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Diagnostic innovation from the veterinary pathology group (VPG)

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Path Line: 01392 247 915 **Email:** tdds@exeter.ac.uk
www.tddslab.co.uk **Fax**: 01392 262 354

Introduction

Real time PCR (qPCR) is a new technology which has recently entered into the mainstream of diagnostic testing in Veterinary Medicine. This manual is designed to provide veterinary surgeons in practice with a concise account of the techniques, pitfalls and proper application of qPCR in veterinary diagnosis. At TDDS we believe that qPCR is at its most powerful when interpreted in the context of an expert cytological, haematological or histopathological opinion. Over the last year we have been developing an innovative approach to diagnosis employing qPCR to identify infectious agents within diagnostic samples and slides. This manual is a summary of our findings and recommendations for harnessing the diagnostic power of this technology in clinical veterinary practice.

DNA and RNA extraction from samples

Nucleic acid (both DNA and RNA) is extracted from the submitted samples using commercially available extraction kits which contain buffers and extraction protocols optimised for use with a number of sample types. Using these kits allows the consistent purification of high quality DNA/RNA, whilst minimising the presence of inhibitors which could interfere with the downstream PCR/RT-PCR. During the extraction procedure a synthetic DNA and/or RNA may be added to the sample to help confirm the successful purification of nucleic acid and the absence of inhibitors in the sample. The extraction procedure routinely uses 100-200µl of blood/body fluid (10-20µl of blood in species with nucleated red cells), 20mg of tissue, or approximately 200mg of faeces. Very dilute samples such as large volume BAL samples or urine may be centrifuged to pellet cells/bacteria in the sample which are then used in the extraction with 200µl of the supernatant.

Polymerase chain reaction in real time

PCR utilises a pair of synthetic oligonucleotide sequences (primers) which are designed to anneal (bind) to a target region of DNA from an organism of interest. Primers, purified sample DNA and Taq DNA polymerase are combined in a suitable buffer and placed in a thermocycler. The thermocycling protocol typically comprises a melting step (~95°C) followed by an annealing/extension step (typically 60°C) which is repeated 40-45 times. The primer sequences play an important role in determining the sensitivity and specificity of the test. Primers are designed so that, in the presence of target DNA only, a single amplicon of known size is produced. With conventional (end-point) PCR the amplicon is analysed by gel electrophoresis at the end of the thermocycling protocol with results being qualitative, not quantitative.

Quantitative (Real-time) PCR (qPCR) differs in that it uses a reporter system which allows amplicon accumulation to be monitored during the thermocycling process. The reporter system may be non-specific (e.g. a double-stranded DNA binding dye such as SYBR Green I). Specific reporter systems incorporate a sequence specific DNA probe in the reaction which produces a fluorescence change in the reaction in the presence of the target amplicon only. These qPCR assays are usually designed to amplify a relatively small region of DNA producing an amplicon less than 200 bases. A real-time PCR machine utilises a thermocyler coupled with a fluorescence monitoring system and analysis software.

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Probes with different fluorescent dyes allow detection of more than one target in the same reaction when two or more primer sets are combined in the same tube (multiplex reaction). This is particularly useful when an assay detecting an organism of interest is combined with an internal control to ensure successful nucleic acid extraction, sample addition and absence of inhibition within a single reaction tube.

Viral agents with an RNA genome (e.g. Feline Coronavirus) undergo an initial nucleic acid extraction step, as above. RNA is then reverse transcribed (RT) to complementary DNA (cDNA) using a retroviral reverse transcriptase. This cDNA is then incorporated into a qPCR reaction as outlined above.

Controls

PCR reactions are run with controls for a variety of reasons. The types of controls depend on the nature of the sample and the target nucleic acid.

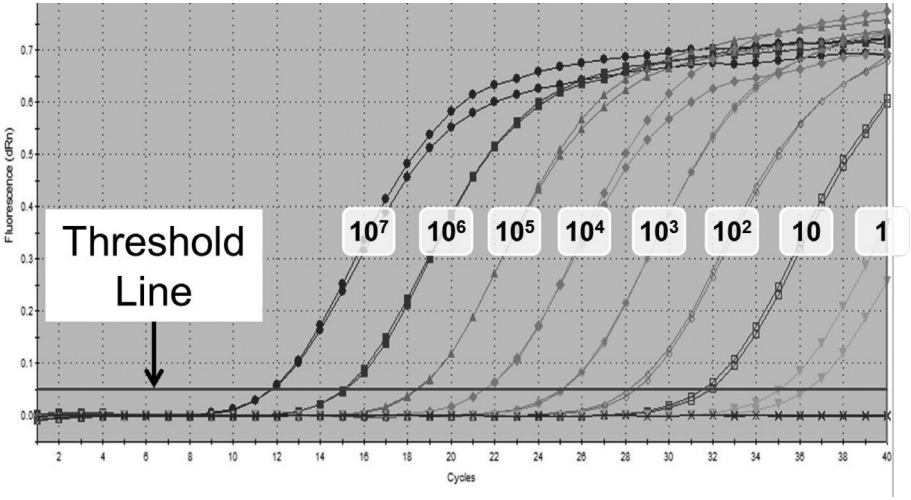
1. Many reactions utilise a primer set for host animal DNA. This not only confirms that the PCR reaction is performing normally but that the sample is of diagnostic quality (i.e. the conjunctival swab contains feline DNA). Reasons for failure of this type of control include inadequate sampling, failure of the PCR reaction, incorrect species (e.g. submission form incorrectly filled-in; samples switched).

2. RNA targets (e.g. Feline Calicivirus) first require reverse transcription so these reactions incorporate a spiked RNA control (which is designed not to cross react with target nucleic acid) to confirm successful RT of the sample.

3. Certain samples, faeces in particular, may contain inhibitors of the PCR reaction but an inconsistent amount of host DNA. Faecal PCRs utilise a synthetic DNA internal control (which does not cross react with target DNA). This control material is added to the faeces sample prior to nucleic acid extraction. Analysis of the amplification of this control material allows an assessment of the presence / degree of inhibition.

Basic interpretation of quantitative results

The analysis software attached to the qPCR machine produces a graph of sample fluorescence vs. the number of thermocycling cycles run with fluorescence proportional to the amount of product present in the reaction. In order to compare the amount of template (target nucleic acid) present in the sample at the beginning of the cycling, the cycle at which the fluorescence in the sample crosses a threshold (threshold line: see graph below) is measured and this cycle number is called the Ct value.



In the graph above, a 10-fold serial dilution of a very concentrated sample was run in parallel PCR reactions. This qPCR assay is approximately 100% efficient with the amount of amplicon in the reaction doubling in each cycle. The sample with 10^7 copies at the start crosses the threshold line at cycle 12 (Ct: 12) whereas the sample with 10^4 crosses at approximately cycle 22 (Ct: 22). Therefore, the lower the Ct value, the more starting template there is in the sample at the start of the qPCR reaction. The starting template is proportional to the viral load, number of bacteria, etc, from which the target nucleic acid was extracted. Another feature of qPCR evident in these results is the reproducibility. Each of the samples is run in duplicate and the results for the samples with greater than 10 copies per PCR are almost identical. A degree of variability is seen in samples with copy numbers less than approximately 10 copies per PCR.

Why choose qPCR?

qPCR differs significantly from serology for detection of infectious disease and is better thought of as similar to culture where the agent itself is detected rather than the immune response to it. Both qPCR and culture provide similar semi-quantitative results but qPCR has some advantages over culture:

- it allows detection of agents for which culture is difficult, slow or requires specialist sampling, submission and processing (e.g. *Chlamydophila*, *Mycobacteria*, *Mycoplasmas*, *Leptospira*, Viruses, Protozoa)

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- it does not require the presence of viable organisms and can therefore be applied where culture cannot (e.g. air-dried smears, EDTA samples, frozen samples)
- it allows detection of a positive control DNA/RNA. Failure to detect target DNA in samples negative for the control is meaningless.
- it may permit detection of virulence genes (e.g. VapA gene in *Rhodococcus equi*).

PCR does not allow extensive antimicrobial sensitivity testing, although genes associated with specific resistance patterns can be detected in some organisms.

The quantitative nature of qPCR may be more useful in certain circumstances than in others. For tests run on blood (e.g. haemotropic mycoplasmas, FIV / FeLV proviral DNA) Ct values provide a good indication of infectious load per ml or µl. For most other samples qPCR results are semi-quantitative due to the variation in sample composition.

Recommended sample types

The type of sample required for qPCR testing varies from test to test and the pathogenesis of the condition under investigation. PCR is a very sensitive technique but correct sample selection is still essential. **The nucleic acid of the target agent must be present in the sample and for this reason blood samples, although convenient, are not the optimum sample type for many agents.** This is akin to culture: a swab from a wound will be a more suitable sample type for microbial culture than a blood sample from the same patient. In the same way, an aspirate from a cat with a granulomatous node will be more suitable for a Feline Coronavirus PCR than a blood sample. Inappropriate sample types will often lead to "false" negatives, false only because the target nucleic acid may not exceed the detectable limit in that sample or may not be present at all.

Samples which can be used include:

- Cytology slides
- Fluids (e.g. peritoneal, pleural, CSF, synovial fluid, washes etc.)
- Blood (EDTA)
- Bone marrow (EDTA)
- Faeces
- Urine
- Plain swabs (+/- VTM). Preferably not charcoal.
- Tissue samples*

* Formalin fixation crosslinks and fragments DNA reducing sensitivity of the test.

Details of samples required can be found in the "test-by-test" section and in the quick reference guide.

Feline Herpesvirus 1 (FHV-1)

Which samples

- Conjunctival / oronasal swabs (plain or VTM, preferably not charcoal)
- Scrapings / cytology slides from conjunctiva or upper respiratory tract
- Tracheal wash / nasal flush fluid

What we detect

The test for feline herpesvirus (FHV-1) amplifies a conserved region of the thymidine kinase gene from FHV-1 but no other types of herpesvirus. It includes a host DNA control.

The meaning of a positive

With a positive PCR, in particular if detected with a high viral load (low Ct) and appropriate clinical signs (see diagnostic planning), FHV-1 is likely the causative agent.

Reactivation of latent virus infection (e.g. after stress or glucocorticoid administration) can also cause viral shedding. In this case a positive result, in particular if detected with a low viral load (high Ct), may be unrelated to the clinical signs.

Epidemiologically, a positive result means that this cat is infected and currently shedding the virus, and may therefore be a source of infection to other cats.

The meaning of "not detected"

Viral DNA is undetectable in the sample and in most cases this means that clinical signs are unrelated to infection with FHV-1. A negative result does not rule out latent infection.

