

# Serology

## Production animals:

### 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, 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 identified 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, 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. 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 respiratory or reproductive tract discharges and is a useful test for acute clinical cases. To assess the size of the antibody response, virus neutralisation titres (VNT – referral test) on paired convalescent sera are required as an additional but more expensive test.

### 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 is a cause of outbreaks of acute primary respiratory disease and contributes to the bovine respiratory disease complex in calves, with a peak incidence at 1-3 months of age. It also predisposes to bacterial pneumonia caused by organisms such as *Mannheimia haemolytica*, *Pasteurella multocida* and *Histophilus somni* by impairing lung defence mechanisms.

**General information about when this test is indicated:** To determine a role for BRSV in bovine respiratory disease investigations.

**Comparison with other related tests:** Additional diagnostic tests include virus isolation (referral test) and histopathology to look for characteristic viral cytopathic changes.

\*NOTE – This test is not performed by Gribbles Veterinary and requires referral to an external reference laboratory.

## 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 a positive GD test confirms *B. ovis* infection. For additional evidence of infection, semen culture of serologically positive rams could be an additional step.

In flocks where *B. ovis* has been detected using CFT and/or GD, it is recommended to test all the sera negative to the CFT using the *B. ovis* ELISA. This test has high sensitivity and 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.

[Brucella ovis Flock Accreditation Scheme guidelines](#)

## 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 Veterinary 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, oesophageal, and intestinal mucosa, diarrhoea and death.

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

- Recommended for cattle older than 10-months-of-age once all colostral immunity has waned. It may also be used in pre-suckling calves to determine whether exposure occurred during late gestation.
- Once infected, cattle will be viraemic for 10-14 days. About 2-4 weeks after infection is cleared, antibodies to BVD will be produced and can be detected.
- Detecting BVD 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 BVD infection. Nevertheless, detecting antibody is significant as it shows there was virus circulating in the herd.
- Pooled antibody testing is possible using bulk tank milk or 9-15 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.

**BVD testing summary:**

BVD tests to use in calves from conception to 10 months of age

Age	PCR	Antigen ELISA	Antibody ELISA	Sample
Conception-40 days	x	x	x	N/A
40-120 days gestation	✓	x	x	Fetal fluid
150 days – birth	✓	x	x	Fetal fluid
Birth-35 days	✓	x	x	Serum/skin
35 days -10 months	✓	✓	x	Serum/skin
10 months and older	✓	✓	✓	Serum/skin

Individual tests for BVDV depending on disease or physiological state

Disease or physiological state	PCR	Antigen ELISA	Antibody ELISA	Sample
Transient infection (TI)	✓	✓	✓	Serum/skin
Conceptus loss	x	x	✓	Serum
Pregnant (Trojan)	x	x	x	N/A
Persistent infection (PI)	✓	✓	✓	Serum/skin
Mucosal disease	✓	✓	✓	Serum/skin

Group test options

Physiological state	PCR	Antigen ELISA	Antibody ELISA	Sample
Milking	✓	x	✓	Bulk milk
Non milking	✓	x	✓	Pooled serum

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 recently 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 has been validated as a quantitative test.

**Comparison with other related tests:** Can be used in conjunction with BVD antibody ELISA, PCR and antigen ELISA testing.

*\*NOTE – This test is not performed by Gribbles Veterinary and requires referral to an external reference laboratory.*

## BVD ANTIGEN ELISA

**Species:** Bovine

**Specimen:** 10 mL serum

**Container:** Red top or gel tube

**Collection protocol:** Venepuncture

**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. A positive result could be TI or PI so retesting is indicated in 3-4 weeks. 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.

## 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 is most prevalent in dairy goats. Arthritis is the most common clinical sign in adult goats while encephalomyelitis can present in young kids. Adults may also develop progressive interstitial pneumonia and chronic mastitis.

**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:** Enzootic bovine leucosis is a rare viral disease of cattle capable of inducing neoplasia. It is transmitted horizontally via body fluids and vertically from dam to calf via infected milk. Less than 3% of infected animals develop neoplasia, with a peak incidence at 6-8 years of age. Affected animals may develop lymphoma in almost any internal organ, and superficial lymph nodes and the retrobulbar region may be affected. 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,

**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 >97% 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, sensitivity is low. However, with clinically affected animals, the sensitivity improves to approximately 66%.

**General information about when this test is indicated:** ELISA is the first test of choice. AGID is used for specific import requirements of some countries and for ancillary testing

**Comparison with other related tests:** ELISA and CFT are other serological tests. Faecal PCR increases sensitivity and may be used to quantify shedding in individual animals or to test pools of up to 5 samples. Faecal Ziehl-Neelsen stains are also possible to check for acid fast organisms, but lack sensitivity.

