

# *ImmunoComb*

## **FELINE CORONAVIRUS (FCoV) [FIP] ANTIBODY TEST KIT**

**INSTRUCTION MANUAL**  
**Sufficient for 12/120 assays**  
**22 APR 2018**



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## **I. INTENDED USE OF THE KIT**

This kit is designed to determine cat serum IgG antibody titer to Feline Coronavirus (FCoV). Cats with Feline Infectious Peritonitis (FIP) typically have high levels of antibody to FCoV. As such, a negative result is helpful in ruling out a diagnosis of FIP.

## **II. GENERAL INFORMATION**

It is estimated that up to 70% of cats, worldwide, are exposed to Feline Coronaviruses (FCoV). Infection is transmitted by the fecal-oral route; the virus can survive in dried secretions for as long as seven weeks. The risk of exposure is higher in catteries and multiple-cat households. FCoV infection in most cats is not associated with clinically apparent disease. In some cats, however, a severe, typically fatal, disease (known as FIP) may develop.

## **III. WHAT IS THE IMMUNOCOMB ASSAY?**

The ImmunoComb test is a modified ELISA, which can be described as an enzyme labeled “dot assay”, that detects antibody levels in serum, plasma or whole blood.

The kit contains all the necessary reagents for developing the test. Results are obtained within 40 minutes.

## **IV. HOW DOES THE IMMUNOCOMB WORK?**

- The ImmunoComb Kit contains 2 main components: a comb shaped plastic card, hereafter referred to as the Comb and a multi compartment developing plate.
- The Comb has 12 teeth – sufficient for 12 tests. Each

tooth will be developed in a corresponding column of wells in the developing plate. Individual or multiple tests are processed by breaking off the desired number of teeth from the Comb.

- Test spots of FCoV antigen are attached to the lowest spots on each tooth of the Comb. The top spot is the Positive Reference . (see figure in section X).

- The first step of the test is to deposit a serum, plasma or whole blood specimen in a well in row A of the multi-compartment developing plate.

- Next, the Comb is inserted into the well(s) with the sample(s) and transferred to the remaining wells (B-F) at timed intervals, according to the step by step instructions (see section VII). Specific IgG antibodies from the specimen, if present, bind to the antigen at the test spots and will be labeled in row C, which contains an enzyme labeled anti-cat IgG antibody.

- At the end of the developing process, a purple-grey color results are developed in all Positive Reference spots and in any positive sample tested spot.

- The intensity of the color result corresponds directly to the antibody level in the test specimen. Results are scored using the Positive Reference spot and CombScale (see section IX).

## **V. CLINICAL SIGNS**

Infection with FCoV is asymptomatic in the majority of cats. In a small percentage of cases, fever, diarrhea and upper respiratory signs such as conjunctivitis can occur. This stage may last for an undefined time and then progress to a severe systemic disease known as Feline Infectious Peritonitis (FIP). FIP manifests clinically in 2 forms: effusive (wet) and non-effusive (dry). FIP is

generally associated with a fatal outcome, even with therapy. Prognosis is grave.

## **VI. DIAGNOSIS:**

Evaluation of antibody titers to FCoV in cats indicates previous exposure to this agent.

It is unclear why clinical disease (FIP) develops only in a small percentage of infected cat. Many of them have a history of recent stress such as relocation to a new home, surgery (e.g., neutering) or illness. Cats with FIP typically have high antibodies titers to FCoV. As such, serology is considered to be useful for helping diagnose individual clinical cases as well as for prevention and control programs in multiple cat households or facilities.

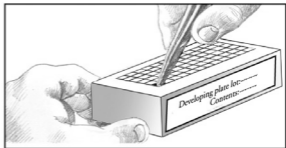
## **VII. STEP BY STEP WITH IMMUNOCOMB**

Before conducting the test, bring the developing plate to room temperature by removing all kit components from the kit carton and place them on the work bench for 60-120 minutes or incubate only the plate at 37°C/98.6°F for 25 minutes.

**Perform assay at room temperature 20° – 25° C / 68° – 77° F.**

**(1)** Obtain blood sample from cat. When testing whole blood, collect sample in EDTA or heparin anticoagulant tube.

**(2)** Mix reagents by gently shaking the developing plate several times prior to use. Use the tweezers to pierce the protective aluminum cover of row A. One well for each sample/specimen.