Special details of this test

The infection is typically localised to the respiratory epithelia (nasal, oral or conjunctival) and these tissues are considered ideal for virus detection.

PCR does not distinguish between vaccinal and wild-type virus, therefore vaccination with modified life vaccine, in particular nasal application (not currently available in UK) may lead to a positive PCR shortly after vaccination in the absence of infection.

Diagnostic planning with the test

For diagnostic work-up of feline respiratory disease this test is ideally used in conjunction with molecular testing for other feline respiratory pathogens (see Feline Respiratory Screen). Co-infections are possible.

Atypical presentations of FHV-1 have been described, predominantly in kittens (severe systemic illness, coughing and acute death). FHV-1 may be associated with skin disease (nasal and/or facial ulcerating and crusting lesions), viraemia, or pneumonia.

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Feline Calicivirus

Which samples

- Oronasal swabs (plain or VTM)
- Scrapings /cytology slides from oral lesions
- Tracheal wash / nasal flush fluid
- (Possibly synovial aspirates)

What we detect

The test for feline calicivirus (FCV) comprises two qPCR assays which target separate conserved regions of the FCV genome from a wide range of FCV isolates. As the genome of FCV is an RNA genome, the sample must be reverse transcribed (RT) prior to performing the PCR; therefore, RNA of non-mammalian origin is added to each sample, and quantified, to confirm successful reverse transcription of the sample. This added RNA does not cross-react with the FCV assays. This control is in addition to a standard host DNA control to check sample quality.

The meaning of a positive

With a positive PCR, in particular if detected with a high viral load (low Ct) and appropriate clinical signs (see diagnostic planning), FCV is likely the causative agent. Subclinical infection is also possible.

Epidemiologically, a positive means that this cat is infected and currently shedding the virus and may therefore be a source of infection to other cats.

The meaning of "not detected"

Calicivirus RNA was not detected in this sample. Due to high variability of the viral genome, RT-PCR may not detect all strains of calicivirus RNA, although we reduce the risk of a false negative by using two separate sets of primers amplifying two separate regions of genome. In addition, a negative result does not entirely rule out infection, as the virus could be present at a level below the detectable limit of the test, but most clinical cases are associated with high viral loads.

Diagnostic planning with the test

For diagnostic work-up of feline respiratory disease this test is ideally used in conjunction with molecular testing for other feline respiratory pathogens (see Feline Respiratory Screen).

Calicivirus infection has been implicated in chronic ulceroproliferative stomatitis, although this has not been conclusively proven. Occasional outbreaks of virulent systemic feline calicivirus infection have been reported with variable but severe clinical signs and high mortality.

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Mycoplasma felis

Which samples

- Tracheal wash, BAL, nasal flush fluid
- Swabs from eyes, nasopharynx, wounds, abscesses (preferably not charcoal)
- Scrapings / aspirates / slides / fresh tissue from similar locations

What we detect

The test for *M. felis* comprises two qPCR assays which target separate conserved regions of the *M. felis* genome, whilst not amplifying other *Mycoplasma* and closely related bacterial species. It utilises a host DNA control.

The meaning of a positive

This animal currently carries *M. felis* and, in the presence of appropriate clinical signs, this is likely to be a contributing factor. Detection in conjunctival or lower airway sites is more significant than detection in oropharyngeal sites (possible commensal oral colonisation).

The meaning of "not detected"

M. felis DNA is undetectable in the sample and in most cases this means that clinical signs are not due to infection with *M. felis*. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test.

Special details of this test

M. felis is difficult to culture; therefore this organism is difficult to study. To date, the clinical significance of isolation/detection of many mycoplasma species is not fully elucidated and commensal species are common in many domestic animals. This PCR has been designed to specifically detect *M. felis*.

Diagnostic planning with the test

There is increasing evidence that *M. felis* contributes to, and may be the sole cause of, ocular and respiratory disease in cats. We have had several strong positive results in BAL samples from cats which have failed to adequately respond to glucocorticoid therapy for presumed feline asthma. Also consider the test in other situations where culture is also appropriate (e.g. wounds, abscesses, lymphadenitis).

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Bordetella bronchiseptica (feline)

Which samples

- Nasopharyngeal swabs (plain or VTM, preferably not charcoal)
- Nasopharyngeal scrapings / cytology slides
- Nasal flush, tracheal wash, BAL fluids

What we detect

The test for *B. bronchiseptica* comprises two qPCR assays which target separate conserved regions of the genome, whilst not amplifying closely related bacterial species. It incorporates a host DNA control.

The meaning of a positive

This animal currently carries *B. bronchiseptica* and in context with appropriate clinical signs this may be causing or contributing to these.

Epidemiologically a positive means that this cat is a potential source of infection to other cats and probably dogs. There may be some zoonotic potential to immunosuppressed individuals.

The meaning of "not detected"

B. bronchiseptica DNA is undetectable in the sample and in most cases this means that clinical signs are unrelated to infection with the organism. While this PCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test.

Diagnostic planning with the test

B. bronchiseptica infection is particularly prevalent in high-density cat populations (e.g. shelters, catteries) and transmission from dogs to cats is possible. *B. bronchiseptica* infection should be considered in coughing and sneezing cats (acute and chronic) as a primary or secondary pathogen along with other infectious agents. For diagnostic work-up of feline respiratory disease this test is ideally used in conjunction with molecular testing for other feline respiratory pathogens.

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Chlamydophila felis (C. felis)

Which samples

- Ocular / conjunctival swabs (plain or VTM, preferably not charcoal)
- Ocular / conjunctival scrapings / cytology slides

What we detect

The test for *C. felis* amplifies a conserved region of the ompA gene from a wide range of isolates of *C. felis*, but no other *Chlamydophila* species or closely related bacterial species. It includes a host DNA control.

The meaning of a positive

This animal currently carries *C. felis*, and with appropriate clinical signs, *C. felis* is likely to be a contributing cause.

The meaning of "not detected"

Chlamydophila DNA is undetectable in the sample and in most cases this means that the clinical signs are unrelated to infection with *C. felis*. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test.

Special details of this test

The primary target tissue for *C. felis* is the conjunctiva. PCR is considered the preferred test for infection with *C. felis*. In the healthy cat population a positive PCR result is only occasionally obtained.

Diagnostic planning with the test

C. felis infection is unlikely in cases of respiratory disease without concurrent ocular signs.

Feline Respiratory Screens

The above PCRs are available individually. You can request more than one PCR for the same sample. The following screens may also be requested on BALs, TW fluid and nasal / oropharyngeal swabs (ideally also a conjunctival swab for *C. felis*):

Feline Respiratory Screen

FHV-1, FCV, *C. felis*, *B. bronchiseptica*, *M. felis*

Feline Respiratory Mini Screen

FHV-1, FCV, *C. felis*

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Feline Haemoplasmas (Feline Infectious Anaemia)

Which samples

- EDTA whole blood

What we detect

The test for feline haemoplasmas comprises three qPCR assays which each target one of the three separate species of *Mycoplasmas* associated with this disease. The three assays individually target *Mycoplasma haemofelis* (formerly *Haemobartonella felis*), '*Candidatus* Mycoplasma haemominutum' and '*Candidatus* Mycoplasma turicensis' but do not amplify the non-target species or other *Mycoplasma* and closely related bacterial species. This allows the tests to definitively identify the species of *Mycoplasma* present in the sample as well as the presence of infection with multiple species. The test incorporates a host DNA control.

The meaning of a positive

DNA from the particular organism is present:

***M. haemofelis*:** in conjunction with a regenerative anaemia, morphological evidence of haemolysis or a positive Coombs' test, *M. haemofelis* is likely the aetiological agent.

***C.* M. haemominutum:** may reflect an incidental carrier status without anaemia, or could be the cause of erythrocyte destruction in the presence of haemolytic anaemia.

***C.* M. turicensis:** less information known about this species but may reflect an incidental carrier status, or has rarely been reported to cause anaemia.

Infections with multiple (including all three) species have been reported.

The meaning of "not detected"

Haemoplasma DNA is undetectable in the sample and in most cases this means that haemoplasma infection is not the cause of the clinical signs. Sampling during antibiotic therapy tends to produce a negative result. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test. This would be more likely in animals being screened as blood donors or for clearance of the organism following therapy rather than those acutely ill.

Diagnostic planning with this test

- Anaemic cats, particularly where there is regeneration and no other cause is identified
- Screening of donors prior to blood transfusion (American College of Veterinary Internal Medicine consensus statement, Feline Advisory Bureau guidelines)

Cats with positive results should have their FeLV and FIV status checked, as infection with these agents are predisposing factors for haemoplasma infection.

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Feline Leukaemia Virus (FeLV)

Which samples

- EDTA whole blood
- Bone marrow aspirate slides or in EDTA

What we detect

The test for FeLV comprises two qPCR assays which target separate conserved regions of the FeLV genome, whilst not amplifying sequenced endogenous retroviral sequences present in the feline genome which can result in false positive results. The qPCR test is performed on the DNA extracted from the submitted sample and is thus targeting the integrated proviral DNA, rather than viral RNA, within the cells from the sample. The test incorporates a host DNA control.

The meaning of a positive

A positive PCR result indicates the presence of FeLV proviral DNA in the peripheral blood leukocytes, regardless of whether viraemia is present or not (latent infection). In addition, the sensitivity of the test can allow detection of low levels of FeLV DNA in cats which are negative by other testing methods.

The meaning of "not detected"

FeLV proviral DNA is undetectable in the sample and in cats which are also antigen test negative, FeLV infection is highly unlikely. In cats which are antigen test positive, the failure to detect FeLV proviral DNA may be due to it being present at a level below the detection limit of the assay; the presence of a viral strain not detected by the PCR due to nucleic acid sequence differences with the primer and/or probe sequences; or a false positive result with the antigen test.

Diagnostic planning with this test

The test can be used in the place of virus isolation to confirm a positive ELISA antigen result. Use the test when there is a suspicion of latent infection in cats that have a negative ELISA result, e.g. animals with lymphoma, bone marrow disease (unexplained anaemia, leukopaenia, thrombocytopaenia or myelodysplasia) or chronic gingivitis, discordant results (i.e. positive ELISA but negative immunofluorescence) possibly related to the stage of infection or a false positive ELISA result.

Blood donors with a negative ELISA may be followed up with PCR (American Association of Feline Practitioners guidelines) prior to transfusion as FeLV provirus is infectious.

