ImmunoComb

FELINE TOXOPLASMA & CHLAMYDOPHILA ANTIBODY TEST KIT

INSTRUCTION MANUAL

Sufficient for 12/120 assays

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Instruction Cat. No: 63FTC711

I. INTENDED USE OF THE KIT

The Feline Toxoplasma & Chlamydophila Antibody Test Kit is designed to determine cat serum IgG antibody titer to *Toxoplasma gondii* and *Chlamydophila 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 *Chlamydophila 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 Chlamydophila. 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, plasma or whole blood.
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 Chlamydophila are attached to each tooth on the Comb. The upper most spot is a Positive Reference. Purified Chlamydophila 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, 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

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.

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

Chlamydophila 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-fluoresence (IF) and ELISA, the dot-ELISA

used by the ImmunoComb is the most user friendly and reliable technique.

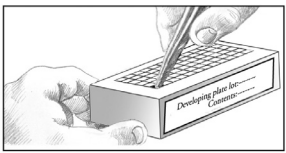
VII. STEP BY STEP WITH IMMUNOCOMB

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.

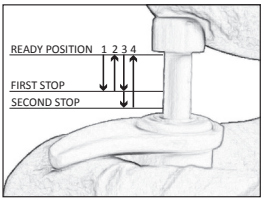
For testing serum or plasma use 5µl.
For testing whole blood use 10µl*.

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 50FTC301 , use the same tip to deposit twice 5µl into the same well in row A.**

Pipetting Technique

Forward Pippeting



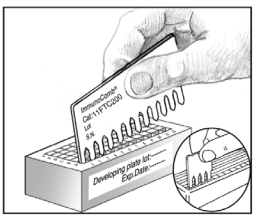
1- Press the operating button to the first stop.

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.

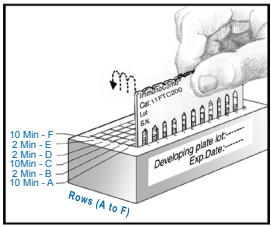
4- Release the operating button to the ready position.

(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 10 minutes. Mix as described above.**

■ Use tweezers to pierce the foil of the next well(s) in row B.

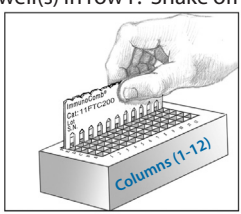
Shake off excess liquid and insert Comb for 2 minutes. Mix as described above.**

■ Pierce the foil of the next well(s) in row C. Shake off excess liquid and insert Comb for 10 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 10 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

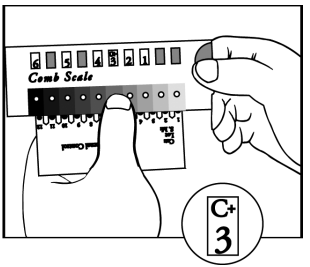
- The upper spot is the Positive Reference and it should give a distinct purple-grey color. This is the same color tone that is generated by a significant positive response of anti Chlamydophila antibodies at 1:32 C.F and anti Toxoplasma antibodies at 1:32 I.F titer. When using the CombScale, this spot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

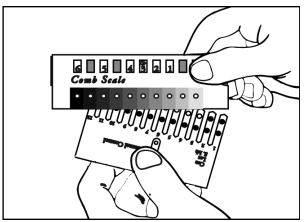
When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit.

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 just found.



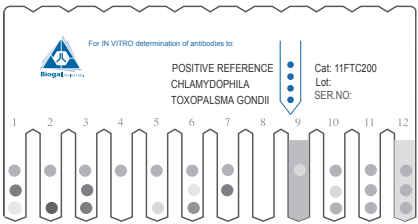
Hold the ruler in this position during the entire reading. This step actually calibrates the C+ to S3, which is the "cut-off" point to which test spots will be compared.

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.



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.

X. EXAMPLE OF A DEVELOPED COMB



Tooth №	Results of Chlamydophila		Results of Toxoplasma	
1	≥S5	High Positive	<S1	Negative
2	S0	Negative	≥S5	High Positive
3	≥S5	High Positive	≥S5	High Positive
4	S0	Negative	S0	Negative
5	S0	Negative	S2	Inconclusive
6	S1	Negative	S4	Positive
7	≥S5	High Positive	S0	Negative
8*		Invalid		Invalid
9**		Invalid		Invalid
10	S2	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.

XI. STORAGE & HANDLING

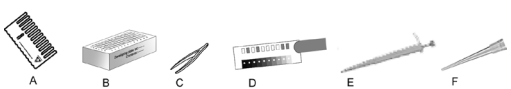
1. Store the kit under normal refrigeration (2° – 8° C / 36° – 46° F). **Do not freeze the kit.**
2. **Do not mix reagents from different kits or from different compartments of the same kit.**
3. The ImmunoComb kit contains inactivated biological material. The kit must be handled and disposed of in accordance 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 (50FTC301)	12 Test Kit (50FTC401)	120 Test Kit (50FTC210)
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



XIV. REFERENCES

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- 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 or by tel: 972-4-9898605 / fax: 972-4-9898690.