IDEXX LABORATORIES

DIAGNOSTIC UPDATE

IDEXX Reference Laboratories • April 2015

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

There are two recognized 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 nonpathogenic FECV biotype and the virulent or pathogenic FIPV biotype, allowing for more definitive diagnosis or exclusion of feline infectious peritonitis (FIP). Diagnosing FIP 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 characterized 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.[1] Cats can present with wet (effusive) or dry (noneffusive) 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. However, the usefulness of coronavirus titers for diagnosing FIP is limited, because 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.[2,3] Until now, only biopsy with immunohistochemistry has provided a definitive diagnosis, but this is rarely done antemortem.

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 nonvirulent 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 FIPV 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.[4] 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.[4] 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

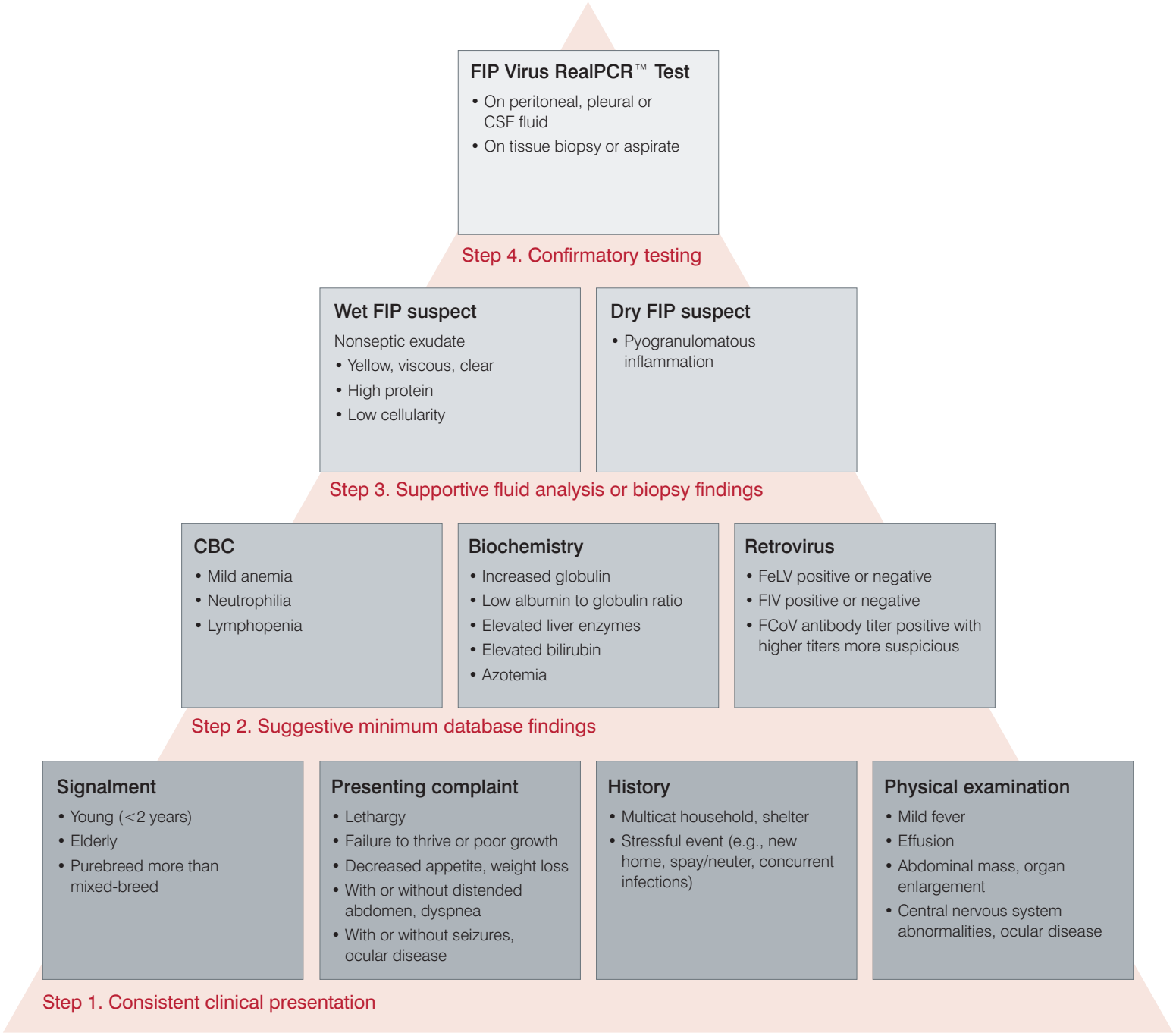
The FIP Virus RealPCR Test is an additional tool that can be used to help confirm the diagnosis of FIP in cats where there are clinical signs and other compatible laboratory findings of this disease (see "Building the diagnosis of FIP" section). The FIP Virus RealPCR Test should be performed on abdominal 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 viremia is too low to permit biotyping. Feces 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 nonvirulent biotype in their feces, 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 the results are positive, the new FIP Virus RealPCR Test will be performed to identify the biotype as either the pathogenic FIPV strain or the less virulent FECV strain.