## JOHNE'S DISEASE COMPLEMENT FIXATION TEST (CFT)

**Species:** Ovine, cervine, bovine, llamoid, 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 >97% 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, the sensitivity is <50%. However, with clinically affected animals, the sensitivity improves to approximately 55%.

**General information about when this test is indicated:** ELISA is the first test of choice. CFT is used for specific import requirements of some countries and for ancillary testing

**Comparison with other related tests:** ELISA and AGID are other serological tests. Faecal PCR increases sensitivity and may be used to quantify shedding in individual animals or to test pools of up to 5 samples. Faecal Ziehl-Neelsen stains are also possible to check for acid fast organisms, but lack sensitivity.

## JOHNE'S DISEASE ELISA

**Species:** Ovine, cervine, bovine, llamoid, 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 and diarrhoea is present in grazing animals

**Comparison with other related tests:** AGID and CFT are other serological tests. Faecal PCR increases sensitivity and may be used to quantify shedding or to test pools of up to 5 samples. Faecal Ziehl-Neelsen stains are also possible to check for acid fast organisms, but lack sensitivity.

## 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 contagious and zoonotic disease 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 leptospirosis may cause 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 in convalescent sera

**Comparison with other related tests:** PCR can be used to detect the presence of leptospira in blood or urine in acute cases.



## LIVER FLUKE ANTIBODY ELISA (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 Veterinary laboratory for details

**Special handling/shipping requirements:** Ship chilled

**General information about the disease:** Liver fluke (*Fasciola hepatica*) 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

## LIVER FLUKE ANTIBODY ELISA (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 (*Fasciola hepatica*) 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. 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

## NEOSPORA ELISA

**Species:** Bovine, ovine

**Specimen:** Serum

**Container:** Plain (red top) or gel tube (*Note: 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, causes abortions in cattle of all ages. 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. The dog is the definitive host.



Some work suggests sheep may be affected too, but the likely seroprevalance 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 recent 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 fetal 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, causes abortions in cattle of all ages. 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. The dog is the definitive host.

Dogs may suffer from a variety of clinical manifestations of *N. caninum* infection), 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. Dermatitis and interstitial pneumonia are also reported.

**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 with titres of up to 1:200 indicates 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  $\geq 1/600$  is indicative of an association between the abortion and Neospora infection. For investigation of reproductive disease in groups use the Neospora ELISA.

## 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 acute abomasal damage. For general parasite screening of adult cattle faecal egg counts are another option.

## 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 in cattle 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

\*NOTE – This test is not performed by Gribbles Veterinary and requires referral to an external reference laboratory

## 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 foetus.

**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:** Virus isolation is available through reference laboratory. Necropsy of aborted piglets and histopathology of fetal tissues can be used to investigate other causes of abortion.

\*NOTE – This test is not performed by Gribbles Veterinary and requires referral to an external reference laboratory.

## Companion animals

### ALLERGY TESTING\*

**Species:** Canine, feline, equine

**Specimen:** Serum (at least 1.5mL)

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

**Collection protocol:** Venepuncture

**Special handling/shipping requirements:** Standard

**General information about the disease:** Atopy is an inflammatory and pruritic allergic skin disease with characteristic clinical features. It is characterised by the tendency to produce IgE antibodies to commonly encountered environmental antigens.

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

The Heska Allercept IgE test can be used to support a clinical diagnosis of atopy or other IgE-mediated disease. The test does not diagnose atopy because some non-atopic, healthy animals may have high circulating IgE levels to some allergens.

The test identifies allergen specific IgE in the serum of dogs, cats and horses using an ELISA technique. The panel tests a total of 48 allergens, including weeds, mites/insects, trees, grasses and moulds, as recommended by specialist dermatologists for Australasian conditions. Knowledge of which allergens are involved can then be used to develop allergen specific immunotherapies\*\*.

This test should be used following consultation with your dermatologist.

\*NOTE – This test is not performed by Gribbles Veterinary and requires referral to an external reference laboratory.

\*\* *Due to license/import restrictions allergen specific immunotherapy products are only available through limited channels such as practices specialising in dermatology. Gribbles Veterinary is currently unable to import or sell these items to veterinarians.*

### 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 (SLE), 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 positive ANA titre needs to be interpreted in light of supportive clinical signs (e.g. polyarthritis, skin disease) and laboratory findings (e.g. proteinuria, haematological abnormalities such as thrombocytopaenia, anaemia or leukopaenia) that may also be seen with SLE. Negative results are uncommon and do not necessarily rule out SLE. Positive ANA titres are non-specific and can also be present in a variety of disease states such as infectious and non-immune-mediated inflammatory diseases. They can also be induced by some therapeutic drugs as well as occasionally being present in normal individuals. These ANA titres are generally weak or transitory. A cat ANA test is also available.

