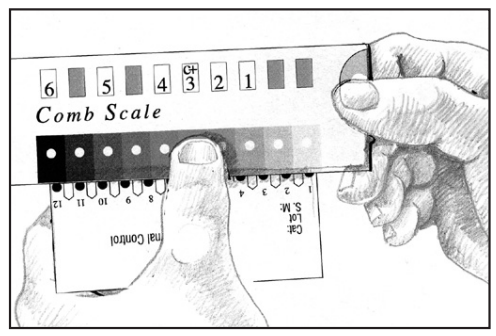
IX. READING AND INTERPRETING THE FCoV ANTIBODY RESULTS

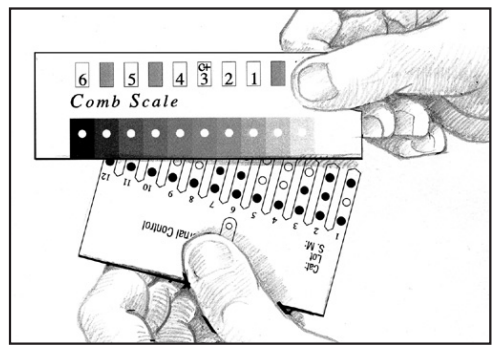
- The upper spot is the internal control - it should give a dark purple grey color.
- The middle spot, the positive reference spot, should give a distinct purple grey color. This is the same color tone that is generated by a significant positive IgG response. This spot should be read as S3 on the CombScale (a scale of S0 to S6).
- The bottom spot on the Comb tests for FCoV antibodies.
- Compare the color tone of the FCoV spot (bottom one) with the positive reference spot (middle one). 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.
- To evaluate the titer, 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 FCoV.

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.



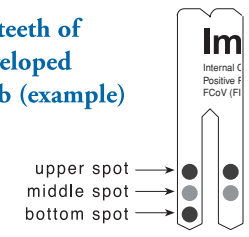
While holding the slide, find the tone of purple grey on the CombScale that most closely matches the **test result spot** (bottom spot). The number that appers in the window above is the CombScale score (S0-S6). Repeat this step with every test spot separately.



- On each tooth of the Comb you should see the internal control spot (upper spot) and the positive reference spot (middle spot). The test spot of FCoV (bottom spot) may appear, depending on the result.

- 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 the CombScale (scale S0-S6; see section x).

Two teeth of a developed Comb (example)



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 manifests clinically in 2 forms: effusive (wet) and non-effusive (dry). FIP is generally associated with a fatal outcome, even with therapy.

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 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 high antibody 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. 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. Use large amounts of water to flush the kit developing solutions down the sewage/drainage system. The kit must be handled and disposed of in accordance with accepted sanitary requirements. It is recommended to incinerate the kit after use.

XII. KIT CONTENTS

Component	Standard Kit (12 Tests) 50FFP201	Lab Kit (120 Tests) 50FFP210
A. ImmunoComb® card (wrapped in an aluminum envelope)	1	10
B. Developing plate*	1	10
C. Disposable tweezers	1	1
D. Calibrated CombScale color card	1	1
E. Unit of 12 capillary tubes & one piston	1	–
Instruction manual	1	1

** The developing plate is illustrated below.*

Fig.1

XIII. 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.

ImmunoComb®

FELINE CORONA VIRUS (FCoV) [FIP] ANTIBODY TEST KIT

INSTRUCTION MANUAL

Product Cat. No: 50FFP201 & 50FFP210

13.2.2008

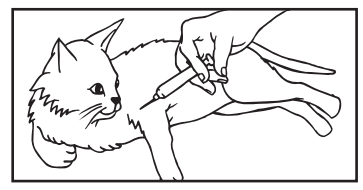
Biogal Galed Laboratories, tel: 972-4-9898605, fax: 972-4-9898690
e-mail: info@biogal.co.il, site:www.biogal.co.il

Instruction Cat. No: 63FFP411

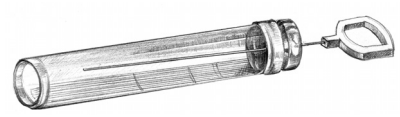
VIII. STEP BY STEP WITH THE IMMUNOCOMB®

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

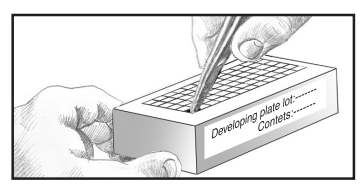
(1) Obtain blood sample from cat.



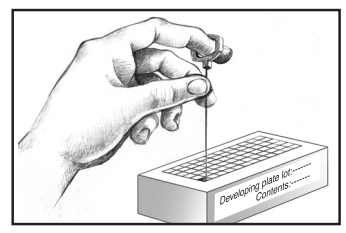
(2) Use a pipette or a capillary tube.
For testing whole blood use 10µl.
For testing serum/plasma use 5µl.



