

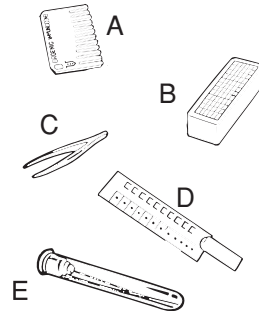
XI. STORAGE & HANDLING

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 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 one 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.

XII. KIT CONTENTS

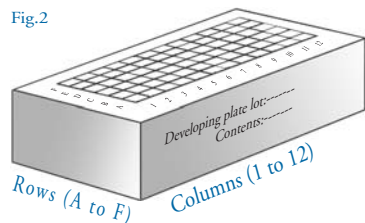
Component	12 Test Kit Cat.50FVV201	120 Test Kit Cat.50FVV210
A. ImmunoComb® card (wrapped in an aluminum envelope)	1	10
B. Developing plate *	1	10
C. Disposable tweezers	1	1
D. Calibrated CombScale color card	1	1
E. Unit of 12 capillary tubes & one piston	1	—
Instruction manual	1	1



* The developing plate is illustrated below.

XIII. REFERENCES

Fig.2



- AAHA Vaccine Task Force. (2006). *JAAHA*, **42**, 80-89.
- Lappin et al. (2002). *J Am Vet Med Assoc*, **220(1)**, 38-42 .
- Mouzin et al. (2004). *J Am Vet Med Assoc*. Jan 1, **224(1)**, 61-66.
- Waner et al. (2006). *J Vet Diag. Invest.*, **18. (3)**, 267-270.

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.

Biogal's
ImmunoComb®

ImmunoComb®

Feline VacciCheck

PANLEUKOPENIA, HERPES VIRUS
& CALICI VIRUS IgG
ANTIBODY TEST KIT

INSTRUCTION MANUAL
SUFFICIENT FOR 12/120 ASSAYS

17.7.2007



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I. INTENDED USE OF THE KIT

This kit is designed to determine cat serum IgG antibody titer for Feline Panleukopenia Virus (FPLV), Feline Herpes Virus (FHV) and Feline Calici Virus (FCV). The main purpose of this kit is to provide a useful tool in assessing immunity status of cats concerning these three pathogens. As such, it can determine the IgG titer of a cat before and after vaccination, as well as the duration of immunity.

II. GENERAL INFORMATION

Feline Panleukopenia Virus (FPLV), Feline Herpes Virus (FHV) and Feline Calici Virus (FCV), are recognized as important causes of illness and death in cats. Kittens are more susceptible to FPLV, FHV and FCV, especially after weaning when protective Maternally Derived Antibody (MDA) levels decrease. Sometimes MDA may actually interfere with vaccinations that are given for immunization.

In many countries, vaccination programs have significantly curtailed, but not eliminated the incidence of these diseases. Thus, FPLV, FHV and FCV continue to be of a great clinical concern among veterinarians worldwide and still present a diagnostic challenge.

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 all Feline Vaccination tests are obtained in less than 20 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 FPLV, FHV and FCV antigens are attached to each tooth of the Comb. The upper most spot is the positive reference spot. The upper antigen spot tests for FPLV, the middle spot tests for FHV and the bottom spot tests for FCV (see fig.1).

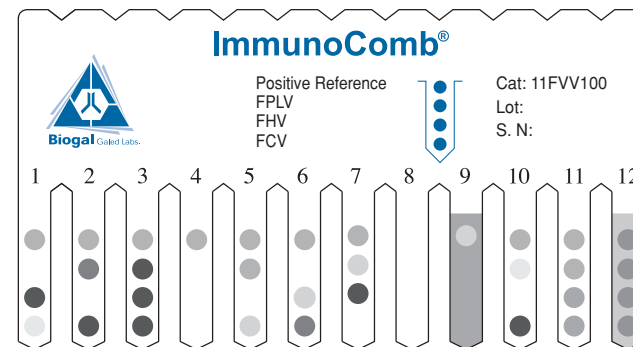
■ The first step of the test is to deposit serum, plasma or blood specimen in a well in row A of the multi-compartment developing plate.

■ 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 step by step instructions (see p.4). Specific IgG antibodies from the specimen, if present, bind to the antigens 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), that contains an enzyme labeled anti-cat IgG antibody, which will bind to the antigen-antibody complexes at the test spots.

