PCR Tests

ASPERGILLUS FUMIGATUS

Species: Bovine, equine, canine and cats

Specimen: Depends on site of infection, e.g. fetal stomach content in abortion cases, swabs from nasal infections, tissue biopsies.

Container: Sterile container with tissue, fluid, swab

Collection protocol: Depends on sample type may involve removal of cotyledons or tissue biopsy, aspiration of fluid, nasal swab.

Special handling/shipping requirements: Dry swab samples are stable at room temperature. Other samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Can cause site-specific mycotic disease e.g. mycotic abortion in cattle, guttural pouch mycosis in horses, nasal infections in dogs and cats. Infection is acquired from environmental sources, generally inhalation or ingestion. It is an opportunistic pathogen depending on impaired, overwhelmed or by-passed host defences to permit hyphal invasion of the tissues

Comparison with other related tests: PCR provides rapid detection and specific identification of the organism. However, due to the ubiquitous nature of the organism histopathology with silver or PAS staining to demonstrate septate hyphae invading tissues, can provides further evidence of the organism's involvement in the disease. Other methods of laboratory diagnosis include culture, which is less sensitive than PCR.

BACILLUS LICHENIFORMIS

Species: Bovine, ovine

Specimen: Fetal stomach contents

Container: Sterile container

Collection protocol: Aspiration of fetal stomach contents into sterile container

Special handling/shipping requirements: Samples be transported and stored chilled. Do not freeze samples.

General information about the disease: Can cause abortion and mastitis in sheep and cattle.

Comparison with other related tests: PCR provides a more rapid and specific method for identification than culture.

BORDER DISEASE VIRUS/ HAIRY SHAKER DISEASE (BDV/HSD)

Species: Ovine

Specimen: Serum, or heart blood or stomach contents from a dead fetus

Container: Plain (red top) or gel tube

Collection protocol: Serum – venepuncture. To identify carriers, take serum and request pooling (5 samples per pool).

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Hairy shaker disease (HSD) is caused by a Pestivirus. Border disease viruses (BDV) 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 BVD 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 BVD infection are seen and vary depending on the age of the fetus 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 occasionally '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. 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: Looking to confirm clinical cases and identify it as a cause of abortion and to identify carriers.

Comparison with other related tests: There is an antibody ELISA is available.

BOVINE ADENOVIRUS 10

Species: Bovine

Specimen: Fresh tissue (intestine), EDTA blood, or formalin-fixed tissues

Container: Sterile container for fresh tissue; EDTA blood tube

Collection protocol: Excision of fresh tissue. Venepuncture

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: BoAdv10 has been reported in New Zealand and overseas to be involved in characteristic small outbreaks of haemorrhagic diarrhoea, recumbency and death in young cattle.

Comparison with other related tests: Histopathology on very fresh specimens is useful.

BOVINE HERPESVIRUS PCR (INFECTIOUS 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 New Zealand, only subtype 2b has been reported. 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 present in New Zealand, are more virulent and 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.

BOVINE VIRUS DIARRHOEA (BVD)

Species: Bovine

Specimen: 10 mL serum, 50 mL milk, ear notch

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

Ear notch – The “AllFlex TSU” is preferred sampling system.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

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, 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:

See the information sheet on our website - Collection of bulk tank milk

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/ear notch
35 days -10 months	✓	✓	x	Serum/skin
10 months and older	✓	✓	✓	Serum/skin

Note: skin/ear notch samples are only suitable for PCR testing

Individual tests for BVD 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

CAMPYLOBACTER FETUS SUBSP. FETUS

Species: Bovine, ovine

Specimen: Stomach contents

Container: Sterile container

Collection protocol: Aspirate fetal stomach contents into sterile container.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Out breaks of abortion in sheep, occasional cause of abortion in cattle.

Comparison with other related tests: The organism although it can be cultured is fragile and the inability to isolate it does not necessarily rule it out, whereas qPCR can provide rapid detection and identification.

