VACCICheck®

FELINE PANLEUKOPENIA, HERPES VIRUS & CALICI VIRUS IgG ANTIBODY TEST KIT

INSTRUCTION MANUAL

Sufficient for 12/120 assays

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Instruction Cat. No: 63FVV711

I. INTENDED USE OF THE KIT

This kit is designed to determine cat serum IgG antibody titer to 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 for assessing immunity status of cats concerning these three pathogens. As such, it can either determine the IgG titer before and following vaccination or the validation 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 most 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 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 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 for the IgG FPLV, FHV and FCV tests are obtained within 23 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 are attached to each tooth on the Comb. The upper most spot is a Positive Reference. Purified FPLV antigen is attached to the upper middle spot, purified FHV antigen is attached at the lower middle spot and purified FCV antigen is attached at the lowest of the 4 spots(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. DESCRIPTION OF DISEASE

Feline Panleukopenia (FPLV also known as Distemper or Feline infectious enteritis) is a highly contagious viral disease that can kill both kittens and unvaccinated adult cats. Symptoms include sudden onset of fever, lack of appetite, dehydration,

depression, vomiting and dizziness. Infected cats may show a decreased number of whole 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:

Diagnosis of FPLV, FHV and FCV is often made 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 severe feline infectious diseases. Yet, since vaccinations 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.

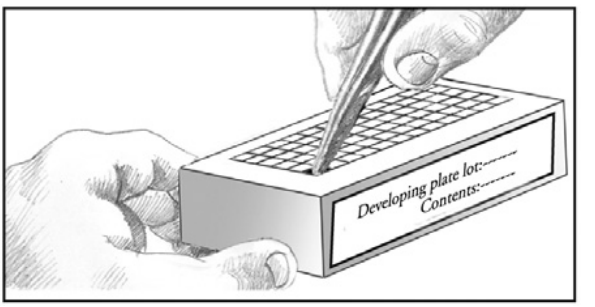
VII. STEP BY STEP WITH IMMUNOCOMB

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.

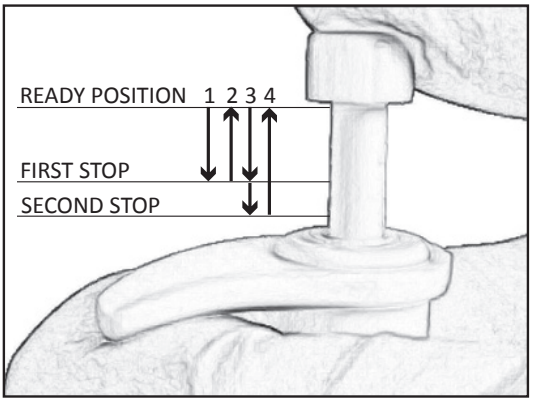
For testing serum or plasma use 5µl.
For testing whole blood use 10µl*.

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 50FVV201 , use the same tip to deposit twice 5µl into the same well in row A.**

Pipetting Technique

Forward Pippeting



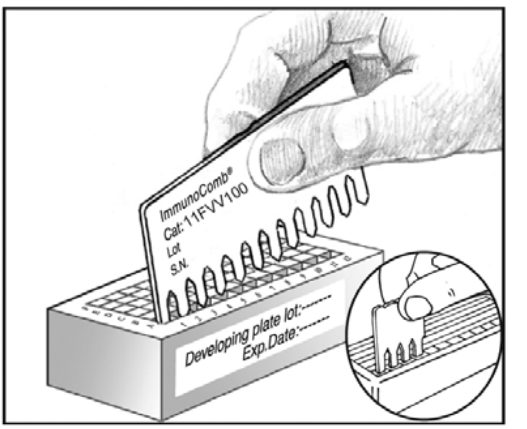
1- Press the operating button to the first stop.

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.

