an animal with inflammatory skin disease, try to sample as early in the course of the disease as possible, before the development of chronic, secondary non-specific changes. Try to sample entire fresh lesions (if small, e.g. vesicles) or the edge of larger fresh lesions.
- Conversely, if the animal has a non-inflammatory skin disease (e.g. suspected endocrine alopecia, a mass), avoid sampling the edge of the lesion or fresh lesions, and instead select the most well-developed area, since this is most likely to have classical histological changes. It should be noted it is uncommon for a skin biopsy to have changes specific to a particular endocrine disorder.
- It is important to note on the accession form any treatment the animal has received in recent weeks. Steroid drugs should optimally be withheld for 2-3 weeks prior to the biopsy. (Depot steroids might need a 6-8 week withdrawal). **Antibiotics before biopsy** may be helpful unless a primary infection is suspected, and culture of the biopsy required. These reduce changes due to secondary infection, important since infections can obliterate the primary lesion and sometimes mimic immune-mediated disease (e.g. pemphigus foliaceus-like dermatophytosis).
- For skin disease investigations, multiple biopsies are always advisable.
- Generally, a 6 mm biopsy punch provides an adequate specimen. Smaller biopsies may be taken from the nose, pinnae and feet. Make sure the instrument is sharp to avoid distortion of the tissue, and take the punch in a continuous rotating motion rather than a “back and forth” twist.
- Ensure if possible that the lesion is in the centre of the punch biopsy, since when processed these are generally sectioned in half by default. An eccentric lesion may therefore be missed if it is not obvious at the time of processing.
- If the lesion is suspected to be deeper in the subcutaneous tissue, a punch biopsy may not penetrate deep enough. Excisional biopsy with a scalpel is indicated in this situation, or where the shearing action of the punch may damage lesions, or where there are larger vesicular and pustular lesions. These biopsies should be elliptical and a minimum of 5 x 15 mm.
- Incisional biopsies (i.e. sampling part of a mass) are often useful in cases of suspected neoplasia, where the lesion may need to be graded or staged before curative surgery is undertaken.
- In horses, full-thickness biopsies of the coronary band result in permanent hoof wall defects and
therefore superficial “shave” biopsies are preferred from that area.

- It is usually not necessary to biopsy normal skin for a “control”, unless the species of animal is unusual or exotic.
- Biopsies can usually be taken with sedation, systemic pain relief and local anaesthesia of the site. If a deep dermal or subcutaneous disease is suspected it is best to avoid local injection, and to use general anaesthesia or ring blocks to prevent damage to the area. It is also important to avoid local anaesthesia if the biopsy will be cultured (lidocaine can inhibit bacterial growth).
- It is best to do as little as possible to clean the biopsy site since disinfectants, scrubbing and clipping will damage the skin and potentially remove the epidermis and valuable information (e.g. acantholytic cells in crusts, vesicles). Gently dabbing the skin with alcohol, and scissor cutting of long hair in the area is the most interference there should be.
- Care is needed in handling the biopsy specimen. It is easy to crush and distort the sample with forceps. The specimen should be gently blotted to remove excess blood. It may then be placed into a tissue cassette if very small (this reduces trauma to the sample floating in formalin during transit, and also reduces handling at the laboratory before processing).
- Punch biopsies do not curl and can be placed directly in formalin. Biopsies taken with a scalpel often need to be attached to cardboard to prevent curling. Adherence of the deep margin should occur within 60 seconds, then place the cardboard and attached sample into 10% buffered formalin. (Do not wait longer than 1-2 minutes, in order to prevent autolysis).
- Unless identification of individual sites is important all the biopsies can be placed in the same container.

**General information about when this test is indicated:**

Any potential neoplasm, persistent ulceration, suspected disease that can only be diagnosed by biopsy (e.g. sebaceous adenitis), any unusual dermatosis, vesicular dermatitis, any suspected disease with an expensive/difficult/dangerous treatment, any dermatosis not responding to apparently rational therapy.

**IMMUNOLOGIC TESTS**

See - Anti-nuclear antibody test.

**PCR TESTS**

**Species:** Cat, Dog.

**Specimen:** Paraffin-embedded formalin-fixed tissue, fixed tissue or fresh tissue.

**Container:** Fixed tissue (1:10 tissue:formalin) or fresh in plain container.

**Collection protocol:** Surgical or necropsy collection of tissue samples.

**General information about the disease:**

Mycobacterial skin disease in cats and dogs is often nodular or diffuse and can be due to *Mycobacterium lepraemurium* or opportunistic mycobacteria. Occasional more serious disease is caused by other species such as *M. bovis*, *M. avium* or the “East Coast” variant of mycobacteria.