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Feline Immunodeficiency Virus

Which samples

- EDTA whole blood
- Bone marrow aspirate slides or in EDTA

What we detect

The test for FIV comprises two qPCR assays which target separate conserved regions of the FIV genome, whilst not amplifying feline genomic DNA. The qPCR test is performed on the DNA extracted from the submitted sample and is thus targeting the integrated proviral DNA, rather than viral RNA, within the cells from the sample. One of the qPCR assays is targeted against a wide range of clade A isolates (the most common clade in the UK), whereas the second assay is targeted against isolates from clades A-D in order to increase the range of isolates detected by this assay. The test incorporates a host DNA control.

The meaning of a positive

The cat has FIV proviral DNA in its genome i.e., it is infected. If the cat is FIV vaccinated (vaccination rarely used in the UK currently) a positive result may occur with certain vaccine strains, but generally vaccination should not produce a positive result.

The meaning of "not detected"

FIV proviral DNA is undetectable in the sample and in most cases this implies that the cat is free of FIV infection. The cat could be infected with a viral strain not detected by the assay due to variations in the targeted sequences within the viral genome.

Diagnostic planning with this test

The test should be used to confirm FIV infection in ELISA FIV antibody positive cats. The test should not be used as a screening test; the ELISA is still recommended as the screening test of choice.

Consider the test when there is a negative ELISA result but disease is strongly suspected (patient may be unable to produce sufficient antibodies to produce a serologic titre especially in the terminal stages of the disease).

In kittens less than 20 weeks of age, with positive ELISA result, PCR allows differentiation of a titre caused by maternally derived antibody from genuine infection.

Use in animals known to have been exposed to FIV infection (e.g. bites) but insufficient time has elapsed for seroconversion. Seroconversion normally occurs within eight weeks but can take up to six months in some individuals

Blood donors with a negative ELISA may be followed up with PCR (AAFP guidelines) prior to transfusion.

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Feline Coronavirus

Which samples

- FIP diagnosis
  - Fine needle aspirates (dry FIP)
  - Intracavitary fluids
  - Aqueous humour
  - CSF
- Enteric coronavirus
  - Faeces - 0.5g (ml) of fresh faeces without contamination (e.g. cat litter)
  - Well-coated faecal swabs

Refrigerate faecal samples or swabs following collection and forward for testing ASAP (preferably within 3 days). Refrigerating the sample prior to submission reduces the chance of a false negative result due to degradation of the coronavirus RNA in the sample.

What we detect

The test for feline coronavirus (FCoV) targets a conserved region of the nucleocapsid (N) protein from a wide range of feline coronavirus and feline infectious peritonitis isolates. It does not discriminate between strains capable of leading to FIP from those which are not. The test incorporates a host DNA control. As the genome of FCoV is an RNA genome, the sample must be reverse transcribed (RT) prior to performing the PCR; therefore, RNA of non-mammalian origin is added to each sample, and quantified, to confirm successful reverse transcription of the sample. This added RNA does not cross-react with the FCoV assay.

The meaning of a positive

In the context of an FNA or fluid showing pyogranulomatous inflammation or evidence for vasculitis, a positive is consistent with the diagnosis of FIP. Positive results from tissue and other samples have been reported in cases of enteric coronavirus during periods of viraemia; therefore, results must be interpreted in context with clinical signs and laboratory data supporting a diagnosis of feline infectious peritonitis.

A positive faecal result is consistent with a shedder of enteric coronavirus. See below (Diagnostic Planning).

The meaning of "not detected"

Feline coronavirus RNA is not detected in the sample. In the context of an FNA or fluid showing pyogranulomatous inflammation or evidence for vasculitis, this is inconsistent with the diagnosis of FIP, but does not completely exclude it. A host DNA control is used and confirms adequate host DNA content in the sample but if the sample contains few monocytes or macrophages, false negative results are possible.

Since faecal FCoV shedding may be intermittent infected cats are not generally considered to have cleared infection until there have been five consecutive monthly negative RT-PCR results on faeces.

Continued overleaf.

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Special details of this test

Coronavirus is usually undetectable in whole blood in cases of enteric coronavirus and either undetectable or only detectable at low levels in whole blood in cases of FIP. Consequently, detection at significant levels in fluids and FNAs shows that the coronavirus has invaded the tissues and has not been passively introduced in blood contamination. In the presence of evidence for pyogranulomatous inflammation or vasculitis this provides very strong evidence for FIP. In principle this is similar to the previously accepted gold standard for FIP diagnosis which is the detection of coronavirus by immunohistochemistry in histopathological sections containing the appropriate pattern of inflammation. The advantage of qPCR and cytology is that they are minimally invasive.

Diagnostic planning with this test:

Use the test in conjunction with serology and the cytology of aspirates or fluids to support the diagnosis of FIP.

In colonies of cats the test may be performed on faecal samples to identify shedders of enteric coronavirus. Shedders are considered a risk factor for cases of FIP in the colony. About 10% of infected cats become persistent shedders. Five consecutive negative monthly RT-PCR results on faeces are required to conclude that an infected cat has eliminated infection (demonstrating that the cat has become seronegative may be more efficient). A cat with nine consecutive monthly positive RT-PCR results is extremely unlikely to eliminate infection and is a likely lifelong carrier (approximately 95% of cats will have cleared infection by this time).

The test may also be used on faeces to screen animals prior to using immunosuppressive therapies that could precipitate FIP.

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Bordetella bronchiseptica (Canine)

Which samples

- Tracheal wash / BAL samples, fluid or slides
- Oropharyngeal swabs

What we detect

The test for *B. bronchiseptica* comprises two qPCR assays which target separate conserved regions of the genome, whilst not amplifying closely related bacterial species. It incorporates a host DNA control.

The meaning of a positive

Tracheal wash/BAL sample: B. bronchiseptica likely to be causing/involved in clinical signs

Oropharyngeal swab: B. bronchiseptica may be causing/involved in the clinical signs; however, B. bronchiseptica can be isolated from the oropharynx of asymptomatic animals.

The meaning of "not detected"

B. bronchiseptica DNA is undetectable in the sample and in most cases this means that clinical signs are unrelated to infection with this agent. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test.

Special details of this test

Effect of vaccination on test: Intranasal vaccines use a live attenuated strain of *B. bronchiseptica* which is detected by the *Bordetella* PCR; therefore, recently vaccinated dogs may give a false positive result with this test.

Diagnostic planning with this test

Use the test for dogs with clinical signs of acute upper respiratory tract disease or a tracheal wash/BAL with neutrophilic inflammation not otherwise explained.

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Aspergillus fumigatus

Which samples

- Nasal flush fluid/cytology slides
- Fresh biopsy material of nasal plaques (preferably not in formalin)
- Nasal swabs

What we detect

The test amplifies a conserved region from the *A. fumigatus* genome but not from other *Aspergillus* species or other types of fungi. It incorporates a synthetic DNA control.

The meaning of a positive

- Asymptomatic animal: means organism is present in nasal passages but as organism is ubiquitous this is not necessarily clinically significant.
- Animal with haemorrhagic nasal discharge +/- evidence for bony destruction on imaging: *A. fumigatus* may be cause of clinical signs
- Animal with haemorrhagic nasal discharge, *pyogranulomatous* (+/- eosinophilic) inflammation and fungal hyphae on cytology:– consistent with *A. fumigatus* infection

The meaning of "not detected"

A. fumigatus DNA is undetectable in the sample. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test. This test doesn't rule out infection with other *Aspergillus* and fungal species, although this is uncommon in the UK

- **Nasal swab/wash**: A. fumigatus infection is unlikely
- **Fresh biopsy material of plaque lesions**: makes infection with *A. fumigatus* very unlikely

Special details of this test

The PCR assay is specific for *A. fumigatus* and it will not pick up other *Aspergillus* or fungal species.

Blood as a sample: whilst blood for PCR can be used to diagnose systemic aspergillosis in humans, in small animals in the UK localised nasal aspergillosis is by far the most common form of the disease, without systemic involvement. PCR from EDTA blood for diagnosis of nasal aspergillosis is not useful and is not recommended

Diagnostic planning with this test

- Any haemorrhagic nasal discharge +/- turbinate destruction
- Any nasal wash fluid with pyogranulomatous/eosinophilic inflammation
- Any nasal wash fluid with fungal hyphae observed cytologically
- Any rhinoscopic examination with fungal-like plaque lesions

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Mycoplasma cynos & canis

Which samples

- **Respiratory disease**
  - Oropharyngeal swab
  - Tracheal wash/BAL
- **Urinary disease**
  - Cystocentesis urine sample
- **Reproductive disease**
  - Deep, guarded vaginal swabs

What we detect

The test for *M. canis and M. cynos* comprises two qPCR assays which amplify separate conserved regions of the relevant genomes, whilst not amplifying closely related bacterial species. The separate qPCR assays are specific to either *M. canis* or *M.cynos* allowing identification of which of the two species are present. The assays incorporate a host DNA control (for respiratory and reproductive samples) and a synthetic DNA control for urine.

The meaning of a positive

- **Oropharyngeal swab**
  - **Asymptomatic dog**: many dogs carry *M. canis/cynos* as normal flora in oropharynx/ upper respiratory tract
  - **Respiratory disease**: *M. canis/cynos* unlikely to be sole cause of clinical signs
- **Tracheal wash/BAL**
  - ***M. cynos*** – may be involved/causing clinical signs but can also be isolated from samples from healthy dogs
  - ***M. canis*** – has not yet been proven to be primary cause of respiratory disease in dogs so significance remains uncertain. May be involved in clinical signs but searching for other associated pathogens (e.g. *Bordetella*) is prudent.
- **Vaginal swab**
  - **Infertility** – mycoplasmas have not yet been proven to be a cause of canine infertility so isolation from the vagina/prepuce remains of uncertain significance.
- **Urinary tract**
  - **Free catch/catheterised urine sample** – probably contaminants from distal urinary/ reproductive tract microflora.
  - **Cystocentesis sample** – presence of mycoplasma may be causing/contributing to clinical signs. Interpret in light of other microbiological findings.

Continued overleaf.

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The meaning of "not detected"

M. canis/cynos DNA is undetectable in the sample and *M. canis/cynos* are unlikely to be involved in the clinical signs. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test.

Diagnostic planning with this test

- Any dogs with unexplained respiratory signs, especially younger dogs with severe/more persistent signs of kennel cough than usual.
- Any dogs with neutrophilic inflammation on tracheal wash/BAL – even if other bacteria are seen/cultured as *Mycoplasma* organisms could be contributing to clinical signs and so testing is worthwhile to help guide antimicrobial choice.
- Dogs with unexplained urinary tract signs, presence of neutrophilic inflammation on cytology but negative standard culture results.

