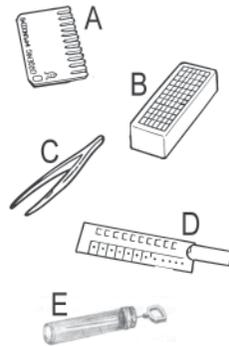


## XI. STORAGE & HANDLING

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

Components	12 Test Kit (50FTC201)	120 Test Kit (50FTC210)
A. ImmunoComb® card (wrapped in aluminum foil)	1	10
B. Developing plate	1	10
C. Disposable tweezers	1	1
D. Calibrated CombScale	1	1
E. Unit of 12 capillary tubes & one piston	1	May be purchased upon request.
Instruction manual	1	1



## XIII. REFERENCES

- Dubey (1986). Feline Practice 16: 12-26, 44-45.
- Montoya & Liesenfeld (2004) Lancet, 363: 1965-1976.
- Molina & Ridley-Dash (2008) USM R & D J: 16(1): 53-55.
- Sykes JE (2005) Feline chlamydiosis. Clin Tech Small Anim Pract. 20(2):129-34.

For further assistance please contact your local Distributor, or Biogal Galed Laboratories directly by E-mail: [info@biogal.co.il](mailto:info@biogal.co.il) or by tel: 972-4-9898605 / fax: 972-4-9898690.

**Biogal's**  
**ImmunoComb®**

# ImmunoComb®

## FELINE TOXOPLASMA & CHLAMYDOPHILA ANTIBODY TEST KIT

### INSTRUCTION MANUAL

Sufficient for 12/120 assays

08 MAR 2011



Biogal Galed Laboratories, tel: 972-4-9898605. fax: 972-4-9898690, e-mail:[info@biogal.co.il](mailto:info@biogal.co.il)

[www.biogal.co.il](http://www.biogal.co.il)

## I. INTENDED USE OF THE KIT

The Feline Toxoplasma & Chlamydomphila Antibody Test Kit is designed to determine cat serum IgG antibody titer to *Toxoplasma gondii* and *Chlamydomphila sp.* The main purpose of this kit is to provide a useful tool to assess immunity status of cats concerning these pathogens and to assist in the diagnosis of clinical cases.

## II. GENERAL INFORMATION

*Toxoplasma gondii* and *Chlamydomphila felis* infect cats all over the world causing illness especially in young cats, but adult cats may also be ill. Toxoplasma especially poses a zoonotic concern. While there is no vaccine against *T.gondii*, vaccination may be applied against Chlamydomphila. Monitoring antibody level by serology may help in diagnosis.

## 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 or plasma.

The kit contains all the necessary reagents for developing the test. Results are obtained within 40 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 Toxoplasma and of Chlamydomphila are attached to each tooth on the Comb. The upper most spot is a Positive Reference. Purified Chlamydomphila antigen

is attached to the middle spot, purified *Toxoplasma gondii* antigen is attached at the lower spot (see figure in section X).

■ The first step of the test is to deposit a serum or plasma 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 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), 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.

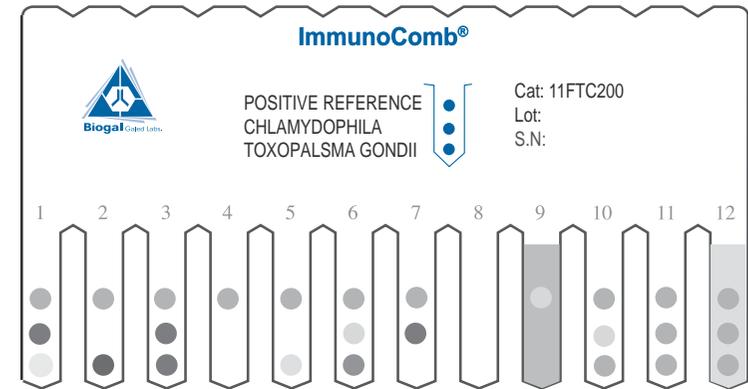
■ The intensity of the color result corresponds directly to the antibody level in the test specimen. Results are scored using the Positive Reference spot and CombScale (see section IX).

## V. DESCRIPTION OF DISEASES

### Toxoplasmosis

*Toxoplasma gondii*, an obligate intracellular parasite that can infect the central nervous system of warm-blooded animals, including humans. Infection is mainly acquired by ingestion of oocysts or tissue cysts. Cats play an important role in the spread of toxoplasmosis because they are the only mammals that secrete resistant oocysts through their feces. Ingested oocytes may migrate to the muscle and brain. *T. gondii* can also be transmitted across the placenta and through the milk so the main sources of infection for a cat are uncooked meat, infected prey, or as kittens in utero or through the milk. Yet in healthy cats, infection will usually be asymptomatic.