Gribbles offers PCR for *Mycobacterium* testing and speciation. (Speciation may require referral to a reference laboratory in some circumstances).

**General information about when this test is indicated:** Cats and Dogs with pyogranulomatous dermatitis or panniculitis and acid-fast bacteria consistent with mycobacteria.
Therapeutics

DIGOXIN MONITORING

Species: Dog, Cat
Specimen: Serum
Container: Plain (red top) or gel tube
Collection protocol: 8 to 12 hours post dosing (i.e., trough sample); fasted sample.
Special handling/shipping requirements: None.

General information about the disease:
Used in cases of congestive heart failure (CHF) with supraventricular tachycardia; cases with reduced fractional shortening. Not indicated in patients with pericardial disease, hypertrophic cardiomyopathy with outflow tract obstruction, or restrictive myocardial disease, unless accompanied by myocardial failure or supraventricular tachycardia.

General information about when this test is indicated:
5 to 7 days after initiation of therapy; ongoing monitoring of therapy. It is recommended to monitor electrolytes and calcium every 2 to 3 months or after any changes in therapy or changes in animal's clinical condition. Hypokalaemia and hypercalcaemia can potentiate digoxin toxicity.

Comparison with other related tests: No.


PHENOBARBITAL / PHENOBARBITONE (PB) THERAPY MONITORING

Species: Dog, cat
Specimen: Serum
Container: Plain (red top) tube (gel tube can interfere with result)
Collection protocol: Fasted overnight (12 h). Trough sample preferred (within 1 hour of next scheduled dose; peak is 4-8 hours post dosing)
Special handling/shipping requirements: None

General information about the disease: Commonly used for initial and long-term management of seizures in dogs and cats

General information about when this test is indicated:
1. 2 weeks and 4-6 weeks after initiating therapy obtain trough sample.
2. Assess any time 2 or more seizures occur between scheduled measurements (peak and trough samples recommended)
3. Assess 2 weeks after any dosage adjustment
4. Assess every 6 months along with CBC, albumin, ALP and ALT, trough sample is adequate for long-term monitoring

Comparison with other related tests: No.
**Interpretation:**

Dogs: effective therapeutic range of 43-194 µmol/L

Cats: effective therapeutic range of 45-129 µmol/L

Mild to moderate increases in ALP and ALT are expected. If hepatotoxicity has occurred there is often a marked increase in ALT relative to ALP. Hepatotoxicity is considered more likely at PB concentrations >133 µmol/L.

Phenobarbital usage may be associated with hepatotoxicity and blood dyscrasias; therefore six-monthly monitoring should include routine haematology, hepatic enzymes, and albumin. Trough samples are adequate for routine monitoring.

**POTASSIUM BROMIDE (KBR) MONITORING**

**Species:** Dog

**Specimen:** Serum

**Container:** Plain (red top) or gel tube

**Collection protocol:** Fasted overnight (12 h). Trough sample preferred (within 1 hour of next scheduled dose; peak is 2 hours post dosing)

**Special handling/shipping requirements:** None

**General information about the disease:**

Used as an anti-epileptic drug commonly in conjunction with phenobarbitone (PB) or as monotherapy in dogs with hepatic dysfunction, in large-breed dogs and working dogs in which side effects from PB are unacceptable.

Signs of bromide toxicity include: stupor or coma, blindness, ataxia, tetraparesis, dysphagia, and megaesophagus.

**General information about when this test is indicated:**

4-6 weeks after initiating therapy (concentrations approximately 50% of steady state) and 8-12 weeks (steady state expected). Or measure if any breakthrough seizures have occurred.

If the dog is on PB therapy when KBr is added to the therapeutic regime then serum PB concentration should also be measured 4-6 weeks after KBr introduction. KBr seems to enhance the excretion or metabolism of PB and frequently the serum PB concentration drops after KBr is introduced.

**Comparison with other related tests:** No.

**Interpretation Combination Therapy:**

4-6 weeks 8 – 12 mmol/L

8-12 weeks 10 – 20 mmol/L

**Interpretation Monotherapy:**

4-6 weeks 12.5 – 15 mmol/L *based on being half of expected steady state dose

8-12 weeks 25 – 30 mmol/L

**Reference(s):**

2. VET POTBROMTHER 06 2006
Toxicology

TOXICOLOGICAL TESTING IN DOGS AND CATS – GENERAL GUIDELINES

Veterinary clinics are often confronted with a dog or cat with clinical signs suggesting exposure to a toxic substance. Clinical signs of many poisonings are similar and may be unable to be reliably differentiated on clinical grounds alone. A careful clinical history can often narrow the differentials. In some cases, owners are concerned that their animal has been deliberately poisoned and are seeking testing to confirm or rule out this possibility. Owners will often request a “toxin screen” in cases of suspected intoxication. Client communication and education is vital so that expectations of testing are not unrealistic. Toxin testing requires a specific toxin to be nominated as there is no suite of tests that covers all possibilities. Toxin testing is inherently expensive, requires specific sample types and false negatives can occur; for instance the toxin may have been eliminated from the body or be undetectable, but clinical signs may persist.