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Angiostrongylus vasorum

Which samples

- Tracheal wash/BAL fluid
- Cytology preps (BAL, TW, lung aspirates)
- Faeces - 0.5g (ml) of fresh faeces without contamination
- EDTA whole blood – see special details section below

Refrigerate faecal samples following collection and forward for testing ASAP (preferably within 3 days). Refrigerating the sample prior to submission reduces the chance of a false negative result due to degradation of the target DNA in the sample. Pooling of 2-3 consecutive faecal samples may be useful to increase the chance of detecting the organism.

What we detect

The test for *A. vasorum* comprises two qPCR assays which target separate conserved regions of the genome, whilst not amplifying closely related parasite species. The assay incorporates either a host DNA or synthetic DNA control, depending on the sample type.

The meaning of a positive

- Faeces: consistent with clinical/subclinical infection. Treatment advisable.
- Tracheal wash/BAL fluid/Cytology preps: likely to be involved/causing respiratory (and other) signs.
- EDTA blood:
  - Asymptomatic dog: likely subclinical infection
  - Respiratory signs: may or may not be contributing/cause of dog's respiratory signs. Interpret in the light of other clinical and diagnostic information.

The meaning of "not detected"

A. vasorum DNA is undetectable in the sample. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test:

- Faeces:
  - Asymptomatic dog: no current evidence for A. vasorum infection. However, note that larvae may be intermittently shed and the infection has a relatively long pre-patent period (38-57 days).
  - Symptomatic dog: A. vasorum less likely to be causing clinical signs but intermittent larval shedding may result in a false negative result.
- Tracheal wash/BAL fluid/Cytology Preps:
  - *A. vasorum* unlikely to be cause of respiratory (and other) signs.
  - In the case of samples where lungworm larvae have been visualised (cytology preparation) then infection with a lungworm species other than *A. vasorum* (e.g. *Crenosoma*, *Capillaria or Filaroides*) should be considered.

Continued overleaf.

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- EDTA blood
  - *A. vasorum* is less likely to be the cause of respiratory/coagulopathy signs.
  - Infection cannot be completely excluded as false negative results can occur. *A. vasorum* DNA tends to be present at relatively low levels in the peripheral blood; therefore, it may be present at a level below the detection limit of the assay.

Special details of this test

This test is specific for *A. vasorum*. It will not cross react with other less common canine lungworms (*Crenosoma*, *Capillaria* and *Filaroides*) or feline lungworms (*Aelurostrongylus*).

We have had a limited number of cases where the test has detected A. vasorum DNA in BAL, TW fluid and / or faeces and EDTA blood has also been submitted. In one case A. vasorum DNA was not detected in EDTA blood. In the remaining cases it was detected but Ct times were much higher than with other sample types. For this reason EDTA is not the preferred sample type when investigating coughing dogs. Remember also that the test is specific for A. vasorum and will not detect other lungworm species.

A. vasorum has the potential to induce bleeding disorders and in these circumstances it may be appropriate to run the test on EDTA blood, alongside a faecal parasite examination and PCR.

Diagnostic planning with this test

- Any tracheal wash / BAL with unexplained (particularly but not exclusively eosinophilic) inflammation or haemorrhage in dogs.
- Tracheal wash / BAL or faecal samples with larvae to confirm species as A. vasorum.
- Screening for subclinical infection – faeces
- EDTA blood - dogs with unexplained coagulopathy (especially increased buccal mucosal bleeding times) with or without increased PT/APTT.

Respiratory Screens

The above PCRs are available individually. You can request more than one PCR for the same sample. The following screens may also be requested on BALs, TW fluid and oropharyngeal swabs:

Canine Respiratory Screen

A. vasorum, B. bronchiseptica, Mycoplasma cynos and canis

Canine Respiratory Mini Screen

B. bronchiseptica, Mycoplasma cynos and canis

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Canine Distemper Virus (CDV)

Which samples

- **Respiratory disease**
  - Oropharyngeal swab
  - Conjunctival scraping
  - Tracheal wash/BAL
- **Neurological disease**
  - CSF
  - Gastrointestinal disease
  - Faeces

What we detect

The test for CDV targets a conserved region of the nucleocapsid (N) protein from a wide range of CDV isolates. The assay incorporates both an RNA control and a host DNA control.

The meaning of a positive

CDV RNA has been detected in the sample and with respiratory disease, CDV may be involved in / causing the clinical signs. A positive result in CSF is consistent with CDV as cause of clinical signs.

The meaning of "not detected"

CDV RNA is undetectable in the sample and is unlikely to be the cause of the clinical signs. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the virus could be present at a level below the detectable limit of the test.

Special details of this test

Recent vaccination with a modified live vaccine may cause false positive results. This should not affect CSF.

Diagnostic planning with this test

Any unvaccinated dog with respiratory $^{+/-}$ GIT signs or neurological signs. Clinical distemper cases in the UK are extremely rare.

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Canine Herpes Virus (CHV)

Which samples

- **Respiratory disease:** Oropharyngeal swabs, tracheal washes, BAL samples
- **Reproductive disease:** Vaginal/penile swabs, fresh PM tissues from puppies (kidney, liver, lungs – not in formalin)
- **Ocular disease**: conjunctival swabs, corneal scrapes, or cytology slides

What we detect

The test for CHV targets a conserved region of the CHV genome from a wide range of CHV isolates. The test incorporates a synthetic DNA control.

The meaning of a positive

- **Reproductive disease**
  - **Infertility:** role as a primary cause of infertility remains controversial. However it can cause resorption of foetuses which may appear to breeders as infertility.
  - **Abortion:** presence of CHV in placental tissues consistent with CHV infection as cause of abortion
  - **"Fading puppies":** presence of CHV within liver, lung or kidney of affected puppies consistent with CHV as cause of death
  - **Asymptomatic bitch prior to breeding:** the presence of CHV in a vaginal swab suggests potential to infect litter when born
  - **Asymptomatic stud dog:** the presence of CHV in a penile swab suggests potential to pass to bitch during breeding

- **Respiratory disease**

Significance as a primary pathogen in canine respiratory disease remains uncertain and assessing for concurrent respiratory pathogens is recommended

- **Ocular disease**

CHV is increasingly being recognised as a primary pathogen causing keratitis in dogs. Presence in dogs showing signs of conjunctivitis/keratitis is likely to be significant.

The meaning of "not detected"

CHV DNA is undetectable in the sample and is unlikely to be involved in clinical signs. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test.

Diagnostic planning with this test

- **Respiratory disease:** can be included alongside testing for other primary pathogens such as *Bordetella*
- "Fading puppies" < 3wks of age
- Pre-breeding for stud dogs and first time breeding bitches
- Dogs with conjunctivitis/keratitis

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Canine Haemoplasmas

(*Mycoplasma haemocanis* & '*Candidatus* Mycoplasma haematoparvum')

Which samples

- EDTA blood

What we detect

The test for canine haemoplasmas comprises two qPCR assays which each target one of the two separate species of *Mycoplasmas* associated with this disease. The two assays individually target *M. haemocanis* and 'C.M. haematoparvum' but do not amplify the non-target species or other bacterial species. The tests can definitively identify the species of *Mycoplasma* present as well as dual infections. The test incorporates a host DNA control.

The meaning of a positive

A positive result confirms the presence of the organism in the blood; however, it may not be responsible for the clinical signs, as subclinical infection is common.

The meaning of "not detected"

Haemoplasma DNA is undetectable in the sample and in the context of a dog with a regenerative anaemia; a negative result is not consistent with haemoplasma infection. Infected animals may be negative during antibiotic therapy. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test. This is most likely to occur in animals being screened as blood donors or following therapy to confirm elimination of the organism.

Special details of this test

Haemotropic *Mycoplasmas* (haemoplasmas) are usually detectable in blood in clinical cases of infectious anaemia as the organism attaches to, and grows on, the surface of erythrocytes. Whilst low numbers may be present initially, by the time they have caused a clinically significant drop in haematocrit there are usually sufficient organisms to give a strong positive (i.e. low Ct) on PCR. However, haemoplasmas are commonly present as a subclinical infection and either splenectomy or immunosuppression is generally required before haemolytic anaemia develops in the dog. Canine haemoplasma infection is uncommon in the UK but occasional cases have been reported.

Diagnostic planning with this test

- Use this test in cases of regenerative anaemia in dogs that have recently been splenectomised or are immunosuppressed.
- Use in cases of regenerative anaemia in dogs that have a history of travel to areas where the proposed vector (*Rhipicephalus sanguineus*) is endemic.
- Use for screening blood donors.

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CEM TRIPLEX qPCR

(Taylorella equigenitalis, Klebsiella pneumoniae, & Pseudomonas aeruginosa)

Which samples

Plain or charcoal swabs from:

- **Mares**
  - Clitoral (fossa and sinus)
  - Endometrial
- **Stallions swabs**
  - Urethra
  - Urethral fossa
  - Penile sheath
  - Pre-ejaculatory fluid (where possible)

What we detect

The triplex qPCR test is able to detect *T. equigenitalis*, *K. pneumoniae*, and *P. aeruginosa*. The assay incorporates a synthetic DNA control. We participate in the twice-yearly VETQAS Horserace Betting Levy Board contagious equine metritis QA Scheme.

The meaning of a positive

A positive result means infection with one or more of the following three bacteria- *T. equigenitalis*, *K. pneumoniae*, *P. aeruginosa*. The triplex qPCR test is used for screening purposes, and any positive result for any of the organisms listed above must be followed up with culture. Any positive qPCR for *T. equigenitalis* must be reported to DEFRA prior to follow up culture. A positive result means suspension of all breeding activities, until the horse is treated and re-tested as clear of infection.

The meaning of "not detected"

The horse is free from infection and breeding activities can proceed.

Special details of this test

With routine culture swabs must reach the laboratory within 48 hours of sampling as the organism is extremely short lived. A major advantage of the qPCR test is that this time restriction no longer applies since qPCR can detect the organism whether it is dead or live. The qPCR test also has a much faster turnaround for samples with results often available within 24 hours, compared to culture which takes 7 days. The qPCR test can be run on either plain or charcoal swabs.

Diagnostic planning with the test

This test protocol forms part of a pre-breeding screening profile for both mares and stallions.

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Rhodococcus equi incl. VapA

Which Samples

- Tracheal aspirates
- Peritoneal fluid in cases of suspected extra-pulmonary R equi infection
- Purulent material from abscess

See special details

What we detect

Samples are tested with two qPCR assays. The first assay specifically amplifies a conserved region of chromosomal monocopy gene (choE), which is conserved amongst *R. equi* isolates. The second assay amplifies a conserved region of the VapA virulence plasmid but no other Vap genes. The assay incorporates a synthetic DNA control.

