

## Diagnose Feline Infectious Peritonitis with More Confidence

**IDEXX Reference Laboratories now offers Feline Infectious Peritonitis Virus (FIP Virus) RealPCR™ Test to aid in the diagnosis of life threatening feline disease**

There are two recognised feline coronavirus biotypes that infect cats, each causing different biological outcomes: feline enteric coronavirus (FECV) and feline infectious peritonitis virus (FIPV).

The FIP Virus RealPCR™ Test differentiates between the less virulent or non-pathogenic FECV biotype and the virulent or pathogenic FIPV biotype allowing for more definitive diagnosis or exclusion of feline infectious peritonitis (FIP). Diagnosing FIP often is extremely difficult and frustrating, and the availability of this new test can help veterinarians reach a diagnosis so that cat owners can make informed decisions regarding treatment and prepare themselves for the ultimate outcome.

### Background

FIP is characterised as an immune-mediated pyogranulomatous vasculitis and is a fatal infection caused by FIPV, a highly virulent form of feline coronavirus. FIP is found worldwide, and primarily affects young cats less than 2 years of age. However, any cat can be infected, and several pure breeds appear to be at higher risk. Stress, recent surgery and overcrowding are additional risk factors.<sup>1</sup> Cats can present with wet (effusive) or dry (non-effusive) forms of the disease.

### Why diagnosing FIP is challenging

Clinical signs can mimic many other systemic illnesses. Until now specific diagnostic tests have been lacking and unable to differentiate between the less virulent FECV and the fatal FIPV biotypes of feline coronavirus. Historically, diagnosis has typically been based on review of history, clinical signs, laboratory findings, and consistent coronavirus antibody titers. A definitive diagnosis even required a biopsy including IHC. However, the usefulness of coronavirus titers for diagnosing FIP is limited because depending on the region approximately 25 % of cats in single-cat households and 75 % – 90 % in multicat households have antibodies to coronavirus; whereas, only 7,8 % – 12 % of feline coronavirus-infected cats actually develop FIP.<sup>2,3</sup>

IDEXX previously introduced the feline coronavirus (FCoV) RealPCR™ Test which allowed detection of feline coronavirus (FCoV) in tissues or fluid, but this test does not differentiate between the low or non-virulent FECV biotype and the lethal FIPV biotype. Consequently, a positive FCoV RealPCR™ Test result on fluid or tissue, while supportive of FIP, does not confirm the diagnosis. Because there was still clearly a need for a true diagnostic test for FIP, the FIP Virus RealPCR™ Test was developed.

### Introducing the FIP Virus RealPCR™ Test

Recently, investigators in the Netherlands identified two mutations in the spike (S) gene of feline coronavirus in cats with FIPV that they did not find in cats infected with FECV.<sup>4</sup> In coronaviruses, the S protein functions in cell entry and is responsible for receptor attachment and membrane fusion. It was postulated that these virulence mutations enable FIPV to efficiently infect and replicate in macrophages and spread systemically, whereas replication of FECV is restricted primarily to the epithelial cells of the gut.<sup>4</sup> Based on these findings and in collaboration with the Utrecht University researchers, IDEXX's molecular diagnostic laboratory developed and validated the FIP Virus RealPCR™ Test which can identify each mutation separately.





### When to Perform the FIP Virus RealPCR™ Test

The FIP Virus RealPCR™ Test is an additional tool that can be used to help confirm the diagnosis of FIP in cats where there are clinical signs and other compatible laboratory findings of this disease (see “Building the FIP Diagnosis”). The FIP Virus RealPCR™ Test should be performed on abdominal or pleural fluid in cats with suspect wet FIP and on tissue biopsy or aspirates on cats with suspect dry FIP. Testing of whole blood specimens is not recommended because often the level of viraemia is too low to permit biotyping. Faeces will not be accepted for biotyping because it is possible for cats with FIP to also have an intestinal infection with FECV and shed this non-virulent biotype in their faeces thus providing misleading results.

### Interpreting FIP Virus RealPCR™ Test Results

All specimens will be first tested for feline coronavirus with the FCoV RealPCR™ Test, and if results are positive, the new FIP Virus RealPCR™ Test will be performed. There are four FIPV biotype results as shown in the following table.

FIPV biotype result	Interpretation
FIPV	FCoV has mutated into the FIPV biotype. In a cat with clinical signs this supports the diagnosis of FIP. If clinical signs consistent with FIP are absent, the FIPV biotype indicates the cat is at high risk for developing FIP and should be monitored closely.
FECV	FCoV has not mutated and the cat is unlikely to have FIP.
Indeterminate	FCoV cannot be typed due to the occurrence of unknown strain variation. FIP cannot be ruled out.
Below limit of detection	FCoV cannot be typed because there were insufficient viral particles to permit biotyping. FIP cannot be ruled out. This result is common with whole blood specimens but can occur with any sample type. Consider submitting an alternate sample type.

