

ImmunoComb®

FELINE TOXOPLASMA & CHLAMYDOPHILA (CHLAMYDIA) ANTIBODY TEST KIT

INSTRUCTION MANUAL SUFFICIENT FOR 120 ASSAYS

I. INTENDED USE

This kit is designed to determine cat serum IgG antibody titers for *Toxoplasma gondii* and *Chlamydomphila sp.* (previously known as *Chlamydia sp.*).

II. WHAT IS THE ImmunoComb® ASSAY?

The ImmunoComb® is a self-contained portable kit. A sensitive test, which detects antibody levels in the blood or serum. The ImmunoComb® provides results within 35 minutes.

III. HOW DOES THE ImmunoComb® WORK?

- Based on a solid phase immunoassay principle, the ImmunoComb® is a plastic card shaped like a comb, on which purified *Toxoplasma* and *Chlamydomphila* antigens are attached.
- Take a serum or plasma specimen. Deposit a sample into well(s) in row A of the multi-compartment developing plate.
- Insert Comb into the sample wells so that antibodies from samples bind themselves to the antigens on the Comb's teeth.
- Non bound antibodies are washed out in the second compartment.
- The next compartment contains an anti-cat IgG antibody labeled with an enzyme. Immerse the Comb in this "conjugate." The bound antibodies will be labeled. Insert the Comb into a compartment where the enzyme reaction takes place. This generates a color change, which indicates the amount of antibodies present.
- Using the CombScale, convert the central spot's color intensity to the anti-chlamydomphila immunoglobulin level, and convert the lower spot's color intensity into the anti-toxoplasma immunoglobulin level.
- A positive reference is located on the highest spot. The color of this spot indicates a positive immune response equal to titer of 1:32 (C.F. or I.F.).
- The ImmunoComb® may be divided to individual teeth. Each segment can be processed independently.

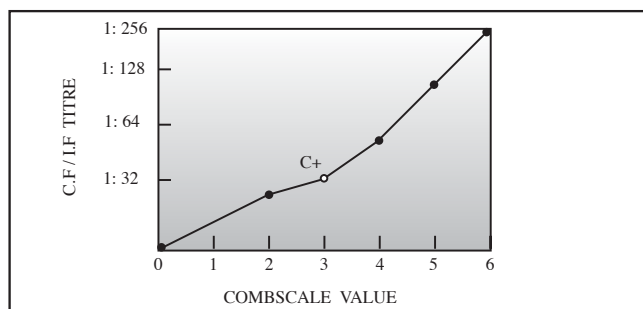
IV. HANDLING & STORAGE

1. Store the kit under normal refrigeration: 2° - 8° C (36° - 46° F). **Do not freeze the kit.**
2. Before conducting the test, all kit elements and specimens must be at room temperature -- preferably for 60-120 minutes (or 22 minutes in 37° C). 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 cover of each compartment while strictly following test procedure instructions. **DO NOT RIP OFF OR REMOVE COVER OF ENTIRE DEVELOPING PLATE ALL AT ONCE.**
8. The ImmunoComb® kit contains inactivated biological material. Kit must be handled and disposed of in accordance with accepted sanitary requirements. Use large amounts of water to flush kit solutions down sewage/drainage system.

V. READING AND INTERPRETING THE RESULTS

- To determine the IgG titer of *Toxoplasma* or *Chlamydomphila* specimens, compare the color intensity of the Comb's appropriate tooth with the color spot series on the enclosed CombScale (see illustrations 8 & 9 for details).
- The bottom spot on the ImmunoComb® tests for *Toxoplasma*. The second spot tests for *Chlamydomphila*. The upper spot is for positive reference.
- Compare the specimen's color intensity with that of the positive reference on the upper spot of each tooth.
- The positive reference is calibrated to a 1:32 titer (C.F. in *Chlamydomphila* or I.F. in *Toxoplasma*). When using the CombScale should be read as S=3.
- Specimens with an identical or higher color intensity than the positive reference are considered positive.
- Specimens with a color intensity lower than the positive reference are considered negative or non-immuned.
- To evaluate the titer, use the CombScale provided in the kit and determine the titer using Fig. 10, as reference. The procedure is detailed in section VI.

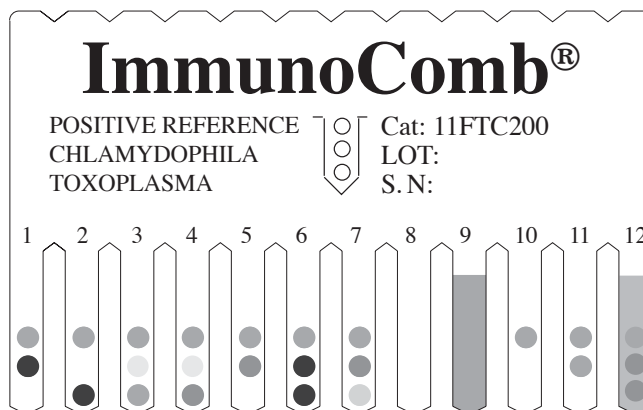
Fig 10. Relationship between the CombScale's "S" Value and the C.F. or I.F.