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



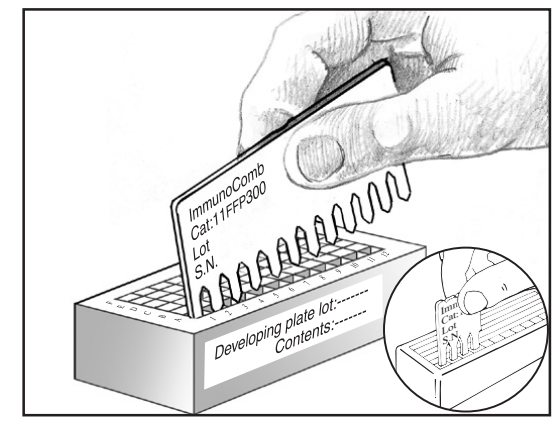
(4) Deposit a sample into well in row A*. Raise and lower the piston /pipette plunger several times to achieve mixing.



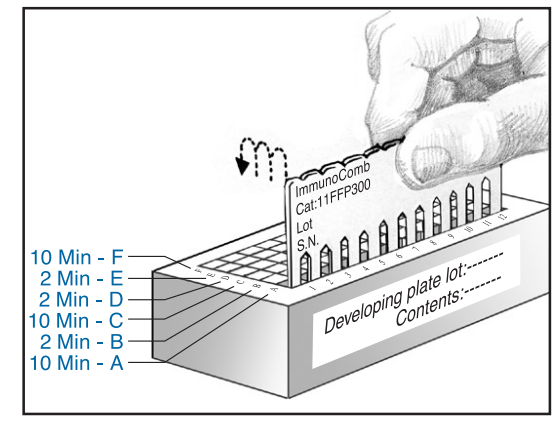
Do not open any wells of row A or other rows which you do not intend to use.

* See Fig 1, page 8.

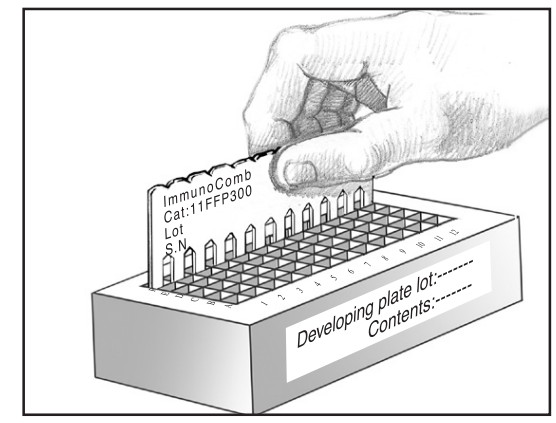
(5) Remove the Comb from its protective envelope. For testing less than 12 samples, cut or fold Comb in allocated notches for the number of tests required. Insert the Comb into the open well(s) in **row A***(printed side facing you) and incubate for **10 minutes.** To improve mixing, gently jiggle the 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 tooth 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 out the Comb and let it dry.



* See Fig 1, page 8.

I. INTENDED USE OF THE KIT

This kit is designed to determine cat serum IgG antibody titer for Feline Corona Virus (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 has been estimated that up to 70% of cats, worldwide, are exposed to Feline Corona Viruses (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?

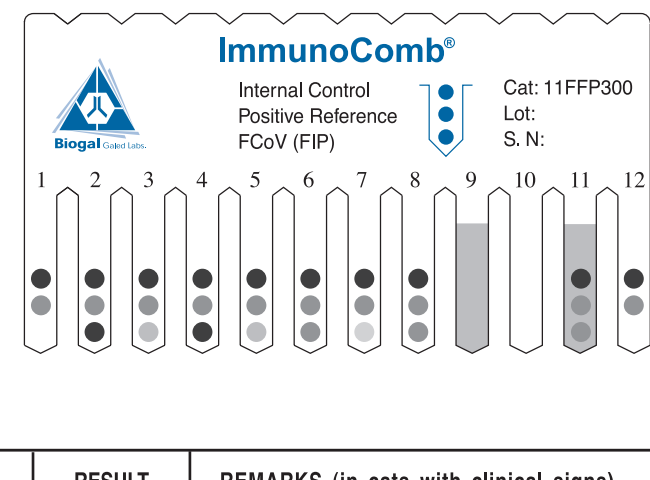
The ImmunoComb® test is a modified ELISA, which has been described as a "dot"-ELISA that detects antibody levels in serum or whole blood. The kit contains all necessary reagents for developing the test and is a self-contained portable kit. Results for the FCoV tests are obtained in less than 40 minutes.

Biogal's ImmunoComb®

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 spot on each tooth of the Comb. The middle spot is the positive reference and the upper most spot is the internal control.
- The first step of the test is to deposit serum, plasma or 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 instructions that follow. Specific IgG antibodies from the specimen, if present, bind to the antigen at the test spots.
- The Comb is transferred to the next well (row B) where non-bound antibodies are washed off.
- The Comb is inserted into the following well (row C), which contains an enzyme labeled anti-cat IgG antibody, which will bind to the antigen-antibody complexes at the test spots.
- After 2 more washes (rows D & E) the Comb is moved to the next well (row F), where a color result develops via an enzymatic reaction.

XI. EXAMPLE OF A DEVELOPED COMB



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

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.

For further assistance please contact your local distributor, or Biogal Galed Labs. directly by e-mail: info@biogal.co.il or by fax: 972-4-9898690.