Gribbles Veterinary Pathology can offer specific testing for a range of toxic substances, however it is important to consider the specific sample requirements and testing limitations for each toxin when advising your clients. Many tests are referred to external laboratories and may have extended turnaround times.

Specific toxin testing is NOT available for the following compounds: Fe-EDTA molluscicides, cholecalciferol based rodenticides, tick paralysis toxin, tetrodotoxin, lily toxin, amatoxin (mushrooms). Please contact the laboratory if you need testing for a specific toxin not listed here; we can often source unusual tests as needed from our network of referral laboratories.

General guidelines for sampling where toxin type is uncertain should aim to provide a wide range of samples for potential testing. Fresh tissue samples should be chilled or frozen for transportation to the laboratory.

Pre mortem sampling:

- Suspected intoxicant (food, bait, water, medication)
- Vomitus
- Urine
- Faeces

Post mortem sampling:

- Fresh liver, kidney (ideally enough to fill a yellow-top pot, or the whole organ minus a small histology sample for smaller animals)
- Urine (yellow top pot)
- Stomach contents, small intestinal contents (yellow top pot)
- Representative histological samples (the most important organs are liver and kidney, upper GIT). Remember formalin to tissue ratio should be at least 10:1 to allow adequate fixation.

Clinicians should also consider syndromes which may mimic intoxication such as hypocalcaemia, hypoglycaemia, hepatic encephalopathy, peripheral neuropathies and primary CNS diseases.

If litigation is threatened then you will need to:

- Have a detailed record of all findings
- Record the identity of the animal(s)
- Collect and label specimens
- Seal specimen containers
- Maintain continuity of possession
- Obtain a receipt of specimens.

Examples of intoxicants that can be tested are provided below. See individual tests for sample requirements.
Biological control agents
- Carbamates
- Metaldehyde
- 1080 (fluoroacetate)
- Strychnine
- Synthetic pyrethroids
- Organophosphates
- Organochlorines
- Anticoagulant rodenticides (warfarin, pindone, coumertyl, bromadiolone, difenacoum, brodifacoum)

Heavy metals
- Arsenic
- Lead

Human medicinals
- Paracetamol
- Aspirin
- Drugs of addiction (opiates, sympathetic amines, benzodiazepines, cannabinoids, barbiturates, cocaine, methadone)
- Antidepressants (Amitryptyline, tricyclic antidepressants)
- Phenobaritone, pentobarbitone

Biological toxins
- Snake venom (not in NZ)
- Cyanobacteria
- Botulism
- Mycotoxins

TOXICOLOGICAL TESTING OF LARGE ANIMALS – GENERAL GUIDELINES

Possible toxic causes of production animal ill health and death are numerous and out of the scope of this publication. When investigating suspected poisonings in animals it is important to be methodical:

Take history, examine the animals (clinically or necropsy), examine the environment, collect samples for laboratory examination and record all the findings. You may need to consult with your local laboratory for advice on appropriate samples, sample volumes and handling details.


If litigation is threatened then you will need to:

- Have a detailed record of all findings
- Record the identity of the animal(s)
- Collect and label specimens
- Seal specimen containers
- Maintain continuity of possession
- Obtain a receipt of specimens.

Examples of more common intoxicants that can be tested are provided below. See individual tests for sample requirements
Biological control agents

- 1080 (fluoroacetate)
- Strychnine
- Synthetic pyrethroids
- Organophosphates
- Organochlorines
- Carbamates
- Metaldehyde
- Anticoagulant rodenticides (warfarin, pindone, coumestryl, bromadiolone, difenacoum, brodifacoum)

Heavy metals

- Arsenic
- Lead
- Copper
- Selenium
- Zinc

Biological toxins (non-plant origin)

- Cyanobacteria
- Clostridium botulinum toxin
- Mycotoxins (aflatoxins, fumonisin, Deoxynivalenol, ochratoxin)
- Urea

Biological toxins (plant origin)

- Mycotoxins (aflatoxins, fumonisin, Deoxynivalenol, ochratoxin)
- Nitrate/nitrite
- Cyanide
- Oxalate
- Ergot alkaloids
- Pyrrolizidine alkaloids
- Indospicine
- Ptaquiliosides
- Pimelea toxin