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 FIP Virus RealPCR™ Test can help to confirm the diagnosis in suspect patients.



There are four possible biotype results as shown in the following table:

FCoV 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 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 specimen type. Consider submitting an alternate specimen 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 receiving FECV biotype result), diagnostic specificity was 100% (no healthy cat receiving FIPV biotype result) and overall accuracy of the test was 99.4%. In 22 cats (12%), a biotype result could not be obtained because of indeterminate typing or viral particle numbers being below the limit of detection.

Ordering information

test code	test name and contents
3630	**FIP Virus RealPCR™ Test** Feline coronavirus (FCoV) RealPCR™ test. If positive for FCoV, the biotype is identified as either the pathogenic FIPV strain or the less virulent FECV strain. **Specimen requirements:** 2 mL (0.1 mL minimum) pleural or abdominal effusion (preferred specimen), tissue aspirates or fresh tissue in an empty, sterile container (preferred specimen) or 1 mL EDTA whole blood (LTT). Keep specimens refrigerated.
3632	**FIP Virus Profile** Chem 25, comprehensive CBC, FeLV antigen and FIV antibody by ELISA, FIP Virus RealPCR™ Test **Specimen requirements:** 2 mL serum, 1 mL LTT, two blood smears (preferred). For RealPCR test: 2 mL (0.1 mL minimum) pleural or abdominal effusion (preferred specimen), tissue aspirates or fresh tissue in an empty, sterile container (preferred specimen) or 1 mL EDTA whole blood (LTT). Keep specimens refrigerated.

Turnaround time

The IDEXX nationwide network of reference laboratories provides daily courier service or IDEXX-Direct® service to pick up your specimens and forward them to our IDEXX Molecular Diagnostics Laboratory in California. IDEXX RealPCR tests are run daily, Monday–Friday. Specimens received on Saturday or Sunday are processed on Monday. You can expect results within 1–3 working days, depending on shipping time.

Contacting IDEXX

Laboratory Customer Support

If you have any questions regarding test codes, turnaround times or pricing, please contact our Laboratory Customer Support Team at 1-888-433-9987.

Expert feedback when you need it

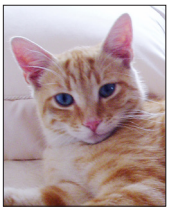
Our team of internal medicine specialists is always available for complimentary consultation. Please call 1-888-433-9987, if you have questions.

References

1. Hsieh B, Burney DP. Feline infectious peritonitis. *Clin Brief.* 2014;12(2):75–80.
2. Rohrbach BW, Legendre AM, Baldwin CA, Lein DH, Reed WM, Wilson RB. Epidemiology of feline infectious peritonitis among cats examined at veterinary medical teaching hospitals. *JAVMA.* 2001;218(7):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(7):594–604.
4. Chang HW, Egberink HF, Halpin R, Spiro DJ, Rottier PJM. Spike protein fusion peptide and feline coronavirus virulence. *Emerg Infect Dis.* 2012;18(7):1089–1095.

A case study: diagnosing feline infectious peritonitis (FIP)

Butterscotch



Patient: Butterscotch, 5-month-old, intact male domestic shorthair

Presenting reason: Butterscotch presented with a one-week history of nasal congestion and diarrhea.

History: Butterscotch's owner reported that Butterscotch was overall acting pretty normal; he seemed happy and was active and purring. They had noticed a nasal discharge the past week with no sneezing and a little diarrhea, but he still had a good appetite and had not vomited.