The meaning of a positive

A positive result in a foal with one or more of the following confirms infection with a pathogenic strain of *R equi*.

1. Clinical signs of lower respiratory tract disease
2. Cytological evidence of septic airway inflammation
3. Radiographical or ultrasonographical evidence of bronchopneumonia

The meaning of "not detected"

R. equi DNA is undetectable in the sample and a negative result is inconsistent with a diagnosis of *R equi* infection. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test.

Special Details of this test

PCR is particularly useful in the diagnosis of *R. equi* infection in foals due to the facts that, unlike culture, it can distinguish between virulent (VapA positive) and avirulent (VapA negative strains) and is less affected by prior antimicrobial treatment. It is important to remember that tracheal aspirates in foals suffering from pneumonia should also be cultured, as *R. equi* is not always the only bacteria involved in these cases.

Although PCR testing can technically be performed on nasal and nasopharyngeal swabs it is not recommended, as outlined in the *ACVIM Consensus statement* on *R. equi* infection in foals. Firstly, false positives may be obtained in asymptomatic cases due to the organism being ubiquitous in some environments and inhalation of dust resulting in contamination of the upper airways and positive results. Secondly, PCR examination of nasal swabs has a sensitivity of around 15%, which is too low to be clinically useful. PCR examination of tracheal aspirates has a sensitivity of 100% and specificity of >90%.

Diagnostic planning with this test

Use the test in conjunction with cytology of aspirates or fluids to confirm the diagnosis of *R. equi* infection in foals.

Infection with *R. equi* is not restricted to equines. It is an infrequent opportunist pathogen in other domestic species in wounds and abscesses, for example.

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Streptococcus equi equi (Strangles) and Streptococcus equi zooepidemicus

Which Samples

- Nasopharyngeal swab
- Guttural pouch washing
- Purulent material from abscessed / ulcerated lymph nodes

What We Detect

The test comprises two assays which used to detect and discriminate between *Streptococcus equi equi* and *Streptococcus equi zooepidemicus* whilst not detecting closely related bacterial species. The assay incorporates a synthetic DNA control.

The Meaning of a Positive

A positive *S. equi equi* result on a nasopharyngeal swab or guttural pouch wash in an asymptomatic animal is diagnostic of a Strangles carrier.

A positive result from purulent material in a clinical case confirms presence of the *Streptococcus equi equi* organism, however please see note below regarding diagnosis of clinical cases.

The Meaning of "not detected"

Streptococcus equi equi DNA is undetectable in the sample. A negative result in conjunction with negative bacterial culture indicates freedom from infection and carrier state in over 90% cases.

Diagnostic Planning With This Test

For use in clinical diagnosis.

Bacterial culture remains the test of choice for the diagnosis of an active Strangles infection as outlined in the *ACVIM Consensus Statement* on *Streptococcus equi* infection in horses. Although PCR is more sensitive than culture and provides results very rapidly, any positive PCR test should ideally be followed up by confirmatory culture. This is because firstly, PCR cannot distinguish between dead and live bacteria, which can be important in determining whether or not the case is infectious; secondly, there may be multiple pathogens involved which could be missed if tested by PCR alone; thirdly, PCR testing does not provide the opportunity for sensitivity testing.

Continued overleaf.

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For use in Strangles Control Programs

PCR, in combination with culture, are the tests of choice to determine the status of exposed and recovered animals. Specific uses are outlined below-

1. The detection of asymptomatic carriers.
2. In combination with Strangles serology for determining infection status prior to moving etc.
3. Determine the success of elimination of S. *equi equi* from the guttural pouch.

Important points for detection of carrier status

A one-off nasopharyngeal (NP) swab is insufficient for diagnosis of carrier status due to the intermittent nature of shedding into the nasopharynx. Due to this three nasopharyngeal swabs taken at weekly intervals are required. Use of concurrent culture and PCR will detect carrier status in over 90% cases. Alternatively, a single bilateral guttural pouch washing is sufficient for diagnosis of carrier status.

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Equine Herpes Virus (EHV) 1 and 4

Which samples

- Nasal or nasopharyngeal swabs
- EDTA whole blood
- Fresh/frozen tissue samples e.g., aborted foetal tissue, placental tissue

What we detect

Each sample is tested with two qPCR assays, one targets the immediate-early (IE) gene from EHV-1 and the other targets a unique region of the EHV-4 genome. The assays are designed against a conserved region from a number of isolates whilst being specific for the target viral type. The assay incorporates a synthetic DNA control.

The meaning of a positive

- **Foal/young horse with clinical signs of respiratory disease**: confirms the role of EHV 1/4 in the pathogenesis of the disease.
- **Aborted foetal/placental tissues:** confirms the cause of the abortion as EHV-1 (very rarely EHV-4)
- **Cases of neurological disease (EHV-1)**
  - Positive EHV-1 test on blood sample indicates viraemia resulting from active infection
  - Positive EHV-1 test on a nasal swab should be interpreted as indicative of the shedding of the infectious virus

The meaning of "not detected"

EHV 1/4 DNA is undetectable in the sample.

- **Foal/young horse with respiratory signs:** inconsistent with involvement of EHV 1/4 in the disease process
- **Abortion foetal/placental tissues:** inconsistent with EHV-1 induced abortion
- **Cases of neurological disease**
  - Negative EHV-1 test result on a blood sample indicates the absence of detectable viraemia
  - Negative EHV-1 test result on a nasal swab indicates the absence of detectable virus shedding

While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test.

Diagnostic planning with the test

Used as part of an investigation of respiratory disease in foals and young horses (older horses are more likely to transmit the virus without showing overt clinical signs). All equine abortions, stillbirths or newborn foal deaths should be tested for EHV (primarily EHV-1 and rarely EHV-4). The test forms part of the diagnostic workup of equine neurological disease.

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Tritrichomonas foetus (feline)

Which samples

- Faeces - 0.5g (ml) of fresh faeces without contamination (e.g. cat litter)
- Well-coated faecal swabs

Refrigerate samples following collection and forward for testing ASAP (preferably within 3 days). Refrigerating the sample prior to submission reduces the chance of a false negative result due to degradation of the target DNA in the sample. Pooling of 2-3 consecutive faecal samples may be useful to increase the chance of detecting the organism.

What we detect

The test for *T. foetus* amplifies a conserved region of internal transcribed spacer (ITS) from a wide range of isolates, but no other closely related protozoal species. The assay incorporates a synthetic DNA control.

The meaning of a positive

A positive result is consistent with *T. foetus* infection

The meaning of "not detected"

T. foetus DNA is undetectable in the sample. A negative result in a cat with diarrhoea does not exclude the possibility of *T. foetus* infection as the protozoa can be intermittently shed in faeces. If clinical signs are strongly suggestive consider retesting on a separate occasion.

Special details of this test

T. foetus may be intermittently shed in faeces so pooling 2-3 consecutive faecal samples may increase the chances of detection. As PCR can detect both live and dead organisms it avoids the problems associated with trying to ensure the organism remains viable when the sample reaches the laboratory. As the diarrhoea associated with *T. foetus* infection can wax and wane, submitting a diarrhoeic sample (rather than a non-diarrhoeic sample) is more likely to yield a positive result. Try to avoid sampling if the cat is currently receiving antibiotics as this may reduce the success of finding the organism.

Diagnostic planning with this test

Use this test in conjunction with routine faecal analysis and culture to investigate cases of diarrhoea, particularly in young cats and colony cats. Co-infections with *Giardia*, *Cryptosporidia* and internal parasites are possible.

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Canine Parvovirus and Feline Panleukopaenia Virus

Which samples

- Faeces - 0.5g (ml) of fresh faeces without contamination (e.g. cat litter)
- Well-coated faecal swabs

Refrigerate samples following collection and forward for testing ASAP (preferably within 3 days). Refrigerating the sample prior to submission reduces the chance of a false negative result due to degradation of the target DNA in the sample.

What we detect

The test for canine parvovirus (CPV) and feline panleukopaenia virus amplifies a conserved region of viral capsid protein gene from a wide range of isolates. The assay incorporates a synthetic DNA control.

The meaning of a positive

A positive result is consistent with the presence of parvovirus in the faeces and, with appropriate clinical signs, is consistent with the diagnosis of parvovirus infection. However, vaccinal virus is also detectable in faeces for approximately 2 weeks post-vaccination. A higher viral load (low Ct) is expected with natural infection (cf. vaccinal virus) but results should be interpreted in light of the vaccinal history.

The meaning of "not detected"

Parvovirus DNA is undetectable in the sample and an animal with appropriate clinical signs a negative result is not consistent with parvovirus infection. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test. This situation is more likely in animals which are being screened for virus following recovery from infection. Our experience of the test is that acutely affected animals often have very high viral loads (low Ct values).

Special details of this test

PCR is more sensitive than other methods of virus detection and is able to detect the virus over a longer period of time post-infection. Maternally derived antibody will not interfere as PCR detects viral DNA (from both viable and inactivated virus) not antibody.

Diagnostic planning with this test

Use this test to investigate cases of acute gastroenteritis in dogs and cats, particularly in unvaccinated animals or those of uncertain vaccine history. In addition, the test can be used to investigate cases of leukopenia (bone marrow suppression) or myocarditis in unvaccinated dogs and cats.

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Giardia

(*Giardia intestinalis*, also known as: *G. lamblia*, *G. duodenalis*)

Which samples

- Faeces - 0.5g (ml) of fresh faeces without contamination (e.g. cat litter)
- Well-coated faecal swabs

Refrigerate samples following collection and forward for testing ASAP (preferably within 3 days). Refrigerating the sample prior to submission reduces the chance of a false negative result due to degradation of the target DNA in the sample. Pooling of 2-3 consecutive faecal samples may be useful to increase the chance of detecting the organism.

What we detect

The test amplifies the *gdh* gene from a wide-range of *Giardia intestinalis (G. lamblia, G. duodenalis)* isolates but not DNA from other infectious agents, including other *Giardia* species. The assay incorporates a synthetic DNA control.

The meaning of a positive

A positive result is consistent with the presence of *Giardia* in the faeces and in the presence of appropriate history and clinical signs, this implies *Giardia* infection. Subclinical giardiasis may occur in clinically healthy animals.

The meaning of "not detected"

Giardia DNA is undetectable in the sample but a negative result does not exclude the possibility of *Giardia* infection as cysts are shed intermittently in the faeces.

Special details of this test

Due to the intermittent shedding of *Giardia* in the faeces, pooling at least 3 consecutive samples is likely to increase the chance of detection. As PCR can detect both live and dead organisms it avoids the problems associated with trying to ensure the organism remains viable when the sample reaches the lab.