LIST OF TOXICOLOGICAL TESTS KNOWN TO BE AVAILABLE

- Mycotoxin testing (aflatoxins, fumonisin, Deoxynivalenol, ochratoxin)
- Ergot alkaloids
- Zeralenone
- Nivalenol
- Pyrrolizidine alkaloids
- Indospicine
- Ptaquiliosides
- Pimelea toxin
- Strychnine testing
- 1080 testing
- Clostridium botulinum toxin by ELISA
- Clostridium perfringen epsilon testing by ELISA
- Nitrate/nitrite (Qualitative)
- Cyanide
- Urea
- Organophosphate pesticides
- N-methylcarbamate pesticides
- Metaldehyde testing
- Rodenticide testing (Warfarin, pindone, coumestryl, bromadiolone, difenacoum, brodifacoum rodenticides)
- Carbamates
- Synthetic pyrethroids
- Organochlorines
- Arsenic
- Lead
- Copper
- Paracetamol
- Aspirin
- Drugs of addiction screen (opiates, sympathetic amines, benzodiazepines, cannabinoids, barbiturates, cocaine, methadone)
- Antidepressant drugs (amitriptyline, tricyclic antidepressants)
- Phenobarbitone
- Snake venom
- Cyanobacteria
- Selenium
- Zinc

MYCOTOXICOLOGY

SPORIDESMIN TOXICITY (Facial Eczema)

Managing this disease involves consideration of the four factors below:

1. Detecting cases of liver damage
   - Species: sheep, cattle, camelids, deer
   - Specimen: serum for GGT and GLDH
   - Container: gel tube
   - General information: Serum GGT and GLDH rise quickly following sporidesmin injury and are almost always high when signs of liver injury (e.g. photosensitivity and jaundice) appear. At 2-4 weeks following ingestion of sporidesmin, there is a good relationship between the GGT level and degree of liver damage. Cases can be graded as mild, moderate or severe at this stage but this has not yet been proven to have prognostic potential. Testing for GLDH helps differentiate other causes of liver disease. In cases of facial eczema, GLDH levels are usually lower than GGT levels.

2. Determining if zinc intakes are adequate for protection
   - Species: sheep, cattle, camelids, deer
   - Specimen: trough water, drench, feed
   - Container: yellow top pot
   - General information: Determination of zinc levels in drinking (trough) water or drenches can detect whether levels are toxic or adequate for facial eczema protection. Zinc drenches can also be tested for proper mixing of the zinc additive. Zinc in feed can also be tested. Determination of zinc levels in serum is not a recommended method for determining whether animals are receiving a protective
dose, as there is a poor relationship between serum zinc and degree of protection. Serum zinc assay is however useful in cases of suspected zinc overdosage/toxicity - see below.

3. Diagnosing zinc toxicity

- **Species:** sheep, cattle, camelids, deer
- **Specimen:** Live animal: Serum in plain or LH tube for zinc and GGT levels, EDTA for a full CBC if there is anaemia
  Dead animal: Fixed abomasum, liver and pancreas for histopathology. Fresh liver and kidney in yellow top pot for zinc determination
- **General information:** Diarrhoea, weight loss, and metabolic disease in cattle are the main clinical signs of zinc toxicity. A few will also develop a haemolytic anaemia. In the live animal, measuring serum zinc concentrations is a valid way to check for possible zinc toxicity. The problem of zinc in rubber stoppers artifactually increasing serum zinc levels is no longer an issue. In those that appear anaemic or have red urine, a CBC is also recommended. Overdosing zinc can suppress copper and iron absorption. At necropsy, the lesions to look for are abomasal ulceration and pancreatic atrophy and fibrosis. Abomasal ulceration occurs when zinc sulphate is used for drenching, as this triggers closure of the oesophageal groove, diverting the drench directly into the abomasum. For this reason, zinc sulphate drenches are not recommended. Pancreatic changes will occur with poisoning due to any form of zinc salt but are a chronic change and often hard to detect. The more common lesion is hepatobiliary necrosis due to sporidesmin toxicity. It is worth stressing that most cases of suspected zinc toxicity turn out to be sporidesmin toxicity due to under dosing with zinc.

4. Spore counting for facial eczema

4a. Pasture spore counting

- **Name of test:** spore counts for facial eczema
- **Specimen:** pasture from at least 5 locations
- **Container:** paper bag
- **General information:** Selecting the sampling site: This depends on the type of farm and the management policy. If all the animals are in one group, e.g. a dairy farm, only the paddock the animals will graze the next day need be sampled. If set stocking is practised, then it is necessary to sample the dangerous paddocks.

