

## XII. LIMITATIONS OF THE KIT

As detectable levels of serum antibodies require 2-3 weeks to develop, a false negative result is most likely to occur when cats are tested shortly after the onset of infection. Any result >S1 but <S3 is considered non-specific. A false positive result occurs when serum antibodies bind non-specifically. This may be caused by a poor quality specimen (i.e. one that is collected and stored improperly) or by antibodies that react with antigen constituents other than those of the virus. False positive results are suspected when positive reactions are unexpected or cannot be reproduced. It is strongly recommended to customers to retest, by an alternative method, samples that give non-specific, invalid reactions or unexpected positive results.

## XIII. STORAGE & HANDLING

1. Store the kit under normal refrigeration: 2° - 8°C (36° - 46°F). **Do not freeze the kit.**
2. Before conducting the test, maintain all kit elements and specimens at room temperature - preferably for 60-120 minutes (or 22 minutes at 37°C or 98.6°F). Perform assay at room temperature of 20° - 25°C (68° - 77°F).
3. Avoid spillage and cross-contamination of solutions.
4. Mix reagents by inverting developing plate several times prior to use.
5. **Do not mix reagents from different kits or from different compartments of the same kit.**
6. **Do not touch teeth of ImmunoComb® card.**
7. When using developing plate, pierce the cover of each compartment according to the test procedure instructions. **Do not remove cover of entire developing plate all at once.**
8. The ImmunoComb® Kit contains inactivated biological material. The kit must be handled and disposed of in accordance with accepted sanitary requirements. It is recommended to incinerate the kit after use.

## 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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**ImmunoComb®**

A solid phase immunoassay for:

# FELINE INFECTIOUS PERITONITIS VIRUS ANTIBODY TEST KIT

For IN VITRO use only

## INSTRUCTION MANUAL

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Distributed by: Modern Veterinary Therapeutics, LLC  
Coral Gables, Florida 33146 - USA

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## I. INTENDED USE OF THE KIT

This kit is designed to aid in the diagnosis of Feline Infectious Peritonitis (FIP). 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 Enteric Corona Viruses (FECV). 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. FECV 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. CLINICAL SIGNS

In Most cats, Infection with FECV is asymptomatic. 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.

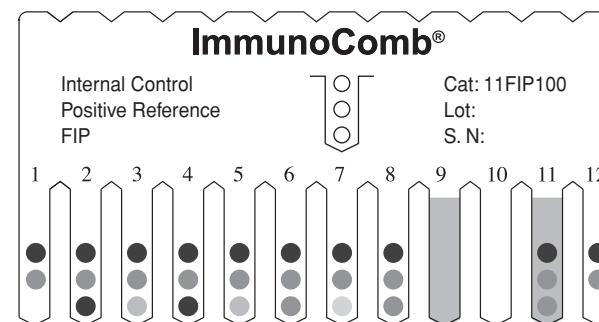
## IV. DIAGNOSIS

FIP Antibodies indicate previous exposure to FECV. It is unclear why clinical disease (FIP) develops only in a small percentage of infected cats. Many of them have a history of recent stress, such as relocation to a new home, surgery (e.g., neutering) or another illness. Cats with FIP typically have FIP antibodies. 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. Yet a definitive clinical diagnosis should not be based entirely on the serological results.

## V. WHAT IS THE IMMUNOCOMB® ASSAY?

The ImmunoComb® test is a modified ELISA, which has been described as a "dot"-ELISA that detects antibody levels in serum or whole blood. The kit contains all necessary reagents for developing the test and is a self-contained portable kit. Results for the FIP tests are obtained in less than 38 minutes.

## XI. EXAMPLE OF A DEVELOPED COMB



TOOTH No.	RESULT	REMARKS (in cats with clinical signs)
1, 12	S0	Negative result - No reaction to FIP.
7	≤S1	A non specific reaction, considered negative.
3, 5	S2	Low non-specific reaction; FIP unlikely.
6, 8	≥S3	Positive reaction; FIP possible.
9	Invalid	High background color - Invalid test.
11	≥S3	High background with Positive reaction; FIP possible.
2, 4	≥S5	Positive reaction, FIP possible.
10	Invalid	No internal control and no positive reference - Invalid test.

Another way to read the results is by using the CombScan 2007. 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 2007 assists labs in reading ImmunoComb® results and conserving the data, and is supplied free of charge upon request.

