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# Molecular Testing Price List 2018

| Feline Tests | Code | £ |
|---|---|---|
| Feline Respiratory Screen (FHV/CFEL/Bord/MFEL) | mt1 | |
| Feline Mini Respiratory Screen (FHV/CFEL/FCV) | mt2 | |
| Feline Extended Respiratory Screen (FHV/CFEL/FCV/Bord/MFEL/MGAT/AEAB) | mt62 | |
| *Bordetella bronchiseptica* | mt3 | |
| *Chlamydophila felis* (CFEL) | mt4 | |
| Feline Calicivirus (FCV) | mt5 | |
| Feline Herpes Virus (FHV) | mt6 | |
| *Mycoplasma felis* (MFEL) | mt7 | |
| *Aelurostrongylus abstrusus* (AEAB) (feline lungworm) | mt59 | |
| *Mycoplasma gateae* (MGAT) | mt60 | |
| + each additional agent | | |
| Feline Leukaemia Virus (FeLV) | mt8 | |
| Feline Immunodeficiency Virus (FIV) | mt9 | |
| Feline Haemoplasmas (Feline Infectious Anaemia) | mt10 | |
| + each additional agent | | |
| Feline Coronavirus (FCoV) | mt11 | |

| Faecal Pathogens | Code | £ |
|---|---|---|
| Canine Diarrhoea Screen (Parvo, CCoV, *Giardia*, *Crypto*) | mt64 | |
| Feline Diarrhoea Screen (TTF, Parvo, FCoV, *Giardia*, *Crypto*) | mt65 | |
| *Tritrichomonas foetus* (TTF) | mt27 | |
| Parvovirus (Panleukopaenia Virus) | mt28 | |
| *Giardia* | mt29 | |
| *Cryptosporidium* | mt30 | |
| + each additional agent | | |
| Canine Coronavirus (CCOV) (enteric samples) | mt54 | |

| Equine Tests | Code | £ |
|---|---|---|
| CEMO Triplex qPCR (*Taylorella, Klebsiella* and *Pseudomonas*) | mt37 | |
| *Taylorella equigenitalis* | mt38 | |
| Stallion CEMO | mt45 | |
| Equine Respiratory Screen (Strangles, *R.eq* and EHV1+4) | mt42 | |
| Equine Strangles | mt39 | |
| *Rhodococcus equi* inc. VapA | mt40 | |
| EHV1 and EHV4 | mt41 | |
| EHV1 only | mt43 | |

Samples: (as appropriate) Plain swabs (+/- VTM) or charcoal (CEMO); faeces; well-coated faecal swabs; fresh biopsy material/FNA;BAL; EDTA blood, bone marrow

| Canine Tests | Code | £ |
|---|---|---|
| Canine Respiratory Screen (AV/CV/Bord/*M.cynos*/CPiV/CCoV) | mt12 | |
| Canine Mini Respiratory Screen (Bord/*M.cynos*/CPiV/CCoV) | mt13 | |
| Canine Extended Respiratory Screen (AV/CV/Bord/*M.cynos*/CPiV/CCoV/CAV-2) | mt63 | |
| Canine Lungworm (AV/CV) | mt66 | |
| *Angiostrongylus vasorum* (AV) | mt14 | |
| *Crenosoma vulpis* (CV) | mt49 | |
| *Bordetella bronchiseptica* | mt3 | |
| *Mycoplasma cynos* | mt16 | |
| Canine Parainfluenza Virus (CPiV) | mt51 | |
| Canine Adenovirus Type 2 (CAV-2) (Respiratory) | mt52 | |
| Canine Adenovirus Type 1 (CAV-1) (Infectious Canine Hepatitis) | mt58 | |
| *Mycoplasma canis* | mt15 | |
| + each additional agent | | |
| Canine Coronavirus (CCoV) (respiratory samples) | mt53 | |

