XI. 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 specimen at room temperature – preferably for 60 – 120 minutes (or incubate only the developing plate for 22 minutes at 37° C / 98.6° F). Perform assay at room temperature 20° – 25° C / 68° – 77° F.
3. Avoid spillage and cross-contamination of solutions.
4. Mix reagents by inverting the 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 the teeth of the ImmunoComb® card.
7. When using developing plate, pierce the cover of each compartment according to the test procedure instructions. Do not remove the aluminum cover of 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.

XII. KIT CONTENTS

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<th>Components</th>
<th>12 Test Kit (50FFP201)</th>
<th>120 Test Kit (50FFP210)</th>
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<tr>
<td>A. ImmunoComb® card (wrapped in aluminum foil)</td>
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<td>B. Developing plate</td>
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<td>C. Disposable tweezers</td>
<td>1</td>
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<td>D. Calibrated CombScale</td>
<td>1</td>
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<td>E. Unit of 12 capillary tubes &amp; one piston</td>
<td>1</td>
<td>May be purchased upon request.</td>
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<td>Instruction manual</td>
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XIII. REFERENCES
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 Coronavirus (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 38 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 spot on each tooth of the Comb. The middle spot is the Positive Reference and the upper spot is the Internal Control (see Fig. 1).
■ The first step of the test is to deposit serum, plasma or whole blood specimen into a well in row A of the multi-compartment developing plate.
■ 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

X. EXAMPLE OF A DEVELOPED COMB

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 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 Laboratories directly by E-mail: info@biogal.co.il or by tel: 972-4-9898605 / fax: 972-4-9898690.
VIII. 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 response (IFA titer ≥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 (middle one). A clear, visible purple-grey dot indicates a positive response to FCoV. A result darker than the Positive Reference indicates a low response to FCoV.
- 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.
- 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 (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.

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.

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®

Perform assay at room temperature 20º-25º C (68º - 77ºF).

(1) Obtain blood samples from cat.

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

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

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

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

(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 at least twice in all of the remaining rows.

(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 (row D & E) for 2 minutes and the last well (row F) for 10 minutes.

(7) Upon completion of 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.