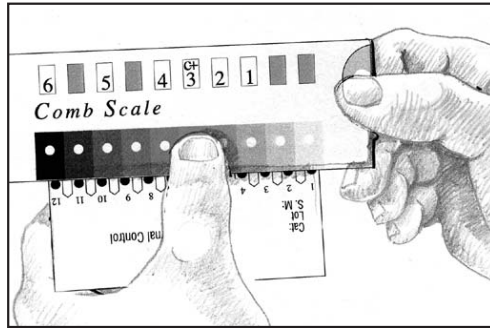
**As with all diagnostic tests, a definitive clinical diagnosis should not be based entirely on the serological results, but should only be made by the veterinarian, after all clinical and laboratory findings have been evaluated.**

## IX. READING AND INTERPRETING THE FIP ANTIBODY RESULTS

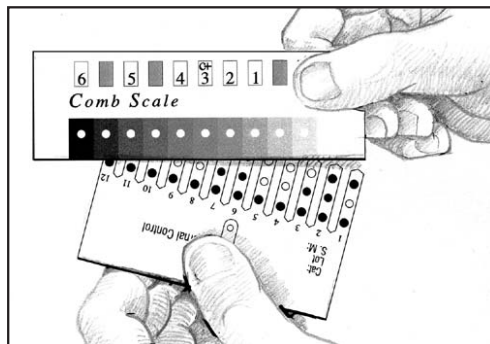
- The upper spot is the Internal Control - it should give a dark purple-grey color.
- The middle spot, the Positive Reference spot, should give a distinct purple-grey color. This is the same color tone that is generated by a significant positive response. This spot should be read as S3 on the CombScale (a scale of S0 to S6).
- The bottom spot on the Comb tests for FIP antibodies.
- Compare the color tone of the FIP spot (bottom one) with the Positive Reference spot (middle one). A clear, visible purple-grey dot indicates a positive response to FIP, so does any result darker than the Positive Reference. Color fainter than the Positive Reference indicates a low response to FIP.
- To evaluate the score, use the CombScale provided in the kit (see section X).
- Cats with FIP usually have high antibody levels.
- A negative result (less than S1) indicates that the cat has not been exposed or had cleared the virus, and is free of FIP.

## X. READING RESULTS WITH THE COMBSCALE

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** (middle spot). Slide the yellow ruler until the C+ mark appears in the window above that color you just found. **Hold the slide 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 slide,** find the tone of purple grey on the CombScale that most closely matches the **test result spot** (bottom spot). The number that appears in the window above is the CombScale score (S0-S6). Repeat this step with every test spot separately.



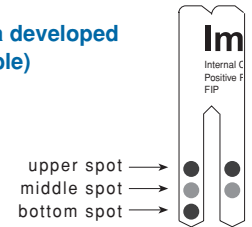
## VI. HOW DOES THE IMMUNOCOMB® WORK?

- 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 FIP antigen are attached to the lowest spot on each tooth of the Comb. The middle spot is the Positive Reference and the upper most spot is the Internal Control (See Fig. 1).
- FIP IgG antibodies if present in serum, plasma or blood specimen, used in row A of the multi-compartment developing plate, react with the FIP antigen on Comb test spots.
- At the end of the developing process, described in section VIII, if positive, a purple grey color result develops via an enzymatic reaction.
- On each tooth of the developed Comb you should see the Internal Control spot (upper spot) and the Positive Reference spot (middle spot). The test spot of FIP (bottom spot) may appear, depending on the result.
- Results are scored using the Positive Reference spot and the CombScale (scale S0-S6; see section X).

## VII. KIT CONTENTS

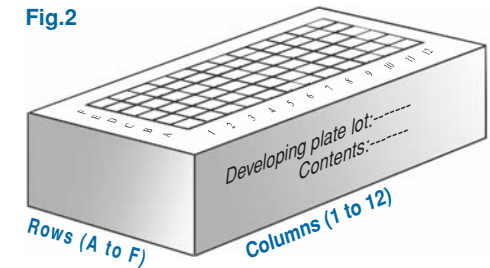
1. **The ImmunoComb® Card** - One Comb sufficient for 12 tests.

Fig 1: Two teeth of a developed Comb (example)



2. **Developing Plate** - One plate, divided into compartments A-F, that are subdivided into 12 wells; The plate compartments are pre-filled with the reagent solutions.

Fig.2

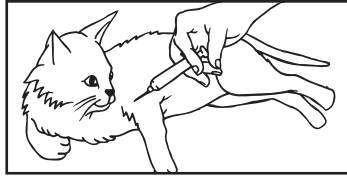


3. **Disposables Tweezers** - For piercing the foil cover of developing plate compartments.
4. **CombScale** - One calibrated CombScale color card for scoring reactions intensities.
5. **Heparinized Capillary Tubes** - A vial with thirteen capillary tubes and a wire piston, intended for transferring whole blood or serum specimen into compartment A of the developing plate. The tubes are calibrated for dispensing 5 microliters serum (lower band), 10 microliters blood (upper band).
6. **Documentation** - Instruction manual with detailed instructions.