CAMPYLOBACTER JEJUNI

Species: Many domestic, wild animals and birds

Specimen: Ovine fetal stomach contents; faeces

Container: Sterile container

Collection protocol: Aspirate stomach contents into sterile container. Collect fresh faeces into sterile container.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Commensal on the oral mucosa and intestinal tract of animals and birds. Pathogenic isolates of *C. jejuni* show several virulence attributes. There is doubt about its ability to cause abortion and diarrhoea in sheep and diarrhoea in cattle. Associated with enteritis and diarrhoea in cats and dogs which can be passed on to humans.

Comparison with other related tests: Can be cultured with definitive identification of isolates by either MALDI-TOF or based on a number of biochemical and other tests. qPCR can provide rapid detection and identification.

CHLAMYDIA PECORUM

Species: Wide host range including cattle sheep and other production animals and wildlife

Specimen: Swab, fluid or tissues from affected areas.

Container: Sterile container

Collection protocol: Collect specimen into a sterile container.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Typically associated with sporadic bovine encephalomyelitis (SBE), polyarthritis and conjunctivitis in ruminants. Has been implicated in respiratory disease and

reproductive diseases including abortion in sheep and cattle. There are reports of *C. pecorum* causing enteritis in sheep

Comparison with other related tests: Cell culture, antigen detection and serology can be used but are of limited availability in New Zealand.

FELINE CALICIVIRUS

Species: Feline

Specimen: Conjunctival and/or a oropharyngeal swab (A single conjunctival or oropharyngeal swab can be submitted and used to test for feline calicivirus, herpesvirus and chlamydophila)

Container: Sterile container 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). 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: Cell culture can be used but is of limited availability in New Zealand.

FELINE CHLAMYDOPHILA

Species: Feline

Specimen: Swab (a single conjunctival or oropharyngeal swab can be submitted and used to test for feline calicivirus, herpesvirus and chlamydophila).

Container: Sterile container 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 *Chlamydophila felis*, infection in cats.

Comparison with other related tests: Cell culture with Giemsa staining or antigen detection can be used but are of limited availability in New Zealand**.**

FELINE HERPESVIRUS-1

Species: Feline

Specimen: Conjunctival and/or oropharyngeal swab (a single conjunctival or oropharyngeal swab can be submitted and used to test for feline calicivirus, herpesvirus and chlamydophila).

Container: Sterile tube 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). 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: Cell culture can be used but is of limited availability in New Zealand.

FELINE IMMUNODEFICIENCY VIRUS (FIV)

Species: Feline

Specimen: 0.5-1 mL of EDTA anticoagulated blood.

Container: EDTA

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.

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, leucopoenia, 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 vaccination status is unknown. 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 vaccinal 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)

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

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.

HELICOBACTER *(rappini-like spp.)*

Species: Ovine

Specimen: Fetal stomach contents

Container: Sterile container

Collection protocol: Aspirate fetal stomach contents

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Mid to late term abortions, perinatal death and weak lambs. Aborted fetuses are characterised by liver lesions that are grossly and microscopically identical to those produced in Campylobacter infections.

Comparison with other related tests: PCR will not detect all Helicobacter spp. but currently detects strains causing disease in the South Island. Culture of the organism is difficult. PCR is a useful primary diagnostic test, or to confirm a histopathological diagnosis.

HISTOPHILUS SOMNI

Species: Bovine, ovine

Specimen: Swabs and fresh tissue but depends on disease or lesions.

Container: Sterile container for tissues, fluids or swabs

Collection protocol: Depends on sample type, may involve removal of cotyledons or tissue biopsy, aspiration of fluid, nasal swab.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Causes a variety of conditions in domestic ruminants including respiratory disease with septicaemia, meningitis/meningoencephalitis (TEME) and genital infections such as endometritis and abortion. The organism can occur in the semen and genital tract of bulls. It is also a commensal of genital tract of sheep and causes epididymitis and orchitis in rams.