**Do not open any wells of row A or other rows which you do not intend to use.**

**Do not remove aluminum cover of developing plate all at once.**

**(3) Deposit a sample into a well in row A.**

**For testing serum or plasma use 5 $\mu$ l.**

**For testing whole blood use 10 $\mu$ l\*.**

Raise and lower pipette plunger several times to achieve mixing. (See Pipetting Technique section). Avoid spillage and cross-contamination of solutions.

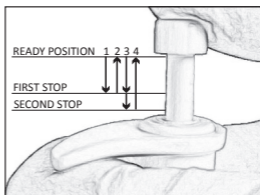
**\*For whole blood only: If dispensing the sample with a fix pipette provided with kits catalogue number 50FFP301 , use the same tip to deposit twice 5 $\mu$ l into the same well in row A.**

## Pipetting Technique

### Forward Pipetting

1- Press the operating button to the first stop.

2- Dip the tip attached to the pipette into the sample to a depth of about 1 cm and slowly release the operating button. Wait for a while, then withdraw it

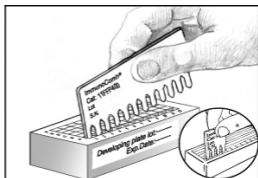


from the liquid touching it against the edge of the reservoir to remove excess liquid adhering to the outer surface of the tip.

3- Dispense the sample into a well in row A by gently pressing the operating button to the first stop. After a second, press the operating button to the second stop. This will empty the tip completely. Remove the pipette from the well.

4- Release the operating button to the ready position.

(4) Remove the Comb from its protective envelope. Do not touch the teeth of ImmunoComb card. For testing less than 12 samples, cut or break the Comb by folding in allocated notches for the number of tests required.



**Note:** Mixing during incubation according to instructions is critical for valid results.

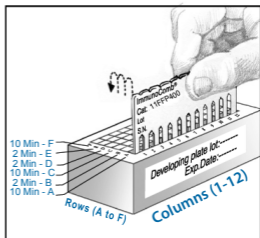
**\*\*To improve mixing, move the Comb up and down 3-4 times. During incubation, repeat the same mixing process 2-3 times.**

Avoid scratching the front active side of the Comb by leaning it to the back while mixing.

Gently shake off excess liquid from Comb teeth onto a tissue before moving it to the next row.

■ Insert the Comb into the open well(s) in row A (printed side facing you) and incubate for 10 minutes. Mix as described above.\*\*

■ Use tweezers to pierce the foil of the next well(s) in row B. Shake off excess liquid and insert Comb for 2 minutes. Mix as described above.\*\*

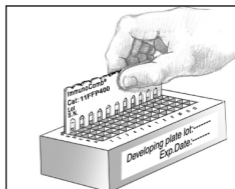


■ Pierce the foil of the next well(s) in row C. Shake off excess liquid and insert Comb for 10 minutes. Mix as described above.\*\*

■ Pierce the foil of the next well(s) in row D. Shake off excess liquid and insert the Comb for 2 minutes. Mix as described above.\*\*

■ Pierce the foil of the next well(s) in row E. Shake off excess liquid and insert the Comb for 2 minutes. Mix as described above.\*\*

■ Pierce the foil of the next well(s) in row F. Shake off excess liquid and insert the Comb for 10 minutes. Mix as described above.\*\*



■ Upon completion of the color development in row F, move the Comb back to row E for 2 minutes for color fixation. Take the Comb out and let it dry for 5 minutes before reading the results.

## VIII. READING AND INTERPRETING THE IgG ANTIBODY RESULTS

■ The top spot, the Positive Reference spot, should give a distinct purple-grey color. This is the same color tone that is generated by a significant positive response (IFA titer  $\geq 1:20$ ). This spot should be read as S3 on the CombScale (a scale of S0 to S6).

■ The bottom spot on the Comb tests for FCoV antibodies.

■ Compare the color tone of the FCoV spot (bottom one) with the Positive Reference spot. A clear, visible purple-grey dot indicates a positive response to FCoV. A result darker than the Positive Reference

means high titer. Color fainter than the Positive Reference indicates a low response to FCoV.

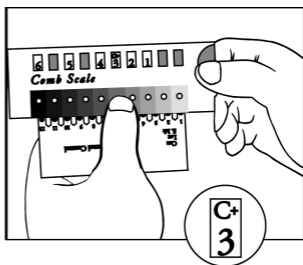
- Cats with FIP usually have high antibody levels.
- A negative result (S0-S1) indicates that the cat has not been exposed or has cleared the virus, and is free of FCoV.
- To evaluate the titer, use the CombScale provided in the kit (see section IX).

## IX. READING RESULTS WITH THE COMBSCALE

The CombScale S value is the number that appears in the yellow window corresponding to the color tone, when Positive Reference color is calibrated to S3.