Diagnostic planning with this test

Use this test in conjunction with routine faecal analysis and culture to investigate cases of diarrhoea particularly in young or immunosuppressed animals. Co-infections with *Cryptosporidia* and internal parasites are possible. This test is not intended as a routine screening test on faeces from clinically healthy dogs and cats.

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Cryptosporidium

Which samples

- Faeces - 0.5g (ml) of fresh faeces without contamination (e.g. cat litter)
- Well-coated faecal swabs

Refrigerate samples following collection and forward for testing ASAP (preferably within 3 days). Refrigerating the sample prior to submission reduces the chance of a false negative result due to degradation of the target DNA in the sample. Pooling of 2-3 consecutive faecal samples may be useful to increase the chance of detecting the organism.

What we detect

The test amplifies the oocyst wall protein gene from a wide-range of *Cryptosporidium* isolates including *C. parvum*, *C. canis*, *C. felis*, and *C. muris* but not DNA from other infectious agents. The assay incorporates a synthetic DNA control.

The meaning of a positive

A positive result is consistent with the presence of *Cryptosporidia* in the faeces and in the presence of appropriate history and clinical signs this implies *Cryptosporidia* infection. Subclinical cryptosporidiosis may occur in clinically healthy animals.

The meaning of "not detected"

Cryptosporidium DNA is undetectable in the sample. *Cryptosporidium* oocyst shedding can be sporadic so a negative result does not completely exclude infection; however, a negative result in an animal with clinical signs of diarrhoea (particularly if a pooled sample is tested) is generally not consistent with a diagnosis of cryptosporidiosis.

Special details of this test

Cats and dogs shed much lower numbers of oocysts compared to cattle and humans. PCR is considerably more sensitive than faecal microscopic detection (even after concentration techniques) as it is more effective at detecting very low numbers of oocysts.

Most ELISA tests detect *C. parvum* (common in humans), consequently they are less sensitive for detection of *C. felis*, *C. muris* and *C. canis*, the more common species affecting cats and dogs.

Diagnostic planning with this test

Use as a second line test following routine faecal analysis and culture. A positive result is more likely to be significant if the animal is immunosuppressed, has pre-existing intestinal disease or has co-infection with e.g. *Giardia*, *Tritrichomonas*, *Toxocara*, *Campylobacter*, parvovirus, etc. The test is not intended as a screening test on faeces from clinically healthy dog and cats.

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Anaplasma phagocytophilum

Which samples

- EDTA whole blood
- Tissue aspirates/smears
- Fresh/frozen tissue
- Synovial fluid
- CSF

What we detect

The test for *A. phagocytophilum* targets a conserved region of the *A. phagocytophilum* genome from a wide range of isolates, but does not amplify closely related *Anaplasma* or other bacterial species. The assay incorporates a synthetic DNA control.

The meaning of a positive

A. phagocytophilum DNA is present in that sample. *A. phagocytophilum* is the probable cause of appropriate clinical history, signs, haematological and cytological findings.

The meaning of "not detected"

A. phagocytophilum DNA is undetectable in the sample and, depending on the sample; this does not entirely exclude *A. phagocytophilum* infection (e.g. PCR on blood may be negative in the face of positive results on splenic tissue, lymph nodes or joints). While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present in that sample at a level below the detectable limit of the test.

Diagnostic planning with this test

Use the test as part of investigation for unexplained fever especially where there is neutropaenia, thrombocytopaenia, reactive / inflammatory lymph nodes or inflammatory joint / CNS disease, coupled with a history suggesting exposure to ticks. A history of overseas travel is not important. The organism is present in the UK.

Path Line: 01392 247 915 **Email:** tdds@exeter.ac.uk
www.tddslab.co.uk **Fax**: 01392 262 354

Bartonella henselae and *Bartonella* spp.

Which samples

- EDTA whole blood
- Fresh/frozen tissue, tissue aspirates or smears.

What we detect

This test comprises two qPCR assays. The first assay targets a conserved region of the *B. henselae* genome from a wide variety of isolates, but not other *Bartonella* species or other bacterial species. The second assay (*Bartonella* spp. assay) is targeted to a wider range of medically important *Bartonella* species including *B. alsatica*, *B. bovis*, *B. clarridgeiae*, *B. doshiae*, *B. elizabethae*, *B. grahamii*, *B. henselae*, *B. koehlerae*, *B. quintana*, *B. taylorii*, *B. vinsonii* subsp. *vinsonii*, *B. vinsonii* subsp. *berkhoffii*, *B. vinsonii* subsp. *arupensis, and B washoensis.* The assays incorporate host DNA controls.

The meaning of a positive

Bartonella spp. DNA is present in the sample. *Bartonella spp.* may be contributing to appropriate clinical history, signs, haematological and cytological findings.

B. henselae DNA is present in the sample. *B. henselae* may be contributing to appropriate clinical history, signs, haematological and cytological findings.

The meaning of "not detected"

Bartonella spp. DNA is undetectable in the sample and in most cases this means that clinical signs are unrelated to infection with *Bartonella spp.*

B. henselae DNA is undetectable in the sample and in most cases this means that clinical signs are unrelated to infection with *B. henselae.*

While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present in a particular sample at a level below the detectable limit of the test.

Special details of this test

Significance in cat is uncertain – there may be asymptomatic bacteraemia

Diagnostic planning with this test

Use the test in dogs as part of investigation for unexplained fever / inflammatory disease and reactive / inflammatory lymph nodes. Also consider testing in cases with hepatitis, retinitis and endocarditis. Species reportedly associated with disease in dogs include *B. vinsonii subsp berkhoffii*, *B. henselae*, *B. claridgeiae*, *B. elizabethae* and *B. washoensis*, each of which will be detected (but not distinguished) by the *Bartonella spp.* assay.

Path Line: 01392 247 915 **Email:** tdds@exeter.ac.uk
www.tddslab.co.uk **Fax**: 01392 262 354

Lyme Disease (*Borellia* spp.)

Which samples

- Dog, possibly horse
  - Synovial fluid or synovium (preferred)
  - Fresh/frozen tissue, tissue aspirates or smears
  - CSF
  - EDTA whole blood (not ideal – see special details)

What we detect

The test for Lyme disease amplifies a conserved region of the genomes from *B. burgdorferi*, *B. garinii* and *B. afzelii*, but not other *Borellia* species or other bacterial species. The assay incorporates a synthetic DNA control.

The meaning of a positive

Borellia spp. DNA detected in the sample. *Borellia* spp. are the probable cause of appropriate clinical history, signs, haematological and cytological findings.

The meaning of "not detected"

Borellia spp. DNA undetectable in sample and in most cases this means that clinical signs are unrelated to infection with *Borellia* spp. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present in that particular sample at a level below the detectable limit of the test.

Special details of this test

"False negatives" are common when testing blood as the organism concentrates in connective tissue.

Diagnostic planning with this test

In the UK Lyme disease is not common in domestic species but is reported in dogs. Horses may also be infected but may not always show signs of disease. Use the test as part of investigation for fever, especially where there are neuromuscular signs, effused and inflamed joints or reactive / inflammatory lymph nodes, coupled with a history suggesting exposure to ticks.

Path Line: 01392 247 915 **Email:** tdds@exeter.ac.uk
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Ehrlichia spp.

Which samples

- Dog, possibly cat
  - EDTA whole blood
  - Fresh/frozen tissue, tissue aspirates or smears (incl. lymph nodes, spleen and bone marrow)
  - Synovial fluid
  - CSF.

What we detect

The test for *Ehrlichia* spp. amplifies a conserved region of the 16S ribosomal RNA gene from *E. canis*, *E. chaffeensis*, *E. ewingii*, *E. muris* and *E. ruminantium* but not from other bacterial species. The assay incorporates a synthetic DNA control.

The meaning of a positive

Ehrlichia spp. DNA detected in that sample. *Ehrlichia* spp. are the probable cause of appropriate clinical history, signs, haematological, biochemical and/or cytological findings.

The meaning of "not detected"

Ehrlichia spp. DNA undetectable in sample and in most cases this means that clinical signs are unrelated to infection with *Ehrlichia* spp. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test.

Special details of this test

This assay does not detect *Neorickettsia risticii* (formerly *Ehrlichia risticii*) which is the cause of equine monocytic ehrlichiosis and has been reported to cause clinical signs in experimentally infected cats.

Diagnostic planning with this test

Use the test in cases with a history of overseas travel. Signs are varied and include chronic fever, wasting, petechiae. Neuromuscular disease is not uncommon (including seizures) and inflammatory joint disease is often reported. There may be lymphadenomegaly and organomegaly. Laboratory findings include thrombocytopaenia, leukopaenia, hyperglobulinaemia and hypoalbuminaemia.

Because of common risk factors, dogs infected with *Ehrlichia* may be co-infected with *Leishmania* and *Babesia*. Therefore, where there is a history of travel to endemic areas combined testing for each of these agents is recommended.

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Leishmania spp.

Which samples

Dog, (rare in the cat)

- Fresh/frozen tissue, tissue aspirates or smears
  - Skin
  - Conjunctival swabs or scrapes
  - Lymph nodes
  - Spleen
  - Bone marrow
- EDTA whole blood

What we detect

The test for *Leishmania* spp. amplifies a conserved region of the kinetoplast DNA from the *Leishmania* species involved in both cutaneous and visceral leishmaniasis including the *L. donovani* complex (*L. donovani*, *L. infantum*, *L. chagasi*) as well as *L. major*, *L. tropica*, *L. aethiopica*, *L. mexicana* and *L. braziliensis*. The assay incorporates a synthetic DNA control.

The meaning of a positive

Leishmania spp. DNA detected in the sample. *Leishmania* spp. are the probable cause of appropriate clinical history, signs, haematological and cytological findings.

The meaning of "not detected"

Leishmania spp. DNA undetectable in sample and in most cases this means that clinical signs are unrelated to infection with *Leishmania spp*. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present in that sample at a level below the detectable limit of the test.

Special details of this test

The effect of the recently licensed CaniLeish vaccine from Virbac, which is composed of purified excreted-secreted proteins of Leishmania infantum combined with an adjuvant, upon the results of qPCR testing is not yet reported. It is important to include relevant vaccination history with all submissions.

Diagnostic planning with this test

Use the test in cases with history of overseas travel (however long ago). Signs are variable and include: chronic fever; chronic wasting; chronic diarrhoea; thickened, crusting or ulcerated cutaneous nodules / chronic skin disease; conjunctivitis / keratoconjunctivitis; uveitis. Affected individuals are commonly hyperglobulinaemic. The PCR detects the species known to cause disease in dogs and cats. Aim to sample affected tissues to minimise risk of false negatives since a negative result in EDTA blood does not entirely exclude *Leishmania* infection.