X. EXAMPLE OF A DEVELOPED COMB



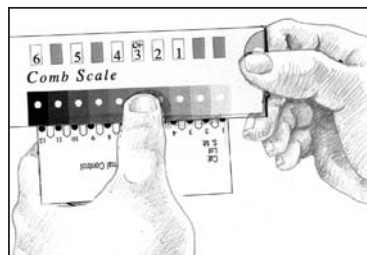
Tooth No.	Results of FPLV	Results of FHV	Results of FCV	Remarks
1	S-0 Negative	≥ S5 High Positive	0 < S1 Negative	
2	S4 Positive	S-0 Negative	≥ S5 High Positive	
3	≥ S5 High Positive	≥ S5 High Positive	≥ S5 High Positive	
4	S-0 Negative	S-0 Negative	S-0 Negative	
5	≥ S3 Positive	S-0 Negative	S 1-2 Inadequate immunity	
6	S-0 Negative	S 1-2 Inadequate immunity	S4 Positive	
7	S 1-2 Inadequate immunity	≥ S5 High Positive	S-0 Negative	
8	Invalid	Invalid	Invalid	No Positive Reference. Repeat test.
9	Invalid	Invalid	Invalid	High Background, Repeat test.
10	0 < S1 Negative	S-0 Negative	≥ S5 High Positive	
11	≥ S3 Positive	≥ S3 Positive	≥ S3 Positive	
12	≥ S3 Positive	≥ S3 Positive	≥ S3 Positive	High Background with positive results.

VIII. READING AND INTERPRETING THE IgG ANTIBODY RESULTS

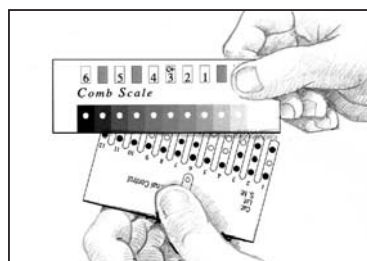
- The upper most positive reference spot has been calibrated to give a cut-off value to each of the 3 antigens, and should give a distinct grey color, that should be read as S3 on the CombScale (a scale of S0 to S6; see section IX).
- The cut-off values for FPLV, FHV and FCV revaccination purposes are HI 1:40, VN 1:16 and VN 1:32, respectively.
- Match and compare the color tone of each test result spot with the color tone of the positive reference spot.
- If the color tone of either of the test spots is equal to or darker than the color tone of the positive reference spot, the cat may be considered to have a protective antibody titer to the specific viral antigen.
- If the color tone of either of the test spots is lighter than the color tone of the positive reference spot, or no color appears in a test result spot, the titer of antibodies to that virus is below the accepted protective level. Revaccination should be considered.
- In order to evaluate the titer use the CombScale provided in the kit.

IX. 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 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 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 grey on the CombScale that most closely matches each **test result spot**. The number that appears in the window above is the CombScale score (S0-S6). Repeat this step with every test spot separately.



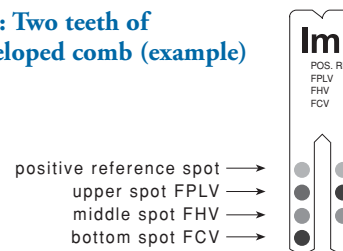
Another way to read the results is by using the CombScan 2007. This is a software program that utilizes a computer and a twain compatible scanner. When a comb is placed on the scanner, the program translates the color results into numerical values. The CombScan 2007 assists labs in reading ImmunoComb® results and conserving the data, and is supplied free of charge upon request.

■ 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.

■ On each tooth of the Comb you should see the positive reference spot (upper most spot). The test spot of any of the tested viruses antigens 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 IX).

Fig. 1: Two teeth of a developed comb (example)



V. DESCRIPTIONS OF DISEASES

FPLV, FHV and FCV cause upper respiratory diseases (nose throat and sinus tracheas).

Feline Panleukopenia (FPLV also known as Distemper or Feline infectious enteritis) is a highly contagious viral disease that can kill both kittens and adult unvaccinated cats. Symptoms include sudden onset of fever, lack of appetite, dehydration, depression, vomiting and dizziness. Infected cats may show a decreased number of white blood cells.

Feline Herpes virus is caused by FHV type 1, also known as Feline Viral Rhinotracheitis. Symptoms include sneezing, coughing, photosensitivity, conjunctival swelling, ocular and nasal discharge. Also seen is: fever, depression, and lack of appetite. Corneal ulcers may develop which can lead to severe infections and even blindness

Feline Calici virus is a respiratory disease similar to a human cold. It is caused by an RNA virus and is more resistant than FHV although its symptoms may appear less severe. Symptoms are similar to FHV but often include ulcers of the tongue. Pneumonia may develop, leading to high mortality rates in kittens.