Physical examination

Butterscotch was bright, alert, responsive and hydrated. His temperature was 103.2°F. He had a clear nasal discharge and moderate ascites.

Diagnostic plan

A complete blood count (CBC), chemistry profile, FeLV and FIV testing and abdominal fluid analysis were performed to screen for metabolic, inflammatory or infectious disease that could be causing Butterscotch's ascites and diarrhea.

Laboratory findings

Hematology—The erythrogram was normal except for a mild decrease in MCV, which is not uncommon in kittens. A leukocytosis with a moderate neutrophilia with no left shift and mild toxicity as well as a mild lymphopenia were present. In face of Butterscotch's fever, these findings were most consistent with an infection, although a purely inflammatory condition was also possible.

Chemistry profile—The low sodium and chloride were likely secondary to loss from diarrhea. The mild hyperkalemia may have been secondary to metabolic acidosis or possible artifact. The most significant finding was the low albumin. Hypoalbuminemia is most commonly a result of decreased production from the liver, renal disease or gastrointestinal (GI) loss. Given Butterscotch also had a mild decrease in glucose and BUN, liver dysfunction secondary to a possible portosystemic shunt was a differential. Although Butterscotch had a normal globulin, which is usually also low with GI loss of protein, his diarrhea still made GI loss a differential. If this was the cause, then perhaps if he were not losing protein through his GI tract, he actually may have had a hyperglobulinemia and a lower albumin to globulin ratio.

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Butterscotch

2014	NOV 9	OCT 31	OCT 29

RBC	8.42	6 - 10 M/µL
Hematocrit	31.2	29 - 45 %
Hemoglobin	10.3	9.5 - 15 g/dL
MCV	37	41 - 58 fL
MCH	12.2	11.0 - 17.5 pg
MCHC	33.0	29 - 36 g/dL
% Reticulocyte	0.2	%
Reticulocyte	17	3 - 50 K/µL
WBC	28.4	4.2 - 15.6 K/µL
Neutrophil	26.412	2.5 - 12.5 K/µL
Lymphocyte	1.136	1.5 - 7 K/µL
Monocyte	0.568	0 - 0.85 K/µL
Eosinophil	0.284	0 - 1.5 K/µL
Basophil	0.0	0 - 0.1 K/µL
Platelet	0	170 - 600 K/µL
Remarks	Slide reviewed microscopically. Neutrophils appear slightly toxic.	

Glucose	68	70 - 150 mg/dL
BUN	12	15 - 34 mg/dL
Creatinine	0.4	0.8 - 2.3 mg/dL
Phosphorus	6.7	3.0 - 7.0 mg/dL
Calcium	8.9	8.2 - 11.8 mg/dL
Sodium	139	147 - 156 mmol/L
Potassium	5.8	3.9 - 5.3 mmol/L
Chloride	112	111 - 125 mmol/L
Total Protein	7.0	5.9 - 8.5 g/dL
Albumin	2.2	2.3 - 3.9 g/dL
Globulin	4.8	3.0 - 5.6 g/dL
Alb/Glob Ratio	0.5	0.4 - 0.8
ALT	13	28 - 100 U/L
AST	29	5 - 55 U/L
ALP	1	0 - 62 U/L
GGT	2	5 - 6 U/L
Bilirubin - Total	0.3	0.0 - 0.4 U/L
Cholesterol	152	64 - 440 U/L

FeLV Antigen by ELISA	NEGATIVE
FIV Antibody by ELISA	NEGATIVE

Abdominal fluid analysis—The fluid analysis was a high-protein, moderately cellular, mixed-cell exudate. This type of ascitic fluid in a kitten is very suspicious for feline infectious peritonitis. However, there was also evidence of possible intracellular bacteria, which would support a septic peritonitis.

Consultation with IDEXX internal medicine specialist

Signalment and physical examination findings were suggestive of FIP. Laboratory changes were not compelling; i.e., no hyperglobulinemia, normal albumin/globulin ratio (but suspicious at 0.5), no anemia and abdominal fluid analysis findings were not convincing but suggestive of FIP. The FIP Virus RealPCR™ Test on peritoneal fluid was recommended. If positive for FIPV biotype, then FIP was very likely. If positive for FECV biotype, then FIP was unlikely.