## 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.

## CHLAMYDIA ANTIGEN TEST

**Species:** Feline, avian

**Specimen:** Conjunctival, nasal/choanal, oropharyngeal or cloacal swabs

**Container:** Dry swab (plastic handle) placed in a sterile container or swab carrier

**Collection protocol:** Swab affected tissue

**Special handling/shipping requirements:** Standard

**General information about the disease:** *Chlamydomphila felis* is a major cause of conjunctivitis in cats and is one of the major pathogens implicated in feline upper respiratory tract disease. Coinfections with other upper respiratory tract pathogens such as feline herpesvirus-1 and/or calicivirus may also occur. *C. felis* is transmitted by direct contact and cats <1 year of age and those from multicat environments are at most risk. The organism can also cause a rhinitis and pneumonia however these disease presentations are uncommon to rare.

*Chlamydomphila psittaci* is the cause of avian chlamydiosis, also known as psittacosis. It has a worldwide distribution and poses a zoonotic risk to humans. Domestic and wild birds infected with the agent may have inapparent subclinical disease (common) or acute, subacute to chronic disease. The respiratory or digestive tracts are primarily affected however systemic illness can also occur.

**General information about when this test is indicated:** The chlamydia antigen test is a lateral flow immunoassay (LFA) test that detects the presence of chlamydial antigens\*

**Comparison with other related tests:** A *C. felis* PCR test is available and deemed more sensitive and specific than the chlamydia antigen test. Cytology of conjunctival smears or impression smears from affected tissues (e.g. spleen, liver, and lung) can be checked for chlamydial inclusions however these are easily confused with other, non-specific basophilic bodies that may be present within epithelial cells.

\* This chlamydia antigen test is used for the detection of *C. trachomatis* in humans however it has been shown to detect all known Chlamydial serovars (ref: Chlamydia Rapid Test Cassette Package Insert)

## CRYPTOCOCCUS ANTIGEN TEST\*

**Species:** Feline, canine

**Specimen:** Serum, CSF

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

**Collection protocol:** Venepuncture, CSF tap

**Special handling/shipping requirements:** Standard

**General information about the disease:** Cryptococcosis is the most common systemic fungal disease of cats. It is caused by the fungus *Cryptococcus neoformans* and *C. gatti*. *C. neoformans* is the most medically important species and it has a worldwide distribution being particularly abundant in soil contaminated with pigeon droppings. Most cases of feline cryptococcosis present as a mycotic rhinitis. Cutaneous, neurological and ocular involvement reflect systemic spread. Although this disease is most common in cats it is also seen in dogs, cattle, horses, sheep, goats, birds, wild animals and people. Dogs usually present with disseminated disease with neurological and ocular involvement.

**General information about when this test is indicated:** The cryptococcus antigen test is a lateral flow immunoassay (LFA) that measures antigen titres in serum or CSF fluid. Antigen titres can be extremely high but even a low titre is considered a positive result.

The cryptococcus antigen test is a useful method of diagnosis in suspected cases in which the organism is not identified by other means. This test can also be used as a method of monitoring response to treatment.

**Comparison with other related tests:** Cytology and histology of suspected lesions may reveal the fungal organisms. Culture is also another method of detection however not currently available due to the zoonotic risk.

*\*NOTE – This test is not performed by Gribbles Veterinary and requires referral to an external reference laboratory*

## 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). Purebred male cats appear to be predisposed. 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 but does not differentiate between enteric coronavirus and FIP virus antibody titres. A positive titre 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. The albumin to globulin (A:G) ratio can also be measured in effusions as well as feline enteric coronavirus/FIP virus titre.

Intraocular and neurological symptoms: 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) and immunohistochemical detection of the virus within these lesions is currently considered the diagnostic gold standard



## FELINE IMMUNODEFICIENCY VIRUS (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 that are born to cats that have been either infected or vaccinated and have transferred maternal antibody to their kittens. The presence of these maternal antibodies can interfere with serological testing for FIV in kittens <6m of age.

**Comparison with other related tests:** FIV PCR testing is another option for disease status investigation



## 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 peripheral blood cytopaenias, neoplasia (especially lymphoma) and/or secondary infection secondary to immunosuppression. These cats usually 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 in New Zealand, 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 must be interpreted in light of clinical and haematological changes that are compatible with FeLV infection.

**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 are 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 they are still positive after 1 year it is likely they will remain viraemic for life.

## 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:** Canine rheumatoid-like polyarthritis is a non-infectious, erosive polyarthritis. Affected dogs have episodes of anorexia, depression and fever with generalised or shifting lameness associated with swelling around the joints. Many of the dogs affected are of the small and toy breeds, with a significant number being Shetland sheepdogs.

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. Serologic testing for RF is expected to be positive in up to 70% of dogs with this disease so a negative result does not rule out the disease. False positive results can also occur in dogs with other systemic inflammatory disease so clinical correlation is required.

**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 Fetal 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.