| Vector Borne Pathogens | Code | £ |
|---|---|---|
| *Anaplasma phagocytophilum* + *platys* | mt21 | |
| *Bartonella henselae* + *B.* spp. | mt22 | |
| Lyme Disease (*Borrelia* spp.) | mt23 | |
| *Ehrlichia* spp. | mt24 | |
| *Leishmania* spp. | mt25 | |
| Canine Babesiosis | mt26 | |
| Canine Haemoplasmas | mt20 | |
| Feline Babesiosis | mt55 | |
| + each additional agent | | |

| Other Agents | Code | £ |
|---|---|---|
| Canine Neuro Screen (CDV/*T. gondii*/*N. caninum*) | mt44 | |
| Canine Distemper Virus (CDV) | mt18 | |
| *Leptospira* spp. | mt34 | |
| *Toxoplasma gondii* | mt35 | |
| *Neospora caninum* | mt36 | |
| *Aspergillus fumigatus* | mt17 | |
| Canine Herpes Virus (CHV) | mt19 | |
| *Chlamydia psittaci* | mt32 | |
| *E. cuniculi* | mt33 | |
| + each additional agent | | |

| Other Tests | Code | £ |
|---|---|---|
| Canine Clonality (PARR) Test | mt46 | |
| Feline Clonality (PARR) Test | mt56 | |
| + additional from same case (canine or feline) | | |

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# Molecular Testing 2018

Iain Peters,
BVMS (Hons), PhD, MRCVS

Our aim at Molecular Testing is to provide a high quality, responsive service which is accessible to all veterinary surgeons facing the challenges of first opinion and referral practice. We pride ourselves on running our extensive range of diagnostic tests twice weekly, allowing a consistent and dependable reporting time. In addition, we are constantly working towards expanding and improving our range of tests to help expand the range of molecular tests available to the veterinary practitioner.

Molecular Testing has been running at TDDS Exeter since January 2012 under the direction of Dr. Iain Peters who qualified as a veterinary surgeon from the University of Glasgow in 1997. Following three years spent in veterinary practice in Devon, Iain gained a PhD in Canine Gastroenterology and Immunology from the University of Bristol in 2004.

Iain remained at the University of Bristol, first as a post-doctoral researcher and latterly as a Lecturer in Veterinary Pathology, until he joined TDDS in 2012. His research interests included the application of molecular techniques, including real-time PCR, to the investigation of canine and feline immune-mediated and infectious diseases. He is an author on over 40-peer reviewed journal articles and of a chapter in the recent second edition of Arthropod-borne Infectious Diseases of the Dog and Cat, covering laboratory diagnosis of arthropod-borne infections. In addition he has presented at a number of veterinary conferences including the BSAVA and ECVIM-CA Congresses. He continues to have an active role in clinical research with colleagues in a number of veterinary schools worldwide.

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# The Canine and Feline Clonality (PARR) Test

## The Science

Clonality testing is a useful tool in the diagnosis of lymphoproliferative disease and is based on molecular identification of clonal populations of lymphocytes, thus differentiating reactive (usually polyclonal) from neoplastic (usually clonal) lymphocytes.
T- and B-cells have unique rearrangements of the V(D)J regions of their receptor genes. PCR is used to amplify these rearranged regions and the amplified products (amplicons) are then separated by capillary electrophoresis. Lymphoproliferative disease arises as a result of neoplastic transformation of a single lymphocyte, which will have a unique V(D)J gene rearrangement. PCR amplicons produced from these cells will all be of the same size and produce a distinct band upon electrophoresis.

## The Application

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.

Clonality testing is recommended when the diagnosis of lymphoma by conventional means is made more challenging, including: early disease; unusual lymphoma subtypes (e.g. small T-cell); poor cell preservation (e.g. equivocal aspirates); persistent lymphocytosis with or without cellular atypia.

## Clonality Testing in Action

The test is highly effective and has been widely used in the Veterinary Pathology Group since 2014 (dog) and 2015 (cat) using a combination of published techniques for B- and T-cell clonality testing. It has provided confirmation of lymphoma in numerous difficult cases when used in conjunction with cytology and/or histopathology.