When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit.

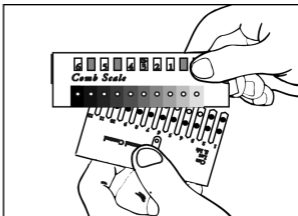
Find the tone of purple-grey on the CombScale that most closely matches the **Positive Reference spot** (upper spot). Slide the yellow ruler until the C+ mark appears in the window above that color you just found.



**Hold the ruler in this position during the entire reading.** This step actually calibrates the C+ to S3, which is the “cut-off” point to which test spots will be compared.

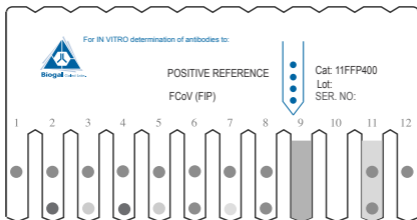


**While holding the ruler, find the tone of purple-grey on the CombScale that most closely matches the desired **test result spot** (one of the lower spots). The number that appears in the window above is the CombScale score (S0-S6). Repeat this step with every test spot separately.**



Another way to read the results is by using the CombScan. This is a software program that utilizes a computer and a TWAIN compatible scanner. When a Comb is placed on the scanner, the program translates the color results into numerical values. The CombScan assists labs in reading ImmunoComb results and conserving the data, and is supplied free of charge upon request.

## **X. EXAMPLE OF A DEVELOPED COMB**



Tooth N°		
1	S0	Negative result - No reaction to FCoV/FIP.
2	≥S5	High positive reaction - Greater likelihood with FIP.
3	S2	Low positive reaction - FIP unlikely.
4	≥S5	High positive reaction - Greater likelihood with FIP.
5	S2	Low positive reaction - FIP unlikely.
6	≥S3	Positive reaction - FIP possible.
7	≤S1	Non specific reaction - Considered negative.
8	≥S3	Positive reaction - FIP possible.
9*		Invalid
10**		Invalid
11***	≤S3	Medium positive reaction - FIP possible.
12	S0	Negative result - No reaction to FCoV/FIP

### Remarks:

- \* High background. Repeat test.
- \*\* No Positive Reference. Repeat test.
- \*\*\* High background with positive results.

## **XI. STORAGE & HANDLING**

1. Store the kit under normal refrigeration (2° – 8° C / 36° – 46° F). **Do not freeze the kit.**
2. **Do not mix reagents from different kits or from different compartments of the same kit.**

3. The ImmunoComb kit contains inactivated biological material. The kit must be handled and disposed of in accordance with accepted sanitary requirements.

## XII. SAMPLE HANDLING AND STORAGE

- Fresh samples are recommended for use.
- Store whole blood at 2-8°C if the test is to be run within 1 day of collection. Do not freeze whole blood samples.
- Store serum and plasma samples at 2-8°C if the test is to be run within 3 days of collection. If test is delayed more than 3 days, freeze samples to -20°C or colder.
- Bring samples to room temperature and mix well before testing.

## XIII. KIT CONTENTS

Components	12 Test Kit (50FFP301)	12 Test Kit (50FFP401)	120 Test Kit (50FFP210)
A. ImmunoComb card (wrapped in aluminum foil)	1	1	10
B. Developing plate	1	1	10
C. Disposable tweezers	1	1	1
D. Calibrated CombScale	1	1	1
E. Junior fix pipette 5µl	1	-	-
F. 10 µl universal grad tip	15	-	-
Instruction manual	1	1	1



A



B



C



D



E



F

## **XIV. REFERENCES**

- Addie, D. D. (1998). The diagnosis and prevention of FIP and recent research into feline Coronavirus shedding. *ESVIM Proceedings: 8th Annual Congress of the European Society of Veterinary Internal Medicine.*
- Addie, D. D. (2000). Guest editorial: Clustering of feline Coronaviruses in multicat households. *The Veterinary Journal*, 159, 8-9.
- Addie, D. D., et al. (2002). Evaluation of the feline Coronavirus antibody ImmunoComb. 2nd International FCoV/FIP Symposium, Glasgow, UK.
- Kiss, I., et al. (2000). Prevalence and genetic pattern of feline Coronavirus in urban cat populations. *The Veterinary Journal*, 159, 64-70.
- Addie D. D. et al. (2004) Evaluation of an in-practice test for feline coronavirus antibodies. *Journal of Feline Medicine and Surgery*, 6, 63-67.

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