**Tips to help predict the most susceptible paddocks for spore counting:**

a) Spore counts on north and west facing slopes are usually higher than east and south facing slopes.
b) Flats generally have lower counts than slopes above them as cool air flows downward at night.
c) Paddocks with a lot of pasture litter and those that are well sheltered often have higher counts.

A useful system is to use a warm slope as an indicator site and to sample it regularly, at least three times a week. When spore numbers on it start to rise, the other paddocks should be checked to define the spore pattern over the farm. Using this system, a bank of information will accrue and susceptible paddocks will be identified. However, it is not safe to assume only the same paddocks will be susceptible every year.

**Method for obtaining a pasture sample for spore counting:**

- Using shears or scissors, cut a handful of pasture leaves from about 1 cm above ground level at not less than five places, which are at least 10m apart, and submit in paper bags not plastic bags so as to avoid "plant sweating".
- Avoid parts of paddocks, which are sheltered by trees or hedges. Take separate samples if you need to know spore numbers under hedges.

- Sample from an area of even slope.

- If you sample the same site regularly, follow the same route across it.

- Take samples at least 3 times a week and more often if the weather favours spore production. Spore numbers will rise in the absence of rain if the weather is humid, particularly late in the season. Numbers do not always rise immediately after rain and the peak may occur up to a week after the last fall.

4b. Faecal spore counting

Faecal spore counting represents more accurately what the animals are ingesting and therefore more accurately reflects FE risk. Faecal samples are also easier to collect. Faecal spore numbers are approximately double the pasture levels but this is very dependent on grazing pressure. The ratio may be less than 2:1 in cattle because of their lower faecal dry matter. As a tentative recommendation levels should be considered dangerous when faecal spore counts approach 75-100,000 spores per gram of faeces.

As you are interested in the spore intake of the herd or flock rather than individual animals, pooling of 5-10 samples is recommended. These are best pooled at the laboratory. Fresh faeces may be removed from the pasture, yards or from the rectum for this purpose.

MYCOTOXIN SCREENING

The Mycotoxin Screen tests for a range of mycotoxins.

**Toxins tested:** Aflatoxins B1, B2, G1, G2, Fumonisins B1, B2, Deoxynivalenol, Nivalenol, HT2, T2, Ochratoxin A, Zearalanone and Vomitoxin.

**Species:** Sheep, cattle, horses, dogs, pigs, chickens

**Specimen:** Silage, grain in yellow top pottle. Pasture in paper bags

**Special handling for pasture:** Using shears or scissors, cut a handful of pasture leaves from about 1 cm above ground level from at least five places, which are at least 10m apart, and submit in paper bags not plastic bags so as to avoid "plant sweating".

**Collection Protocol:** The feed needs to be as representative as possible to the feed that was being fed at the time of the problem occurring

**General information about the diseases:**

**Zearalenone** is produced by several species of Fusarium fungi. It is an oestrogenic mycotoxin that causes infertility in sheep and pigs and has been incriminated as a cause of infertility in cattle. Most Fusarium growth occurs in late summer and autumn at the base of pasture on dead leaf litter and in poorly stored grain. Green and growing grasses are less affected.

**Tricothecenes - Nivalenol (NIV) and Deoxynivalenol (DON) and T2 toxin**

Tricothecenes are thought to be involved in some cases of ruminant ill thrift. The toxins are produced by Fusarium and other fungal species.

**NIV and DON** usually infect grain, especially maize, and may cause a problem in pigs fed such maize. The effects are dose related:

a) < 5mg/kg is associated with feed refusal and decreased weight gain.

b) > 5 mg/kg can cause vomiting, diarrhoea, abortion, nervous signs and death.
**T2 toxin.** T₂ toxicity is predominantly associated with mouldy maize and is mostly a problem in pigs and poultry. In pigs, besides ill thrift this toxin can also cause an irritant contact dermatitis.

**Aflatoxin** is a member of the bisfuranocoumarin group of compounds, produced as metabolites mainly by *Aspergillus flavus*, *Aspergillus parasiticus* and *Penicillium puberulum*. Sheep and adult cattle are quite resistant to the toxin, horses moderately resistant whereas dogs, pigs and calves are sensitive and may be fatally intoxicated at a dose rate of < 1 mg/kg body weight. Colonisation and toxin production can occur in grains such as maize, cottonseed and peanuts in all phases from growth through harvest. They can be produced in peanuts, soybeans and other small grains mainly during storage. Toxin production is encouraged when warm, moist ambient conditions are combined with crop damage (drought or storm).