Because of common risk factors dogs infected with Leishmania may be co-infected with Ehrlichia and Babesia and where there is a history of travel to endemic areas combined testing for each of these agents is recommended.

Path Line: 01392 247 915 **Email:** tdds@exeter.ac.uk
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Babesiosis (Canine)

Which samples

- EDTA whole blood
- Spleen, lymph node, bone marrow aspirate smears

What we detect

The test for canine babesiosis amplifies a conserved region of the 18S ribosomal RNA gene from *B. gibsoni*, *B. canis canis*, *B. canis vogeli and B. canis rossi* but not from other protozoal species. The assay incorporates a host DNA control.

The meaning of a positive

Babesia spp. DNA detected in the sample and are the probable cause of appropriate clinical history, signs, haematological and/or cytological findings.

The meaning of "not detected"

Babesia spp. DNA undetectable in sample and in most cases this means that clinical signs are unrelated to infection with *Babesia* spp. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test.

Special details of this test

This test does not detect *B. felis* and is therefore not useful in feline cases. Please contact us if you have a feline case as we have a primer set which has been successfully used with this organism.

Diagnostic planning with this test

Use the test in cases with a history of overseas travel. Haemolytic anaemia is the commonest acute presentation. This may be of variable severity and may be accompanied by thrombocytopaenia. Lymphadenomegaly and splenomegaly are common. A wide range of chronic complications is reported including CNS disease, acute respiratory distress syndrome, renal failure and pancreatitis. Also consider the test as part of work-up for unexplained fever.

Because of common risk factors dogs infected with *Babesia* may be co-infected with *Ehrlichia* and *Leishmania* and where there is a history of travel to endemic areas combined testing for each of these agents is recommended.

Path Line: 01392 247 915 **Email:** tdds@exeter.ac.uk
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Toxoplasma gondii

Which samples

- CSF
- Fresh tissue / fluid aspirates (e.g. BAL, effusions, aqueous humour)

What we detect

The test amplifies the repeat region of a wide-range of *Toxoplasma gondii* isolates and not DNA from other infectious agents, including other protozoan species. The assay incorporates a synthetic DNA control.

The meaning of a positive

A positive result detects the presence of the organism in infected animals but will not necessarily distinguish acute from chronic, subclinical encysted infection. *Toxoplasma* is the probable cause of the clinical signs with appropriate clinical history, haematological, biochemical and/or cytological findings.

The meaning of "not detected"

Toxoplasma DNA undetectable in sample and in most cases this means that clinical signs are unrelated to infection with *Toxoplasma*. A negative result does not entirely rule out toxoplasmosis, as false negative results may occur in chronically infected patients, if the selected tissue does not contain the organism or is present at a level below the detection limit of the assay.

Special details of this test

Detection of *Toxoplasma* by PCR should be interpreted in combination with the clinical signs and serology results. Once infected, animals retain *Toxoplasma* tissue cysts for life, and a positive PCR result may indicate a chronic subclinical infection.

Diagnostic planning with the test

In equivocal cases, use the PCR test in conjunction with serology to help distinguish acute from chronic infection.

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Neospora caninum

Which samples

- CSF
- Tissue / fluid aspirates
- Formalin-fixed tissues (fresh tissue preferred)
- Faeces
- EDTA whole blood

What we detect

The test amplifies the *Neospora*-specific Nc5 gene locus of a wide-range of *Neospora caninum* isolates and not DNA from other infectious agents, including other protozoan species. The test incorporates a synthetic DNA control.

The meaning of a positive

A positive result detects animals infected with *Neospora* but will not necessarily distinguish acute or chronic subclinical encysted infection. *Neospora* is the probable cause of the clinical signs with appropriate clinical history, haematological, biochemical and/or cytological findings.

The meaning of "not detected"

Neospora DNA undetectable in the sample and in most cases this means that clinical signs are unrelated to infection with *Neospora*. However, the organism may be present at levels below the detection limit of the assay.

Special details of this test

Shedding of oocysts may be intermittent or absent in faeces of clinically affected cases and the organism may be present transiently in the blood of infected animals.

Diagnostic planning with the test

Use the PCR result in combination with serology and the clinical signs to confirm the diagnosis of neosporosis.

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Mycobacterium qPCR (*M. tuberculosis* and *M. avium* Complexes)

Which samples

- Fixed and stained fine needle aspirates / smears of effusions or BAL samples
- Formalin-fixed tissues (reduced sensitivity)

What we detect

This test comprises two qPCR assays. The first assay amplifies a conserved region of the genomes from the species within the *Mycobacterium tuberculosis* complex [MTC] (*M. tuberculosis*, *M. bovis and M. microti*, *M. canettii*, *M. africanum*, *M. pinnipedii*) but not from the *Mycobacterium avium* Complex [MAC] or other bacterial species. The second assay amplifies a conserved region of the genomes from the species within the *Mycobacterium avium* Complex [MAC] (*M. avium* subsp. *avium*, *M. avium* subsp. *paratuberculosis*, *M. intracellulare)* but not from the MTC or other bacterial species. In addition, the MAC assay amplifies DNA from *M. malmoense* and *M. scrofulaceum*, which are both implicated in human disease. Both assays incorporate a synthetic DNA control.

The meaning of a positive

In the context of a (pyo)granulomatous lesion or disseminated granulomatous disease, a positive result is consistent with mycobacteriosis due to one or more of the bacterial species detected by the MTC or MAC assays. In lesions with cytological evidence of acid fast bacteria this confirms that the acid fast bacteria belong to one or more of the bacterial species detected by the MTC or MAC assays.

The meaning of "not detected"

MTC and/or MAC DNA is undetectable in the sample and in most cases this excludes infection with these organisms but does not rule out infection by other mycobacterial species e.g., *M. lepraemurium* (feline leprosy). While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test. This is particularly important with formalin-fixed tissues as the cross-linkage and degradation of DNA increases the likelihood of false negative results.

Special details of this test

Acid-fast staining of slides degrades the DNA and prevents successful qPCR. This qPCR can be performed on cytology slides stained with Wright's stain (our frontline stain, which includes methanol fixation).

Continued overleaf.

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Diagnostic planning with the test

We anticipate that this test is used at the pathologist's discretion having examined fixed cytological and histopathological samples and having identified an appropriate pattern of inflammation or suspicious micro-organisms. At this point it may be appropriate to request a ZN stain, mycobacterial PCR or both. An advantage of PCR over culture is that it may be performed on fixed and stained cytological and histopathological samples which are not suitable for culture. Speciation still requires culture which, in turn, requires fresh tissue, effusion or wash fluid. Fresh samples intended for culture should be submitted to the AHVLA. Where there are acid fast organisms and / or where there is a positive PCR in biopsied or aspirated samples the AHVLA will perform culture and the cost will be met by DEFRA. Mycobacterial infection is potentially zoonotic and if disease is identified in dogs or cats the Local Health Authority should be informed.

Isolation of *M.bovis* from cats and dogs is notifiable to DEFRA. **Be sure to include the owner's details and, most importantly, address when notifying DEFRA.**

Path Line: 01392 247 915 **Email:** tdds@exeter.ac.uk
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Leptospira spp.

Which samples

- EDTA whole blood
- Urine
- Aqueous humour
- CSF
- Fresh/frozen tissue (liver/kidney)

What we detect

The test for *Leptospira* amplifies a conserved region of the genome from a number of pathogenic *Leptospira* isolates including *Leptospira interrogans* (including serovars Icterohaemorrhagiae, Canicola, Pomona, Grippotyphosa, and Bratislava), *Leptospira noguchii* and *Leptospira borgpetersenii*. The assay incorporates a synthetic DNA control.

The meaning of a positive

In the context of supportive clinical signs and laboratory data, a positive PCR result is consistent with the diagnosis of leptospirosis**.** The assay may detect organisms being shed by a subclinical carrier.

The meaning of "not detected"

Leptospira DNA (pathogenic serovars of *Leptospira*) is undetectable in the sample. A negative PCR result does not exclude a diagnosis of leptospirosis, especially if there are supportive clinical signs and laboratory data. While this qPCR is very sensitive, a negative result does not entirely rule out infection, as the organism could be present at a level below the detectable limit of the test. Note that the use of antimicrobials may lead to false negative results due to organism clearance.

Special details of this test

This test only detects known pathogenic serovars.

Diagnostic planning with the test

PCR can be used in combination with serologic tests to improve the sensitivity of the diagnosis. Because the timing of infection is uncertain, both blood and urine from suspect animals should be tested. To date, studies have failed to detect *Leptospira* DNA in the blood of dogs in the period post-vaccination. Therefore, it would be surprising if false positive results were found in the urine of vaccinated animals.

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Encephalitozoon cuniculi

Which samples

- Fresh post-mortem tissues especially brain and kidney
- Lens material from uveitis cases
- Urine

What we detect

The test amplifies the ITS gene from *E. cuniculi* but no other *Encephalitozoon* or other infectious agents, including other fungal species. The test incorporates a synthetic DNA control.

The meaning of a positive

E. cuniculi DNA is present in the sample. A positive PCR result is supportive of the diagnosis of encephalitozoonosis in the context of supportive clinical signs. However, spores may be present in the urine of symptomatic and asymptomatic rabbits. The brain, kidney and lens tissue tend to have the greatest number of spores and may be the best tissues to detect the organism.

The meaning of "not detected"

E. cuniculi DNA is undetectable in the sample. Spores are shed in the urine of affected rabbits from 3-5 weeks post seroconversion and only sporadically, therefore their detection in urine is not always possible. For the aforementioned reasons, a negative urine PCR result should be interpreted in combination with the clinical signs. Detection of the organism in CSF has proven unreliable for detection and is thus not recommended.

Diagnostic planning with the test

A minimally invasive method for showing that *E. cuniculi* is being shed in the urine of affected rabbits. Use in conjunction with serology to confirm active cases.

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The Canine Clonality (PARR) Test

PARR – PCR for Antigen Receptor Rearrangement

In the veterinary literature this technique was first described in a preliminary study of canine lymphoma by Vernau and Moore in 1999, then later by the same group in 2003. Since then it has been developed and refined in a number of research studies as an ancillary tool in the diagnosis of canine lymphoproliferative disease based on molecular detection of a clonal population of lymphocytes. This test aids in the differentiation of reactive (usually polyclonal) from neoplastic (usually clonal) population of lymphocytes.