FIP Virus RealPCR Test result

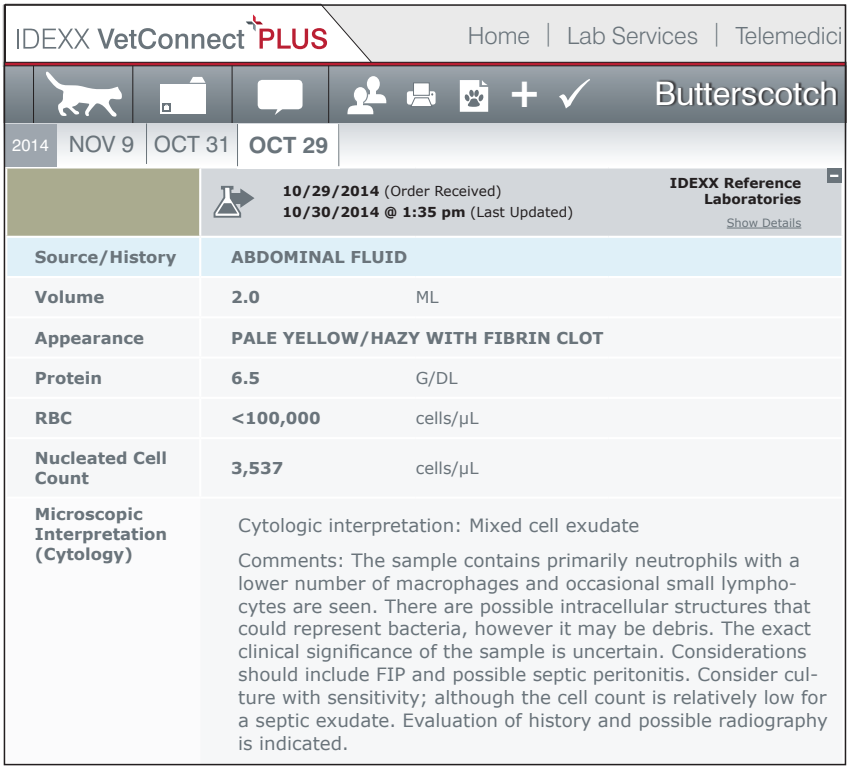
The FIP Virus RealPCR Test was positive for feline coronavirus. The biotyping result indicated that the FCoV had mutated into the FIPV biotype. Because Butterscotch presented with clinical signs, this supported the diagnosis of FIP.

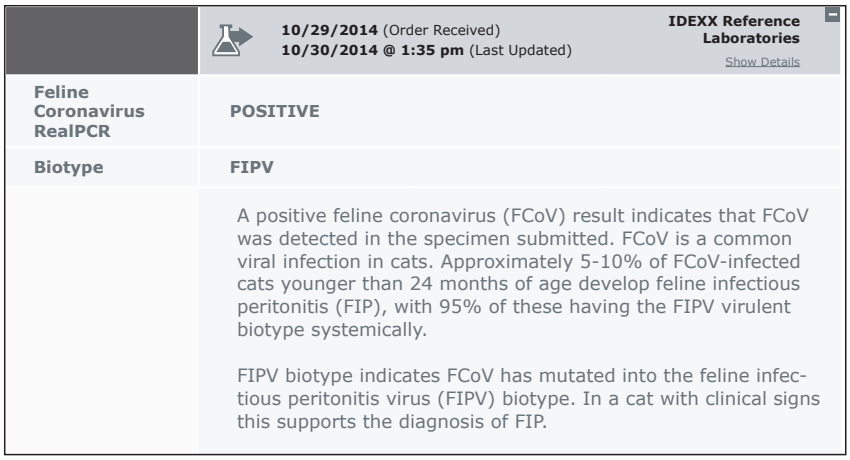
Case outcome

Butterscotch was treated symptomatically with supportive care but deteriorated quickly after diagnosis. He was humanely euthanized two weeks later. A necropsy was performed and specimens from several organs were submitted. The microscopic interpretation was severe generalized 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.





The information contained herein is intended to provide general guidance only. As with any diagnosis or treatment, you should use clinical discretion with each patient based on a complete evaluation of the patient, including history, physical presentation and complete laboratory data. With respect to any drug therapy or monitoring program, you should refer to product inserts for a complete description of dosages, indications, interactions
and cautions.

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