## X. EXAMPLE OF A DEVELOPED COMB



Tooth No.	Results of Chlamydomphila		Results of Toxoplasma	
	Score	Result	Score	Result
1	≥S5	High Positive	<S1	Negative
2	S0	Negative	S6	High Positive
3	≥S5	High Positive	≥S5	High Positive
4	S0	Negative	S0	Negative
5	S0	Negative	S1-2	Inconclusive
6	S1-2	Inconclusive	S4	Positive
7	≥S5	High Positive	S0	Negative
8*		Invalid		Invalid
9**		Invalid		Invalid
10	S1-2	Inconclusive	≥S3	Positive
11	≥S3	Positive	≥S3	Positive
12***	≥S3	Positive	≥S3	Positive

### Remarks:

\* No Positive Reference. Repeat test.

\*\* High background. Repeat test.

\*\*\* High background with positive results.

## VIII. READING AND INTERPRETING THE IgG ANTIBODY RESULTS

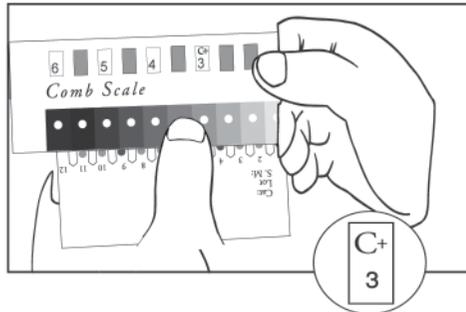
- The upper spot is the Positive Reference and it should give a distinct purple-grey color. This is the same color tone that is generated by a significant positive response of anti Chlamydomphila antibodies at 1:32 C.F and anti Toxoplasma antibodies at 1:32 I.F titer (see Fig. 1). When using the CombScale, this spot should be read as S3 (see section IX).
- The middle spot on the Comb gives the result of Chlamydomphila IgG antibodies in the specimen.
- The bottom spot on the Comb gives the result of Toxoplasma IgG antibodies in the specimen.
- Compare the color tone of Chlamydomphila and Toxoplasma 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 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).

## 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.

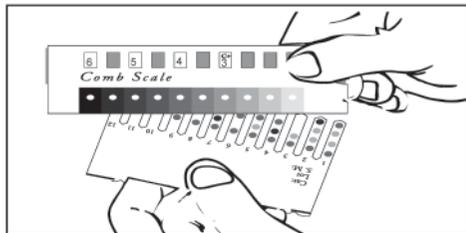
When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit. Find the tone of purple-grey on the CombScale that most closely matches the **Positive Reference spot** (upper spot). Slide the yellow ruler until the C+ mark appears in the window above that color you have 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 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.



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 assists labs in reading ImmunoComb® results and conserving the data, and is supplied free of charge upon request.

The signs of toxoplasmosis in pets are nonspecific: fever, loss of appetite, depression. Further signs may occur depending on whether the infection is acute or chronic, and where *T. gondii* is found in the body, the most severe are found in the nervous system.

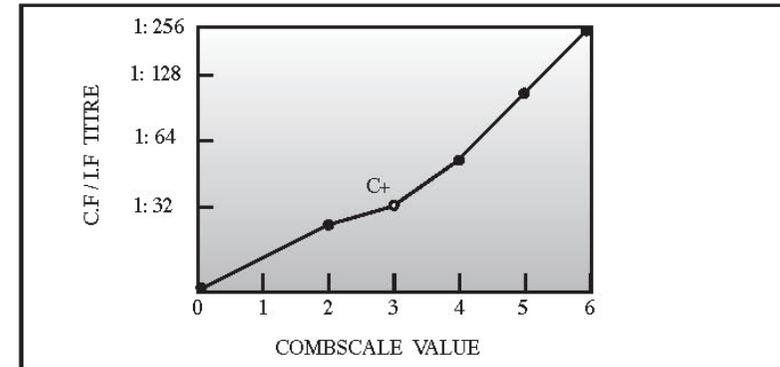
## Chlamydiosis

*Chlamydomphila felis* (previously *Chlamydia psittaci* var. felis) is an obligate, intracellular bacteria, with cell walls resembling those of Gram-negative bacteria. *C. felis* is primarily a conjunctival pathogen, capable of causing acute to chronic conjunctivitis, with blepharospasm, chemosis, congestion and a serous to mucopurulent ocular discharge. Transient fever, inappetence and weight loss may occur shortly after infection, although most cats apparently remain well and continue to eat. Clinical signs improve after a few weeks but mild conjunctivitis often persists for months.