Comparison with other related tests: *Histophilus* species are fragile organisms and difficult to culture. PCR provides a rapid, specific method of detection and identification in a range of sample types.

JOHNE'S DISEASE

Species: Bovine

Specimen: faeces

Container: Sterile container

Collection protocol: Faeces collected per rectum using single use disposable gloves and faeces placed in sterile container. Up to 5 faecal samples can be pooled in the laboratory prior to testing.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Johne's disease is caused by an infection with the bacterium *Mycobacterium avium paratuberculosis* (MAP) of the gut in cattle and other ruminants. Gradual thickening and inflammation of the intestinal wall eventually prevents uptake of nutrients. Clinical Johne's disease is characterised by ill-thrift, progressive weight loss and profuse diarrhoea. No cure is available and the condition is eventually fatal. Calves and young stock are particularly susceptible to infection. However, the disease has a very long 'incubation period' so that clinical signs of Johne's disease typically appear several years later in the adult cow. Shedding of Johne's bacteria (primarily in faeces) also increases with age and advanced stage of infection. Shedding may be intermittent during early stages of the disease so faecal samples may need to be taken at intervals (e.g. every 6months) and tested.

Comparison with other related tests: *Mycobacterium avian paratuberculosis* culture is undertaken by reference laboratories and the organism can take up to 16 weeks to grow. Ziehl-Neelsen (ZN) staining of faecal or ileocaecal valve mucosal smears is no longer recommended. Various serological test are available for diagnosis including ELISA, complement fixation test (CFT) and agar gel immunodiffusion test (AGID). The ELISA and CFT are useful for confirmation of infection of clinically affected animals. The semi-quantitative PCR has an advantage in that it is a rapid test used to detect the presence of the organism and to assess the degree shedding from an animal.

LEPTOSPIRA PCR

Species: Ovine, cervine, bovine, caprine, llamoid, canine, equine, porcine

Specimen: Whole blood, urine

Container: EDTA tube, sterile container

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.

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 fetuses can be submitted where leptospirosis is suspected.

LISTERIA MONOCYTOGENES AND IVANOVII

Species: Bovine, ovine and other ruminants

Specimen: Fetal stomach contents in abortion cases. In other diseases, take fresh tissues/fluid from affected areas.

Container: Sterile container

Collection protocol: Aspirate fetal stomach contents or other fluids. Excise tissue or lesion.

Special handling/shipping requirements: Samples be transported and stored chilled. Do not freeze samples.

General information about the disease: *Listeria monocytogenes* and *L. ivanovii* are both pathogenic for animals but *L. monocytogenes* is more significant causing multiple disease conditions including septicaemia, abortion, mastitis, CNS infections and diarrhoea outbreak resembling salmonellosis in ruminants. *L. monocytogenes* can infect humans and is of public health significance. *L. ivanovii* can cause abortions but not diarrhoea in sheep.

Comparison with other related tests: Listeria is readily cultured but PCR can provide rapid specific detection.

MALIGNANT CATARRHAL FEVER (MCF) PCR

Species: Bovine, cervine, swamp buffalo

Specimen: Whole blood or clots from serum tube. Heart blood from dead animals.

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: A common lesion in the eye is corneal opacity. Affected animals are also likely to be pyrexic with markedly elevated rectal temperatures as high as 42^{o}C. 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: Clinical signs and histopathology can be used to diagnose the disease

MORAXELLA (*M. bovis, M. ovis, M. bovoculi*)

Species: Bovine, ovine and other ruminants

Specimen: conjunctival or ocular swab or swab of ocular secretions

Container: Sterile container, vacutainer

Collection protocol: If possible, take a swab of lacrimal secretions deep in the inner canthus of the eye, or swab of fluid from the conjunctiva and ocular surfaces of the eye. Send swab in sterile container e.g. red top vacutainer. Swabs can also be sent in microbiological transport media. Pooling up to 5 swabs in the same sample container is acceptable.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: *Moraxella bovis* is considered the main causative agent of Pinkeye in cattle. There is also a suggestion that *Moraxella bovoculi* and other pathogens such as *Mycoplasma spp.* and infectious bovine rhinotracheitis (IBR) may facilitate *Moraxella bovis* – associated ocular colonization or spread.