**ENDOPHYTE TOXINS**

**Ergot Alkaloids**

**Species:** Sheep, cattle, pigs  
**Specimen:** Pasture in paper bag, grain in yellow top pot  
**Special handling/shipping requirements:** The feed needs to be as representative as possible to the feed that was being fed at the time of the problem occurring.  
**Collection protocol:** Using shears or scissors, cut a handful of pasture leaves from about 1 cm above ground level at not less than five places, which are at least 10m apart, and submit in paper bags not plastic bags so as to avoid "plant sweating".

**General information about the disease:** Ergot Alkaloids are mainly involved in heat stress, gangrene of the extremities and animal ill thrift. It is found in 'wild-type' ryegrass and fescue. Many newer releases of pasture cultivars have been bred to reduce the level of these toxins.

**Lolitrem B and ergovaline**

**Species:** sheep, cattle, horses, deer, camelids  
**Specimen:** Pasture  
**Container:** Paper bag  
**Special handling/shipping requirements:** The feed needs to be as representative as possible to the feed that was being fed at the time of the problem occurring.  
**Collection protocol:**  
Using shears or scissors, cut a handful of pasture leaves from about 1 cm above ground level at not less than five places, which are at least 10m apart, and submit in paper bags not plastic bags so as to avoid "plant sweating".

**General information about the disease:**  
Lolitrem B, a potent tremorgen, is the predominant alkaloid involved in rye-grass staggers. Ergovaline, a vasoconstrictor, is also present. If you have ryegrass, both ergovaline and lolitrem B can be present. If you want to have only one test done, a test for lolitrem B is recommended. If you have fescue, an ergovaline test is recommended. Other problems reported include ill-thrift, heat stress, scours, infertility and lowered milk production.

**Other mycotoxins** (contact the laboratory for information on availability and price)

- Lolitrem B for Perennial Ryegrass can also be tested in fat & faeces as an indication of exposure.
- Ergotamine / ergocristine / ergocryptine / ergocornine / ergosine arising from mould infestation in pasture & grain
- Peramine in Perennial Ryegrass
- Lolines in Fescue & other pastures
- Formononetin / genistein / diadzein / biochanin A / coumarin / coumestrol in clovers & pastures
- Volatile acids & Lactic acid in water & silages
- Tyramines / Tryptamines in phalaris (in development)
Viral Isolation

BOVINE ADENOVIRUS ISOLATION

Species: Bovine
Specimen: Fresh lung, intestine or serum
Container: Pottle or red top tube

Collection protocol: Fresh tissues at necropsy, serum from live animals

Special handling/shipping requirements: Standard

General information about the disease:
Adenovirus infection of cattle primarily presents in yearlings in the winter and virus targets the gastrointestinal tract and kidneys.

General information about when this test is indicated:
For confirmation of the infectious agent or investigation of disease in live affected animals.

Comparison with other related tests:
Histopathology is a useful test to screen for the presence of adenoviral infection. Basophilic intranuclear inclusions can be seen in the endothelium of affected tissues confirming the diagnosis. A PCR test is also available.

BOVINE HERPES MAMMILLITIS VIRUS ISOLATION

Species: Bovine
Specimen: Swabs, fresh tissue
Container: Virus transport media for swabs (contact the laboratory to source these from the referral laboratory), tissues in sterile pottles

Collection protocol: Swab of lesions

Special handling/shipping requirements: Ship all samples chilled

General information about the disease:
An ulcerative condition of the teats caused by bovine herpes virus 2. Pain from the infection can result in decreased milk production. Vesicles form and rupture, leaving a scab covered ulcer. An aspirate of the fluid in the vesicle or collection of the scabs are suitable samples to use for virus isolation.

General information about when this test is indicated:
This test is undertaken by specialist referral government laboratories and would require pre-arranging transport media and notification of the laboratory to expect it

Comparison with other related tests: Nil
BOVINE PSEUDO COWPOX VIRUS ISOLATION

Species: Bovine

Specimen: Swabs, fresh tissue

Container: Virus transport media for swabs (contact the laboratory to source these from the referral laboratory), tissues in sterile pottles

Collection protocol: Swab of lesions

Special handling/shipping requirements: Ship all samples chilled

General information about the disease:

A mild popular condition of the teats caused by bovine parapox virus. Parapoxvirus is related to orf and popular stomatitis virus. Vesicles form and rupture, leaving a scab covered ulcer. Scabs may be abundant and can be collected without causing pain. An aspirate of the fluid in the vesicle or collection of the scabs are suitable samples to use for virus isolation.