## VIII. STEP BY STEP WITH THE IMMUNOCOMB

Perform assay at room temperature of 20°- 25°C (68°- 77°F).

(1) Obtain blood sample from cat.

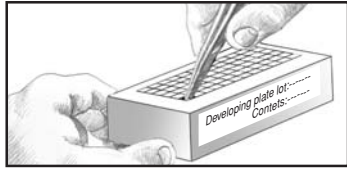


(2) Use a pipette or a capillary tube.

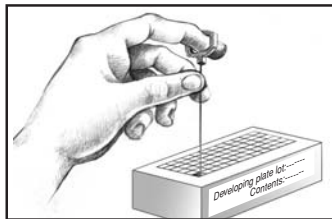
For testing whole blood use 10µl.  
For testing serum/plasma use 5µl.



(3) Use the tweezers to pierce the protective aluminum cover of well in row A\*. One well for each sample/specimen.



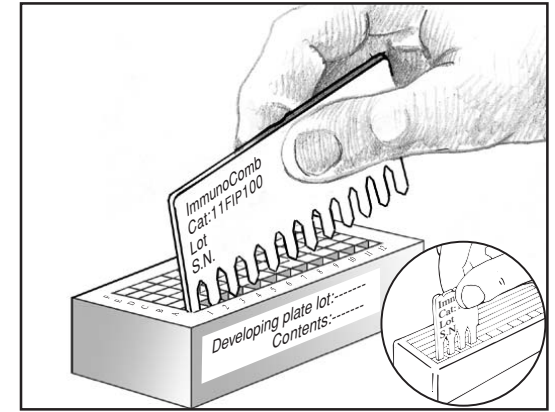
(4) Deposit a sample into well in row A\*. Raise and lower the piston /pipette plunger several times to achieve mixing.



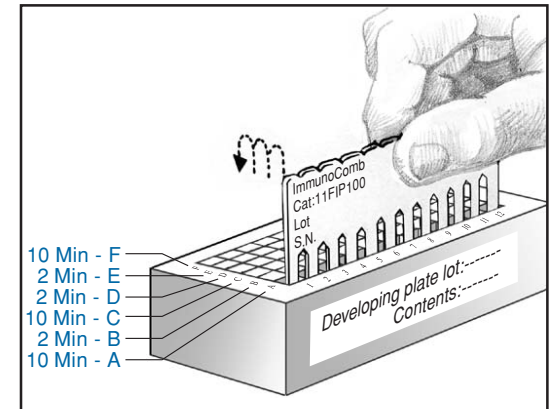
Do not open any wells of row A or other rows which you do not intend to use.

\* See Fig 2, page 3.

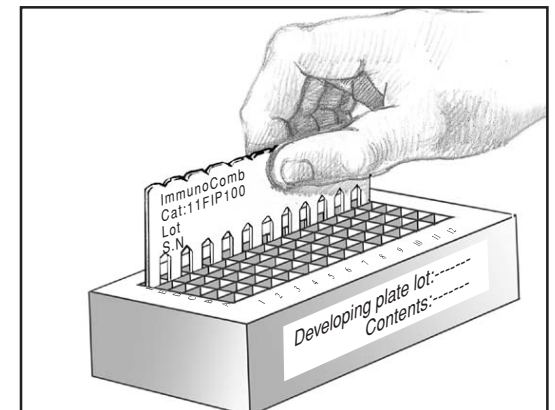
(5) Remove the Comb from its protective envelope. For testing less than 12 samples, cut or fold Comb in allocated notches for the number of tests required. Insert the Comb into the open well(s) in **row A\*** (printed side facing you) and incubate for **10 minutes**. To improve mixing, gently jiggle the Comb up and down at the start of each incubation. Repeat this motion every 2-3 minutes in all rows for achieving best results.



(6) Use tweezers to pierce the foil of the next well (**row B\***), and insert Comb for **2 minutes**. Before transferring the Comb from one well to the next, pierce the foil of the next well. Gently shake off excess liquid from the Comb teeth onto a tissue and insert Comb into the next well (**row C\***) for **10 minutes**. Then, place Comb in the remaining wells (**rows D\* & E\***) for **2 minutes** and the last well (**row F\***) for **10 minutes**.



(7) 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 **1-10 minutes**.



\* See Fig. 2, page 3.