Comparison with other related tests: *Moraxella* spp. can be cultured but qPCR apart from providing a rapid alternative is particularly useful in cases where culture results in a heavy mixed growth of organisms (contaminants/normal flora) with no significant isolates identified.

MORTIERELLA WOLFII

Species: Bovine

Specimen: Fetal stomach contents. Cotyledons or infected tissue. Serum or blood from animals with suspect mycotic pneumonia.

Container: Sterile container

Collection protocol: Aspirate fetal stomach contents.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Causes abortion in cattle. Unlike other fungal causes of abortion, 20% of cows that abort because of *M. wolfii* will subsequently develop fatal mycotic pneumonia. It is likely that fungi establish a primary focus of infection in the lungs. The fungi then travel through the blood to the uterus, resulting in endometritis and abortion. After abortion, in 20% of cows, large numbers of fungi then re-enter the circulation, resulting in embolic pneumonia and/or occasionally encephalitis.

Comparison with other related tests:

M. wolfii can be grown in culture but the qPCR provides a more rapid and specific method of detection.

MYCOPLASMA HEMOFELIS

Species: Feline

Specimen: 1 mL whole blood

Container: EDTA (purple top) tube

Collection protocol: Venepuncture

Special handling/shipping requirements: Standard

General information about the disease*:* *Mycoplasma 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 *M. 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.

MYCOPLASMA BOVIS

Refer to MPI guidelines for commercial testing of *M. bovis*

Species: Bovine

Specimen: Milk/colostrum; nasopharyngeal swabs, tonsillar crypt swabs, preputial swabs; Semen; Exudates/dry swabs of lesions.

Container: Sterile container

Collection protocol: See guidelines published by Dairy Cattle veterinarians' branch of the NZVA and by MPI - Nasopharyngeal swabs / Preputial swabs

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: *Mycoplasma bovis* is a bacterium that causes illness in cattle, including udder infection (mastitis), abortion, pneumonia, and arthritis. *Mycoplasma bovis* is the most common mycoplasma of cattle, and the most important of its family. The closest relative to *M. bovis* is *M. agalactiae* found in sheep. *M. bovis* was first reported in New Zealand in 2017. The clinical symptoms of affected cattle in New Zealand so far includes: mastitis in dry and milking cows, arthritis in cows, late-term abortions and the birth of premature calves. It is expected that calves on affected farms will have an increased incidence of pneumonia and otitis media. This will increase the morbidity and mortality on affected farms and if this disease establishes, an increased replacement rate for cattle will be required. The organism has not been detected in New Zealand prior to 2017 despite serious attempts to find it via surveillance. A 2009 survey of bulk milk (vat) samples randomly selected from New Zealand dairy farms found no evidence of infection using polymerase chain reaction (PCR) and bacterial culture techniques. *M. bovis* is listed as an Unwanted Organism under the Biosecurity Act 1993.

Comparison with other related tests: The serum ELISA is one of the most sensitive tests for *M. bovis* but is unsuitable for use in single animals and small mobs and has the potential to produce false positive

(possibly due to cross reaction with other species of mycoplasma) and false negatives in animals that have not seroconverted at the time of testing. It is good at detecting historic exposure and as herd test.

MYCOPLASMA CONJUNCTIVAE

Species: Ovine

Specimen: conjunctival or ocular swab or swab of ocular secretions

Container: Sterile container or vacutainer

Collection protocol: If possible take a swab of lacrimal secretions deep in the inner canthus of the eye, or swab of fluid from the conjunctiva and ocular surfaces of the eye. Send swab in sterile container e.g. red top vacutainer. Swabs can also be sent in microbiological transport media. Pooling up to 5 swabs in the same sample container is acceptable.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Pink-eye (Infectious ovine keratoconjunctivitis; IOK) is characterised by a severe ocular discharge, conjunctivitis and a corneal opacity that can eventually lead to perforation of the cornea and permanent blindness. It can affect all ages of sheep and goats and the predisposing causes include exposure to dust, flies and close contact during yarding. In New Zealand Cooper (1967) found outbreaks occurred throughout the year but increased in autumn.