## VI. DIAGNOSIS

Measurement of antibodies to *T. gondii* in the blood is the best method to diagnose toxoplasmosis. Sometimes the oocysts can be found in the feces but they look so similar to some other parasites that this is not a reliable method of diagnosis. Also, cats shed the oocysts for only a short period of time (about 2-3 weeks) and often are no longer shedding when they are showing signs of disease. PCR may be used to confirm infection and for monitoring the efficiency of treatment. Out of all existing serology techniques: latex agglutination test (LAT), Immuno-fluorescence (IF) and ELISA, the dot-ELISA used by the ImmunoComb® is the most user friendly and reliable technique.

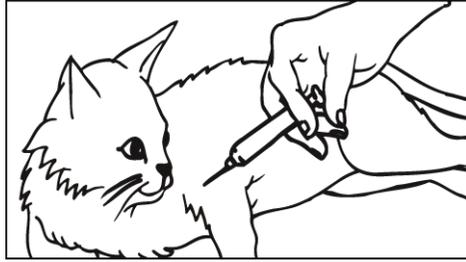
Fig. 1 Relationship between the CombScale's "S" value and the C.F. or I.F.



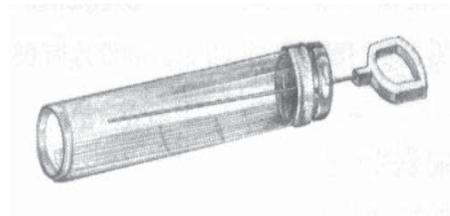
## VII. STEP BY STEP WITH IMMUNOCOMB®

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

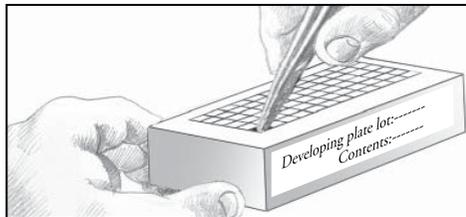
- (1) Obtain blood sample from cat.



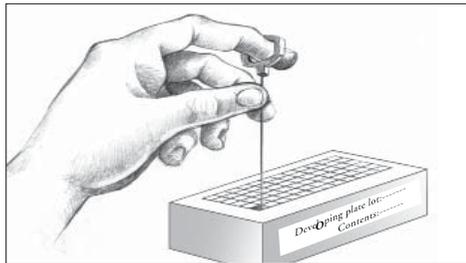
- (2) Use a pipette or a capillary tube\* to apply 5µl serum/plasma.



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



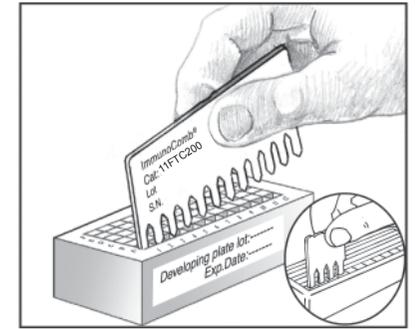
- (4) Deposit a sample into a well in row A. Raise and lower pipette/piston plunger several times to achieve mixing.



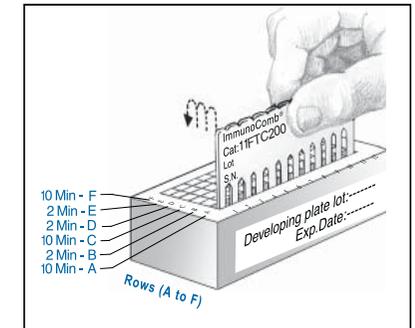
Do not open any well of row A or other rows which you do not intend to use.

\* Unit of 40 capillary tubes & one piston may be purchased upon request.

- (5) Remove the Comb from its protective envelope. For testing less than 12 samples, cut or break the Comb by folding 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 dip Comb **up and down** at the start of each incubation (each row). 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 Comb from one well to the next, pierce the foil of the next well. Gently shake off excess liquid from Comb teeth onto a tissue. Insert Comb into the remaining wells (**row D & E**) for **2 minutes** each 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.