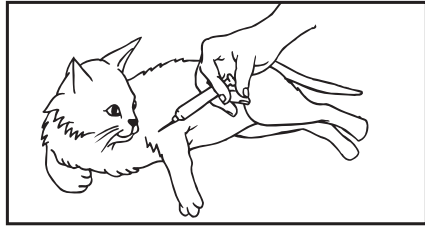
VI. DIAGNOSIS

Veterinarians typically make a presumptive diagnosis of FPLV, FHV and FCV infection based on clinical signs. Some of the signs are common to the two or three diseases. Laboratory tests can be helpful for confirming the diagnosis. In addition to hematology and blood chemistry, serology is becoming a more widely accepted diagnostic tool. Serology, by measuring the amount of specific IgG antibodies circulating in the blood, provides the mean to monitor a cat's immunity status following infection and or vaccination, Proper vaccination of kittens and cats will allow them to be protected against sever feline infectious diseases. Yet, since vaccination does not always confer proper immunity, and over vaccination is not recommended, it is advisable to monitor the serological status of the cat in order to only vaccinate when necessary.

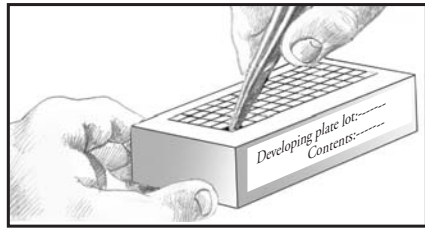
VII. STEP BY STEP WITH THE IMMUNOCOMB®

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

(1) Obtain blood sample from cat.



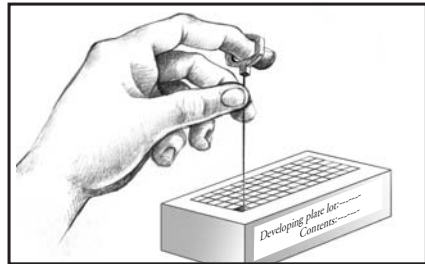
(2) Use the tweezers to pierce the protective aluminum cover of row A*. One well for each specimen.



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



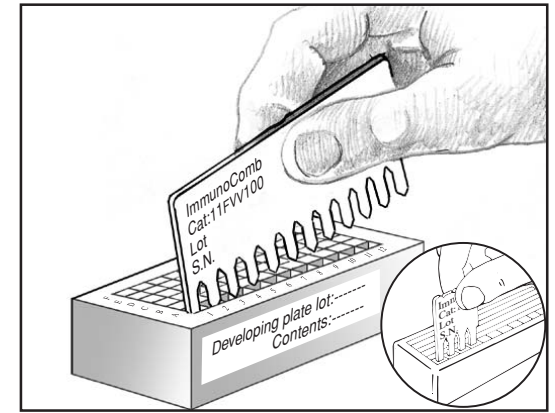
(4) Deposit a sample into well A*. Raise and lower the piston /pipette plunger several times to achieve mixing.



* See Fig. 2 page 8.

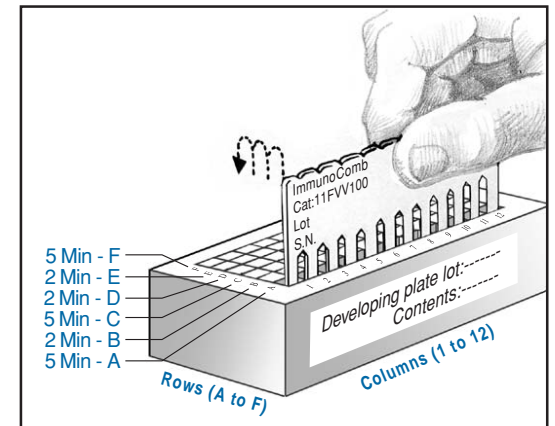
Do not open any wells of row A or other rows which you do not intend to use.

(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 **5 minutes**. To improve mixing, gently dip 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 **5 minutes**.

Then, place Comb in the remaining wells (**rows D & E***) for **2 minutes** and the last well (**row F***) for **5 minutes**.



(7) Upon completion of the color development in **row F**, move the Comb back to **row E** for **2 minutes** to fix color. Take the Comb out and let it dry.