### Diagnostic Accuracy of the FIP Virus RealPCR™ Test

The diagnostic accuracy of the FIP Virus RealPCR™ Test was determined from 186 cats who were either healthy or had confirmed FIP based on biopsy. For the 164 cats where a biotype result was obtained, the diagnostic sensitivity was 98.7 % (1 cat with FIP returning the FECV biotype result), diagnostic specificity was 100 % (no healthy cat returning the FIPV biotype result) and overall accuracy of the test was 99.4 %. In 6 cats (3.2 %), a biotype result could not be obtained (indeterminate typing) due to unknown viral strain. In 16 samples (8.6 %), viral load was very low and resulted in a ‘below limit of detection’ interpretation.

### Specimen Requirements

Recommended specimens for FIPV biotyping include peritoneal, pleural or CSF fluid or tissue aspirate or biopsy. Whole blood specimens are acceptable but commonly have insufficient viral particles to allow biotyping. Biotyping will not be performed on faeces.

### Turnaround time

You can expect results within 3 – 5 working days from receipt of the sample at the lab.

#### References

1. Hsieh B, Burney D. Feline Infectious Peritonitis. Clinician’s Brief 2014; 75-80.
2. Rohrbach BW, Legendre AM, Baldwin CA, et al. Epidemiology of feline infectious peritonitis among cats examined at veterinary medical teaching hospitals. JAVMA 2001; 218:1111-1115.
3. Addie D, Belák S, Boucraut-Baralon C, et al. Feline infectious peritonitis. ABCD guidelines on prevention and management. J Feline Med Surg 2009; 11:594-604.
4. Chang H, Egberink HF, Halpin R, Spiro DJ, Rottier PJM. Spike protein fusion peptide and feline coronavirus virulence. Emerg Infect Dis 2012; 18:1089-1095.

**Case example:**

Butterscotch, 5 month, male intact, domestic shorthair

**Presenting complaint:**

Nasal congestion and diarrhoea for 1 week. No vomiting.  
Purring, walking around, overall acting pretty normal.

**Physical examination:**

Temperature: 39.4°C, clear nasal discharge, moderate ascites.

**CBC:**

Leukocytosis (WBC: 27.8 G/l) with neutrophilia (26.400/ $\mu$ l),  
no left shift and slight toxicity, and lymphopenia (950/ $\mu$ l)

**Chemistry panel:**

Mild hypoglycaemia (Glucose: 3.33 mmol/l), hyponatraemia  
(sodium: 142 mmol/l), hyperkalaemia (potassium: 6.0 mmol/l),  
hypoalbuminaemia (26 g/l), normal globulin (43 g/l), normal alb/  
glob ratio (0.6)

**FeLV Antigen and FIV Antibody:** Negative

**Abdominal fluid analysis:**

Mixed cell exudate

Protein: 65 g/l

Nucleated cell: 3.537/ $\mu$ l

Microscopic Interpretation: The sample contains primarily neutrophils with a lower number of macrophages and occasional small lymphocytes are seen. There are possible intracellular structures that could represent bacteria, however it may be debris. The exact clinical significance is uncertain. Considerations should include FIP and possible septic peritonitis. Consider culture with sensitivity; although the cell count is relatively low for a septic exudate.

**Consultation with IDEXX Internal Medicine Specialist:**

Signalment and physical examination findings suggestive of FIP. Laboratory changes not compelling; i.e. no hyperglobulinaemia, normal albumin/globulin ratio (but suspicious at 0.6), no anaemia, and fluid analysis not convincing but suggestive of FIP. Recommend FIP Virus RealPCR™ Test on peritoneal fluid. If test is positive for FIPV biotype, then FIP is very likely. If test is positive for FECV biotype, then FIP is unlikely.

**FIP Virus RealPCR™ Test Result: FIPV**

FIPV biotype indicates that the FCoV has mutated into the FIPV biotype. In a cat with clinical signs this supports the diagnosis of FIP.

**Case Outcome:**

Butterscotch was treated symptomatically with supportive care but deteriorated quickly after diagnosis. He was humanely euthanised two weeks later. A necropsy was performed and samples from several organs were submitted. The microscopic interpretation was severe generalised fibrinopurulent peritonitis with focal pleuritis typical of FIP. Immunohistochemistry staining identified coronavirus antigen in the inflammatory foci confirming the diagnosis of FIP.