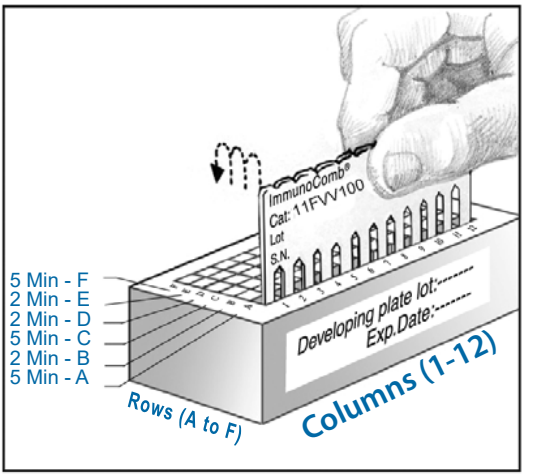
4- Release the operating button to the ready position.

(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.**

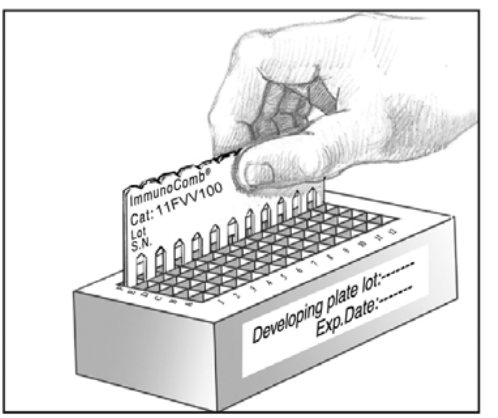
Avoid scratching the front active side of the Comb by leaning it to the back while mixing.
Gently shake off excess liquid from Comb teeth onto a tissue before moving it to the next row.



- Insert the Comb into the open well(s) in row A (printed side facing you) and incubate for 5 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 5 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 5 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.

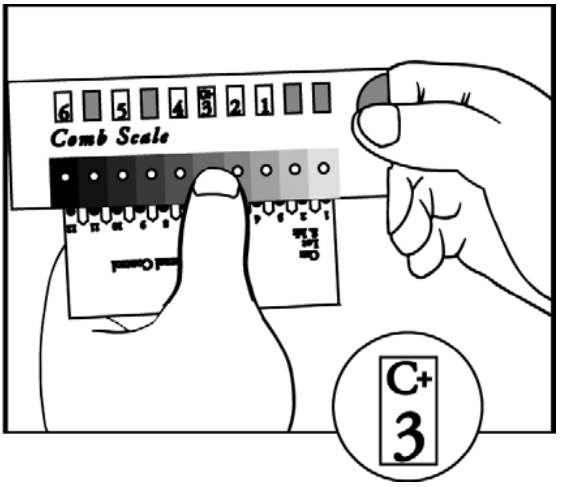
VIII. READING AND INTERPRETING THE IgG ANTIBODY RESULTS

- The upper most spot is the Positive Reference spot and it should give a distinct purple-grey color. This is the same color tone that is generated by a significant positive response of anti FPLV antibodies at 1:80 HI, anti FHV antibodies at 1:16 titer of VN test or of anti FCV antibodies equal to 1:32 VN. When using the CombScale, this spot should be read as S3 (see section IX).
- The upper middle spot on the Comb gives the result of FPLV IgG antibodies in the specimen.
- The lower middle spot on the Comb gives the result of FHV IgG antibodies in the specimen.
- The bottom spot on the Comb gives the result of FCV IgG antibodies in the specimen.
- Compare the color tone of FPLV, FHV and FCV test spots with the Positive Reference spot (separately).
- A color tone that is equal or darker than the reference spot is considered a positive response.
- A color tone that matches with S2 is considered a weak positive result.
- A faint color tone of S1 or less is considered a negative result.
- To evaluate the antibodies score use the CombScale provided in the kit (see section IX).
- A test spot with a washed blue appearance is invalid. Refer to Biogal for further advice.
- The dry Comb may be kept as record.