General information about when this test is indicated:

This test is undertaken by specialist referral government laboratories and would require pre-arranging transport media and notification of the laboratory to expect it

Comparison with other related tests: Nil

BOVINE VIRAL DIARRHOEA VIRUS ISOLATION

Species: Bovine

Specimen: Swabs, fresh tissue, serum or blood

Container: Plain tube for sera, EDTA tube for blood, virus transport media for swabs (contact the laboratory to source these from the referral laboratory), tissues in sterile pottles

Collection protocol:

Venepuncture, post mortem, swab of lesions

Special handling/shipping requirements:

Ship all samples chilled

General information about the disease:

Bovine viral diarrhoea virus ("pestivirus") is one of the most significant viral diseases in cattle. Clinically, there are three forms of the disease:

- A persistently infected (PI) form which may/may not have clinical signs
- An acute transient form characterised by fever and diarrhoea and short term immunosuppression. These animals will mount an immune response and clear the virus in 10-14 days.
- Mucosal disease (MD) only occurring in PI animals. PI animals are infected by a noncytopathogenic strain of the virus. A subsequent spontaneous mutation of the virus to a cytopathogenic strain within the PI animal results in MD, characterised by seromucoid nasal secretions, severe erosive lesions in the oral and intestinal mucosa, diarrhoea and death.
General information about when this test is indicated:
This test is undertaken by specialist referral government laboratories and would require pre-arranging transport media and notification of the laboratory to expect it.

Comparison with other related tests:
An ELISA test is available to test for the virus either as an antigen or antibody, PCR testing for virus identification and VNT testing for serological responses to infection are also available.

CANINE VIRUS ISOLATION

Species: Canine
Specimen: Swabs, fresh tissue, serum or blood
Container: Red top tube for sera, EDTA tube for blood, virus transport media for swabs (contact the laboratory to source these from the referral laboratory), tissues in sterile pottles

Collection protocol: Venepuncture, post mortem, swab of lesions
Special handling/shipping requirements: Ship all samples chilled

General information about the disease:

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sample</th>
<th>Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canine herpes virus</td>
<td>Lung, liver</td>
<td>Sterile pottle</td>
</tr>
<tr>
<td>Canine adenovirus infectious canine hepatitis</td>
<td>Lung, liver, kidney</td>
<td>Sterile pottle</td>
</tr>
<tr>
<td>Parainfluenza virus</td>
<td>Nasal scraping</td>
<td>Sterile pottle</td>
</tr>
<tr>
<td>Distemper virus</td>
<td>Serum</td>
<td>Plain tube</td>
</tr>
</tbody>
</table>

General information about when this test is indicated:
This test is undertaken by specialist referral government laboratories and would require pre-arranging transport media and notification of the laboratory to expect it.

Comparison with other related tests:
An ELISA test is available to check if canine parvovirus and distemper virus antibodies are present to assess the efficacy of vaccination. Herpes virus and adenovirus infections can be confirmed with histopathology as the lesions are pathognomonic.
CAPRINE HERPES VIRUS ISOLATION

Species: Caprine

Specimen: Vaginal or preputial scraping or swabs,

Collection protocol: Swab of lesions

Special handling/shipping requirements: Ship all samples chilled

General information about the disease:
Caprine herpes virus can cause ulcerative balanoposthitis vulvovaginitis, respiratory disease and abortion in goats.

General information about when this test is indicated:
This test is undertaken by specialist referral government laboratories and would require pre-arranging transport media and notification of the laboratory to expect it

Comparison with other related tests:
A virus neutralisation test is also available to check for antibody in affected goats.

CERVINE PARAPOX VIRUS ISOLATION

Species: Cervine

Specimen: Scrapings or swabs in sterile pottles

Collection protocol: Scrape or swab of lesions

Special handling/shipping requirements: Ship all samples chilled

General information about the disease: Parapoxvirus infections can occur in deer causing ulcerative lesions on velvet.

General information about when this test is indicated:
This test is undertaken by specialist referral government laboratories and would require pre-arranging transport media and notification of the laboratory to expect it

Comparison with other related tests:
Histopathology can be used to examine affected tissues as characteristic lesions are found.