T and B cells have unique rearrangements of the V(D)J regions of their T-cell and B-cell receptor genes, respectively. During rearrangement there is also addition and deletion of variable numbers of DNA nucleotides within variable regions of these genes, resulting in a range of rearranged DNA sequence lengths. Primers are designed to bind to conserved regions of the genes and amplify across the variable regions of the uniquely rearranged V(D)J segments of T and B lymphocyte DNA. The amplified products (amplicons) are then separated based upon size by capillary electrophoresis. Although, in response to infection, lymphocytes may be selected for clonal proliferation, in most cases, these will have resulted from the rearrangement of multiple V(D)J genes with slightly different additions and deletions of DNA nucleotides. A sample of this type produces a range of amplicons of differing length which form a smear and upon electrophoretic separation due to the wide range of receptor gene rearrangements (polyclonal).

Lymphoproliferative disease arises as a result of neoplastic transformation of a single lymphocyte, which will have a unique V(D)J gene rearrangements, resulting in the proliferation of a clonal population, which in theory should all have the same unique V(D) J genes. Amplicons produced from these cells should all be of the same size and would produce distinct peak or band upon electrophoresis.

TDDS is using published techniques for B and T cell clonality testing in conjunction with a novel B-cell primer set. Initial in-house validation has utilised approximately 80 cytology cases encompassing lymphoma, lymphoid reactivity, non-lymphoid neoplasms and inflammatory disease. Assuming clonality equates with lymphoma initial results with these cytology samples support a sensitivity for canine lymphoma of 94% and a specificity of 93%, values similar to those published elsewhere.

Clonality testing may be applied to stained cytology preparations, blood, fluids, bone marrow, tissue aspirates and air-dried smears which contain the abnormal cell population (currently formalin-fixed tissues are not routinely analysed). Cellularity of the sample is important and, as with many infectious disease PCRs, a host DNA control is used initially to assess sample quality and quantity.

We recommend that clonality assessment is not used as a sole diagnostic test but is run in conjunction with one or more other diagnostic techniques including haematology, cytology, histopathology, immunohistochemistry and flow cytometry. This is particularly

Continued overleaf.

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important as false positive results have been reported with chronic inflammation associated with some infectious agents and cytological assessment is useful in ensuring that the abnormal population is present in the sample prior to analysis.

In most cases of lymphoproliferative diseases a clonal rearrangement of either the BCR or TCR gene locus will be found but clonal rearrangements of both loci are reported. It is recommended that clonality testing is not relied upon as the sole means of differentiating B-cell from T-cell lymphoproliferative disease, due to this potential for dual rearrangement, and as clonal rearrangements in myeloid neoplasms can occur.

Using Clonality Testing

Haematological, cytological and histopathological diagnosis of advanced lymphoma is often straightforward and requires no additional testing. Diagnosis by conventional means may be more challenging for a variety of reasons and clonality testing may be recomended in these situations:

Case Example: 5yo FN WHWT with generalised lymphadenomegaly.

- Three lymph node smears initially submitted.

Cytological interpretation: reactive hyperplasia with subtly expanded intermediate sized lymphoid population on one smear. Suggest blood film examination, check for skin lesions / organomegaly, aspirate other nodes and this node in 1-2 weeks, consider lymph node biopsy and clonality testing.

- 10 days later three nodes aspirated and biopsied.

Cytological interpretation: mildly reactive nodes with expanded intermediate sized lymphoid population. Histopathological diagnosis: lymphoid hyperplasia with distorted architecture, possible atypical hyperplasia.

- 3 weeks later lethargic, losing weight, nodes larger, strongly suspect lymphoma. Two lymph node smears.

Cytological interpretation: lymphoid reactivity, expanded intermediate sized lymphoid population, as before. This could reflect infiltration from disease elsewhere or a slowly progressive indolent lymphoma. Consider clonality testing.

- 6 months later: Lymphadenomegaly resolved with chemotherapy. Lymph nodes enlarged again. Lymph node smears submitted.

Cytological interpretation: Lymphoma.

- Clonality Test run on the initial three smears: TCR Polyclonal; BCR Clonal

This test was carried out as part of the development of the clonality testing (after submission of the final samples) and would have supported a diagnosis of lymphoma from the first submitted samples.

Continued overleaf.

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- Small clonal population:
  - Early disease
  - Unusual lymphoma sub-type (e.g. follicular lymphoma)
- Tissue sampled not primary site of disease (e.g. mesenteric node VS GI tract)
- Low diagnostic yield
- Poor cell preservation
- Persistent lymphocytosis with or without cellular atypia

Our clinical experience with this test is very encouraging. In our daily work a number of lymphoma aspirates are submitted which, although highly cellular, contain few intact or well preserved cells. Sometimes preservation is repeatedly poor on reaspiration. In such cases interpretation is often tentative. Detection of a clonal population in these situations provides further strong support for the diagnosis of lymphoma and has avoided the need for clinicians to readmit patients and take further samples.

There are also less obvious situations in which the test may prove very useful e.g., the differentiation of plasmacytomas and cutaneous lymphoma from other round cell tumours.

We are continuing to evaluate the use of canine clonality testing, particularly with regard to detection of early lymphoma and lymphoma subtypes which are more challenging to diagnose by conventional means (as with the case above). These are often indolent cases and it can take a considerable period of time before disease progresses to the point where it can be confidently diagnosed with cytology or histopathology. We therefore anticipate this to be an area of ongoing research for months and years to come.

We have found that canine clonality testing provides an invaluable adjunct to cytology in the modern diagnosis of lymphoma by minimally invasive methods.

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www.tddslab.co.uk **Fax**: 01392 262 354

Quick guide to optimal sample types

The following list is designed for quick reference but is not an exhaustive summary of all possible sample types for all available tests. In many cases choice of sample should depend on the localisation of clinical signs.

Anaplasma phagocytophilum
EDTA whole blood; FNA smears lymph node, spleen, bone marrow; synovial fluid

Angiostrongylus vasorum
Tracheal wash/BAL fluid or smears, faeces, lung aspirate smears, EDTA whole blood*

Aspergillus fumigatus
Nasal flush fluid / cytology smears, nasal plaque biopsies, nasal swabs

***Bartonella* spp.**
EDTA whole blood, tissue aspirates / smears

Bordetella bronchiseptica

Cat	Nasopharyngeal swabs / scrapings / cytology smears, nasal flush
Dog	Tracheal wash / BAL fluid or smears

Canine babesiosis
EDTA whole blood, FNA smears from spleen or bone marrow

Canine Clonality Test
EDTA whole blood, bone marrow; FNA smears, cavity fluids, biopsy material of any tissue type where lymphoma suspected

Canine Distemper Virus
Oropharyngeal swab, conjunctival scraping, tracheal wash / BAL fluid / smears

Canine Haemoplasmas
EDTA whole blood

Canine Herpes Virus

Resp	Oropharyngeal swabs, tracheal wash / BAL fluid / smears
Repro	Vaginal/penile swabs, PM tissue from puppies (kidney, liver, lungs)
Ocular	Conjunctival swabs, corneal scrapes / smears

Canine Parvovirus
Fresh faeces, well-coated faecal swab

CEM TRIPLEX qPCR.
Plain or charcoal swabs - Mares: Clitoral (fossa & sinus), endometrial. Stallions: Urethra, urethral fossa, penile sheath, pre-ejaculatory fluid

Chlamydophila felis
Ocular / conjunctival swabs, ocular / conjunctival scrapings / cytology smears

Cryptosporidium
Faeces, well-coated faecal swabs

Ehrlichia
EDTA whole blood; FNA smears spleen, bone marrow, lymph node, synovial fluid, CSF

Encephalitozoon cuniculi
Urine, PM tissue (esp. brain/kidney), lens material from uveitis cases

Equine Herpes Virus 1 and 4
Nasal / nasopharyngeal swab, EDTA whole blood, placental / aborted foetal tissue

Feline Calicivirus
Oronasal swabs, scraping / smears from oral lesions, tracheal wash / nasal fluid

Feline Coronavirus
FNA smears eg liver, lymph node (dry FIP); intracavitary fluids, aqueous humour, CSF, faeces

Feline Haemoplasmas (FIA)
EDTA whole blood

Feline Herpesvirus 1
Conjunctival / oronasal swabs, scrapings / cytology smears (conjunctiva / upper respiratory tract), tracheal wash / nasal flush fluid or smears

Feline Immunodeficiency Virus
EDTA whole blood, bone marrow (aspirates or in EDTA)

Feline Leukaemia Virus
EDTA whole blood, bone marrow (aspirates or in EDTA)

Feline Panleukopaenia virus
Fresh faeces, well-coated faecal swab

Giardia
Faeces, well-coated faecal swabs

***Leishmania* spp.**
EDTA whole blood, FNA smears (skin, conjunctival swabs / scrapes, lymph nodes, spleen, bone marrow) or biopsies from similar tissue

***Leptospira* spp.**
EDTA whole blood, urine, kidney FNA or biopsy

Lyme Disease (*Borellia* spp.)
Synovial fluid (EDTA or smears), CSF, EDTA whole blood*

***Mycobacterium* (*M. tuberculosis* & *M. avium* complexes)**
FNA smears (effusions, BAL, lymph nodes, masses etc), similar tissues formalin fixed.

Mycoplasma cynos* & *canis

Resp	Pharyngeal swab, tracheal wash / BAL fluid or smears
Urinary	Cystocentesis urine sample
Repro	Deep, guarded vaginal swabs

Mycoplasma felis
Tracheal wash / BAL fluid or smears, swabs (eyes, nasopharynx, wounds, abscesses), scrapings, aspirates and smears / tissue from similar locations

Neospora caninum
CSF, faeces, tissue biopsies

***Rhodococcus equi* inc. VapA**
Tracheal wash / BAL fluid or smears, peritoneal fluid, swabs / smears from abscesses

Streptococcus equi equi* (Strangles) & *Streptococcus equi zooepidemicus
Nasopharyngeal swab, guttural pouch washings, swabs / smears from abscessed / ulcerated lymph nodes

Toxoplasma gondii
CSF, smears of BAL, effusions, aqueous humour, tissue biopsies

Tritrichomonas foetus
Faeces, well-coated faecal swabs

Path Line: 01392 247 915 **Email:** tdds@exeter.ac.uk
www.tddslab.co.uk **Fax**: 01392 262 354

*EDTA whole blood may be used for these agents but is not the optimal sample type and may have reduced sensitivity.
For samples where swabs are suitable try to avoid charcoal swabs. For PCR on excised or biopsied tissue remember that formalin fixation will reduce nucleic acid recovery.