IX. READING RESULTS WITH THE COMBSCALE

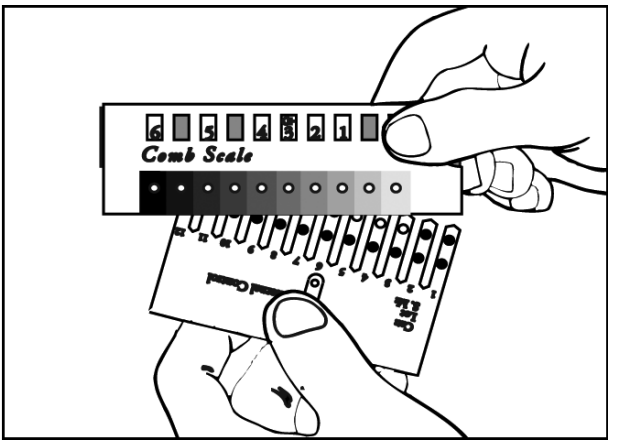
The CombScale S value is the number that appears in the yellow window corresponding to the color tone, when Positive Reference color is calibrated to S3.

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



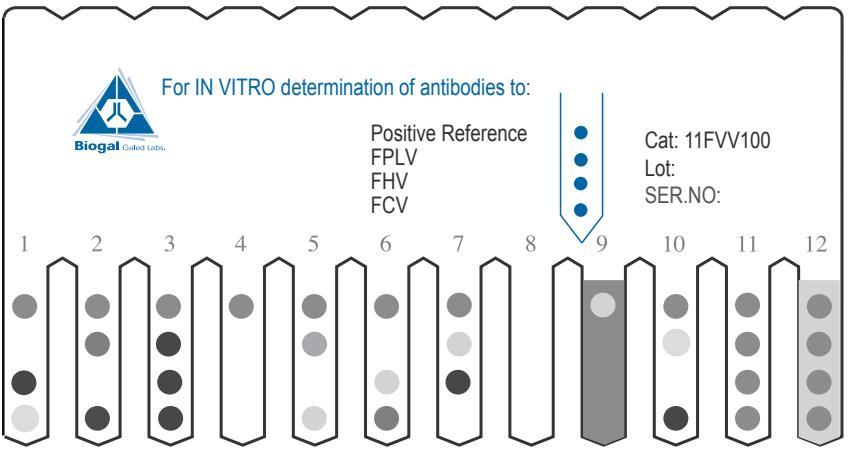
Find the tone of purple-grey on the CombScale that most closely matches the **Positive Reference spot** (upper spot). Slide the yellow ruler until the C+ mark appears in the window above that color you just found.

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.

X. EXAMPLE OF A DEVELOPED COMB



Tooth Nº	FPLV RESULTS		FHV RESULTS		FCV RESULTS	
1	S0	*Negative*	≥S5	*High pos.*	<S1	*Negative*
2	S4	*Positive*	S0	*Negative*	S6	*High pos.*
3	≥S5	*High pos.*	≥S5	*High pos.*	≥S5	*High pos.*
4	S0	*Negative*	S0	*Negative*	S0	*Negative*
5	≥S3	*Positive*	S0	*Negative*	S2	**Weak pos.*
6	S0	*Negative*	S2	**Weak pos.*	S4	*Positive*
7	S2	**Weak pos.*	≥S5	*High pos.*	S0	*Negative*
8**		*Invalid*		*Invalid*		*Invalid*
9***		*Invalid*		*Invalid*		*Invalid*
10	<S1	*Negative*	S0	*Negative*	≥S5	*High pos.*
11	≥S3	*Positive*	≥S3	*Positive*	≥S3	*Positive*
12****	≥S3	*Positive*	≥S3	*Positive*	≥S3	*Positive*

Remarks:

*Considered inconclusive in case of disease suspicion.
**No Positive Reference. Repeat test.
***High background. 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 (50FVV201)	12 Test Kit (50FVV401)	120 Test Kit (50FVV110)
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

AAHA Vaccine Task Force. (2006). JAAHA, 42, 80-89.
DiGangi et al. (2011). J Feline Med Surg. 13(12): 912-918.
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 Laboratories directly by E-mail: info@biogal.com or by tel: 972-4-9898605 / fax: 972-4-9898690.