EQUINE VIRUS ISOLATION

Species: Equine

Specimen: Swabs, fresh tissue, serum or blood

Containers:
Red top tube for serum, EDTA tube for whole blood, virus transport media for swabs (contact your local Gribbles Veterinary laboratory to source these from the referral laboratory), tissues in sterile pottles.
**Collection protocol:** Venepuncture, post mortem, swab of lesions

**Special handling/shipping requirements:** Ship all samples chilled

**General information about the disease:** This will vary depending on the disease

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sample</th>
<th>Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calicivirus</td>
<td>Lung, liver, kidney, serum</td>
<td>Sterile pottle, plain tube</td>
</tr>
<tr>
<td>Coital exanthema (EHV-3)</td>
<td>Vaginal scrapings</td>
<td>Sterile pottle</td>
</tr>
<tr>
<td>Equine rhinopneumonitis (EHV-1)</td>
<td>Serum, fetal lung or liver</td>
<td>Plain tube, sterile pottle</td>
</tr>
<tr>
<td>Equine adenovirus</td>
<td>Nasal scrapes, serum, tonsil</td>
<td>Sterile pottle, plain tube</td>
</tr>
<tr>
<td>Equine rhinovirus</td>
<td>Nasal secretions, serum</td>
<td>Sterile pottle, plain tube</td>
</tr>
</tbody>
</table>

**General information about when this test is indicated:**

This test is undertaken by specialist referral government laboratories and would require pre-arranging transport media and notification of the laboratory to expect it

**Comparison with other related tests:**

Check if an ELISA test is available to test for the virus either as an antigen or antibody.

**PORCINE VIRUS ISOLATION**

**Species:** Porcine

**Specimen:** Swabs, fresh tissue, serum or blood

**Container:** Plain tube (red top) for serum, EDTA tube for whole blood, virus transport media for swabs (contact the laboratory to source these from the referral laboratory), tissues in sterile pottles

**Collection protocol:** Venepuncture, post mortem, swab of lesions

**Special handling/shipping requirements:** Ship all samples chilled

**General information about the disease:**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sample</th>
<th>Container</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encephalomyocarditis virus</td>
<td>Heart, spleen, brain</td>
<td>Sterile pottle</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>Swab</td>
<td>Sterile pottle</td>
</tr>
<tr>
<td>Parvovirus</td>
<td>Sow serum, fetal spleen, lung or heart</td>
<td>Plain tube, sterile pottle</td>
</tr>
</tbody>
</table>

**General information about when this test is indicated:** This test is undertaken by specialist referral government laboratories and would require pre-arranging transport media and notification of the laboratory to expect it
Comparison with other related tests: Check if an ELISA test is available to test for the virus either as an antigen or antibody.
Vitamin analyses

VITAMIN A /CAROTENE
Species: Production animals and companion animals
Specimen: Serum (or fresh tissue)
Container: Plain (red top) tube (tissue in yellow top pot)
Special handling/shipping requirements: Serum should be separated promptly from the clot, and samples need to be protected from light (wrap in aluminium foil). Serum must be frozen if testing is delayed >24 hours after collection.

VITAMIN B1/THIAMINE
Specimen: Whole blood, frozen
Container: Lithium heparin or EDTA
Special handling/shipping requirements: Sample wrapped in foil and frozen
General information about the disease: Polioencephalomalacia is confirmed at necropsy by demonstrating typical histopathology in formalin fixed cerebral cortex. In the live animal, response to vitamin B1/thiamine administration is also a valid confirmatory test.

VITAMIN B6
Specimen: Serum
Container: Plain (red top) tube

VITAMIN B12 /COBALT
Species: Production animals and companion animals
Specimen: Serum, plasma or fresh liver
Container: Plain (red top) or gel or lithium heparin tube; liver in yellow top potte
Collection protocol: Liver can be collected by biopsy in the live animal, at meatworks or following on-farm slaughter of production animals.
Special handling/shipping requirements: Samples must be frozen if testing is delayed >48 hours after collection.
General information about the disease: Liver Vit B12 levels accurately indicate body reserves of the mineral. Serum Vit B12 levels can be influenced by day to day fluctuations.
General information about when this test is indicated: See production animal mineral testing for further information.
VITAMIN C

Specimen: Serum
Container: Gel tube
Special handling/shipping requirements: Sample wrapped in foil and frozen

VITAMIN D

Species: Production animals and companion animals
Container: Plain (red top) or gel and fixed tissue in formalin

General information about the disease: Production animals are usually poisoned due to incorrect feed formulation. Companion animals are poisoned with excessive Vitamin D supplements or cholecalciferol. In the live animal the serum sample is for biochemistry (especially urea, creatinine, calcium, phosphate as well as Vitamin D). In the dead animal, the fixed tissues investigate the presence of tissue mineralisation and renal damage.

VITAMIN E

Species: Mainly pigs
Specimen: Serum, plasma or fresh tissue
Container: Plain (red top) tube or lithium heparin tube; tissue in yellow top pot
Special handling/shipping requirements: Serum should be separated promptly from the clot and samples need to be protected from light (wrap in aluminium foil).

General information about the disease: in the dead animal, fixed heart can also be submitted to check for typical histopathology of Vitamin E deficiency.

For all other vitamin analyses, contact your local Gribbles Veterinary Laboratory for availability.