**Benefit of FIP Virus RealPCR™ Test:**

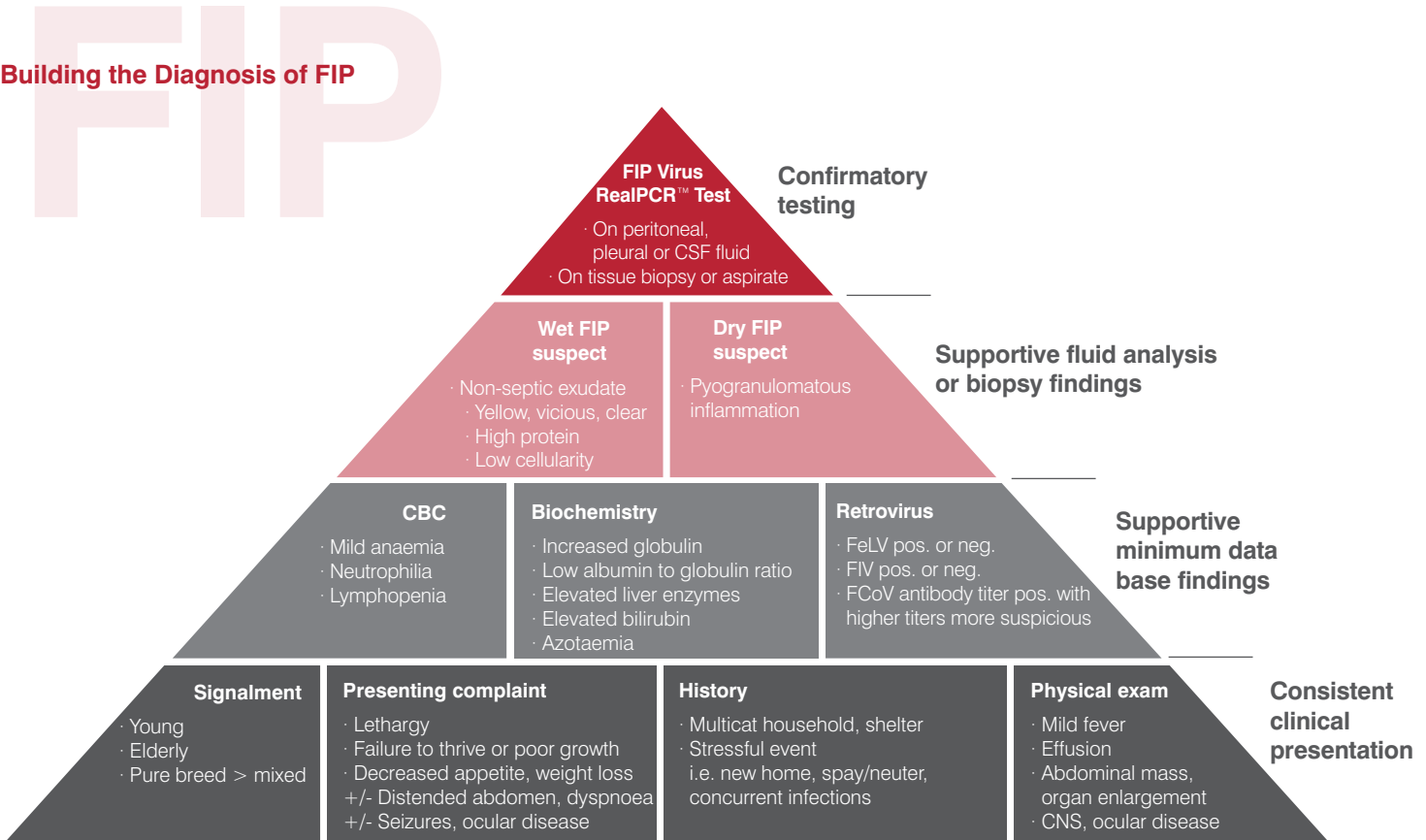
Butterscotch had some physical examination and laboratory abnormalities supportive of the diagnosis of FIP, but as with many cats with this disease, some findings did not support this diagnosis. The FIP Virus RealPCR™ Test really improved Butterscotch's veterinarian's and owner's confidence that he had FIP. It avoided Butterscotch having to undergo other procedures, and the owners could focus on him during his remaining time without unnecessarily incurring additional costs for diagnostics and treatments that were not going to be effective.



*Butterscotch*



## Building the Diagnosis of FIP



Diagnosing FIP is like building a pyramid. The patient's clinical presentation as well as findings from multiple tests must be considered to reach a diagnosis. The FIPV RealPCR Test can help to confirm the diagnosis in suspect patients.