Validation using appropriate canine cytology cases provided a sensitivity for canine lymphoma of 94% and a specificity of 93%, values similar to those published elsewhere.

Validation using appropriate feline cytology cases provided a specificity of >90% and sensitivity of 75% which is similar to the experiences of other international groups developing feline clonality testing in that the specificity of the test is similar to canine clonality (usually greater than 90%) but the sensitivity of the test is significantly lower than in dogs and may be as low as 65%. In most cases a positive result confirms but a negative result does not rule out lymphoproliferative disease.

Due to its lower sensitivity than the test in dogs, we currently recommend the use of feline clonality testing only under specific guidance from one of our pathologists for use under specific circumstances to assist diagnosis of difficult lymphoma cases.
Samples previously examined at another laboratory should be accompanied by the previous report at the time of submission.

## Further Clinical Recommendations – the Dos and Don'ts of Clonality Testing

The test is most effective when used on stained cytology preparations or other sample types known to contain the abnormal cell population. Formalin-fixed, paraffin embedded tissues can be used in most cases, but the deleterious effects of formalin fixation can make analysis of this sample type more problematic.

Staining procedures with an acidification step (e.g. ZN and haematoxylin/eosin) hydrolyse DNA and can prevent the use of specimens for PARR testing.

The test is at its most powerful when used with the recommendation and interpretation of a cytopathologist or histopathologist for confirming suspected cases of lymphoproliferative disease.

Some false negative results occur; therefore the test should never be used to rule out lymphoproliferative disease without including expert cytological/ histopathological examination.

Clonality testing and CSF samples: The test can demonstrate that a documented lymphocytic pleocytosis is clonal (i.e. neoplastic), if sufficient cells can be recovered from the sample. The test is ineffective if used on low or acellular CSF samples or on blood in cases of suspected CNS lymphoma (as with lymphoma in other anatomic sites), without evidence of atypical lymphocytes or an abnormal lymphocyte count in the circulation.

### Case Example: 5yo FN WHWT with generalised lymphadenomegaly

Aspirates taken on three different occasions over a three week period for cytology all yielded an equivocal "reactive hyperplasia with a suspiciously expanded intermediate-sized lymphoid population". Histopathology was also equivocal reporting "lymphoid hyperplasia with distorted architecture, possible atypical hyperplasia". Eventually treatment for lymphoma was elected in spite of the lack of a definitive diagnosis. The dog responded to chemotherapy but the lymphadenopathy recurred and further cytology smears yielded the diagnosis of lymphoma. Thus repeated cytological and histopathological investigation over a seven month period was required to make the diagnosis in this case.

Clonality Testing run retrospectively on the first three cytology smears from the case showed B-cell clonality and would have supported a diagnosis of B-cell lymphoma from the first submitted cytology samples.

### Case Example: 3 yo FN Domestic Shorthair with ascites and a large intra-abdominal mass (spleen)

Peritoneal fluid: a large population of relatively homogeneous small cleaved lymphocytes, not overtly neoplastic but a small cell lymphoma cannot be ruled out.

Splenic aspirate: the same population of lymphocytes is noted but present in insufficient numbers to confirm splenic lymphoma.

Feline Clonality Testing (PARR): Peritoneal fluid and splenic aspirate – clonal B-cell receptor rearrangement detected.

Diagnosis: Small B-cell lymphoma

# Minimal residual disease testing in lymphoma

Our major molecular testing project of 2017-2018 is the technical validation of a method for developing tumour specific qPCRs for individual B-cell lymphomas so that tumour regression can be monitored quantitatively during and after chemotherapy from a blood sample or lymph node aspirate. This extraordinary, ground-breaking advance in the management of lymphoma is currently undergoing clinical validation with the help of our specialist colleagues in oncology referral practices. When we are satisfied with the clinical validation this will become a key component of our minimally invasive lymphoma protocols.

**Further detailed information on Clonality testing is available in our PCR manual and on our website (www.tddslab.co.uk)**