In NZ *M. conjunctivae* is considered the primary causative agent in sheep. Overseas research has shown that *Moraxella ovis* and *M. conjunctivae* are the most commonly isolated organisms in IOK although both these organisms can also be isolated from apparently healthy eyes.

Comparison with other related tests: *M. conjunctivae* is difficult to culture so qPCR provides a rapid method of detection and specific identification

MYCOPLASMA OVIS

Species: Ovine

Specimen: EDTA blood

Container: EDTA (purple top) tube

Collection protocol: Venepuncture. It is advisable to take multiple blood samples from the mob.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: Causes ovine haemoplasmosis, which is characterized by poor weight gain, severe anaemia, and even mortality in lambs and, occasionally, young adult sheep. Chronic sustained infection can occur asymptomatically in older sheep. M. ovis also causes a more severe disease in goats

Comparison with other related tests: A CBC and a blood smear made at the time of blood collection provides useful additional information for a diagnosis.

NEOSPORA CANINUM

Species: Bovine

Specimen: Fresh brain (including from mummified fetus) 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*, is an apicomplexan protozoan parasite infection that may cause 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.

General information about when this test is indicated: The PCR is most suitable for abortion investigations to detect *N. 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.

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.

STREPTOCOCCUS EQUI SUBSPECIES EQUI

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 container

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. It is important to be aware that even if the nasal samples are shown to be free 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 detect 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.

THEILERIA ORIENTALIS IKEDA

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 *Theileria 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.

TOXOPLASMA GONDII

Species: Ovine

Specimen: Brain tissue even from mummified fetuses. Stomach contents represents a subsample of the amniotic fluid, fetal fluid and fetal plasma, hence is an ideal sample to test for aetiological agents by microbiological culture and PCR testing.

Container: Sterile container for brain tissue and fetal stomach contents

Collection protocol: Excision of brain tissue and aspiration of fetal fluid.

Special handling/shipping requirements: Samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease: *T. gondii* is a common cause of abortion in sheep. Clinical ovine toxoplasmosis occurs following primary infection of pregnant sheep through ingestion of sporulated oocysts. Infected animals may subsequently abort or produce still-born and/or weak lambs, often along with a small mummified fetus.

Comparison with other related tests: Use the PCR result in combination with LAT, histopathology of brain and cotyledons and the clinical signs for investigating possible *Toxoplasma gondii* abortion

TRITRICHOMONAS FETUS - 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: *Tritrichomonas fetus* is a flagellate, pyriform protozoan parasite that can cause infertility in cattle.

T. 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 *T. 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.

TRITRICHOMONAS FETUS - FELINE

Species: Feline

Specimen: A small sample (approximately 50g - a lima bean-sized sample) of litter-free faeces or an In-Pouch™ 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-Pouch™ culture system (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.*

UREAPLASMA DIVERSUM

Species: Bovine

Specimen: Swabs, fetal stomach contents

Container: Sterile container

Collection protocol: Swab affected area, aspirate fetal stomach contents.

Special handling/shipping requirements: Dry swab samples are stable at room temperature. Other samples are to be transported and stored chilled. Do not freeze samples.

General information about the disease*:* *Ureaplasma diversum is a* common commensal in cattle both overseas and in New Zealand. Pathogenicity depends on the strain of organism and host resistance. Clinical disease in ruminants is characterised by granular vulvitis, purulent vaginitis and infertility. Ascending infections may cause abortion with distinctive, but not pathognomonic, placental and fetal lesions

Comparison with other related tests: *Ureaplasma diversum* is difficult to grow in culture so PCR provides a rapid and specific method for identification of this organism.