Important

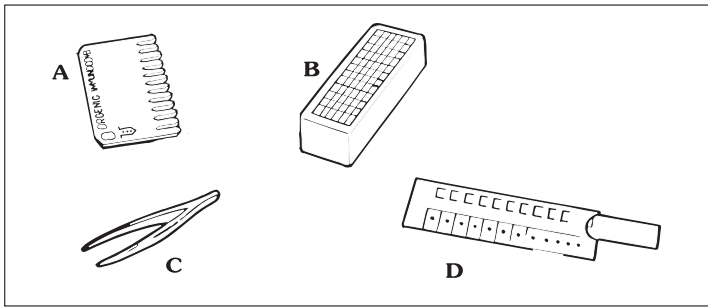
The margin of error is similar to that of other enzyme immunoassay kit procedures. Therefore, an error in one color scale window will not result in a wrong diagnosis.

Example of a Developed Comb



TOOTH No.	RESULT & REMARKS
1	High positive reaction for Chlamydomphila. Negative reaction to Toxoplasma.
2	High positive reaction for Toxoplasma. Negative reaction to Chlamydomphila.
3, 4	Very low reaction for Chlamydomphila - considered negative. Positive reaction for Toxoplasma.
5, 11	Positive reaction for Chlamydomphila, negative for Toxoplasma.
6	High positive reaction for Chlamydomphila and for Toxoplasma.
7	Medium positive reaction for Chlamydomphila. Low reaction for Toxoplasma.
8	No positive reference - Development failed.
9	High background colore - No valid test.
10	Negative reaction for Chlamydomphila and for Toxoplasma.
12	High background color, positive results to Chlamydomphila and Toxoplasma, although S value is inconclusive.

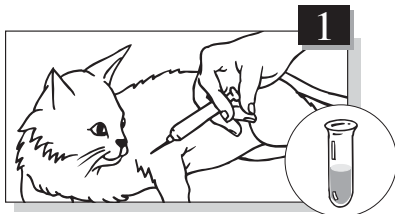
STEP-BY-STEP WITH IMMUNOCOMB[®]



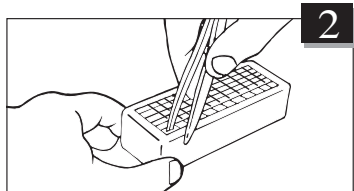
The ImmunoComb[®] kit includes: **A.** Ten ImmunoComb[®] card, wrapped in an aluminum envelope; **B.** Ten developing plate; **C.** One disposable tweezers; **D.** One calibrated CombScale color card; and a user manual.

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

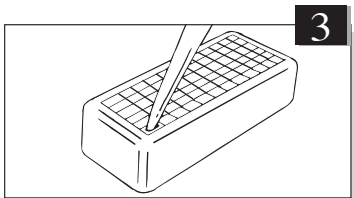
When using a serum



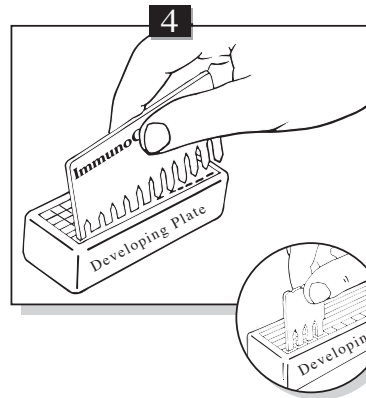
Use a pipette or a capillary tube. For testing a serum sample use 5µl.



Slit open the protective aluminum cover of compartment A with the tweezers. One well for each sample.

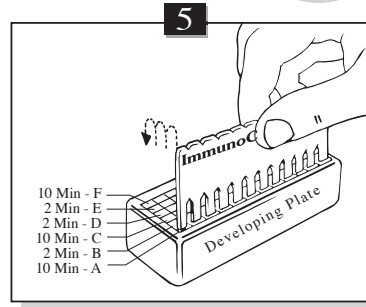


Dispense a sample into each well. When using the capillary tubes raise and lower the piston several times to achieve mixing. When using a pipetor, mix by depressing the plunger a number of times.

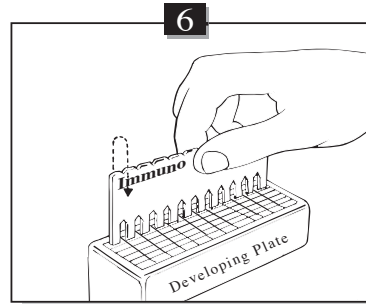


Remove the ImmunoComb[®] from its protective wrapping and insert (printed side facing you) in the required compartments of Row A. Gently move Comb up and down several times, then let incubate in Row A's compartments for **10 minutes**.

If necessary break the Comb by folding back its notches. You can separate each tooth of the Comb.



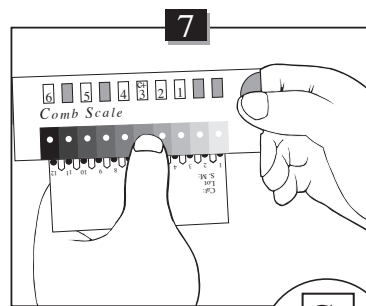
Pierce the cover of the appropriate section of compartment B with the tweezers. Follow same procedure for remaining rows at end of each incubation period. Gently shake off excess liquid onto a tissue. Insert ImmunoComb[®] in row B's compartment and let incubate for **2 minutes**. Shake-off and transfer ImmunoComb[®] to Row C and incubate for **10 minutes**. Similarly, the ImmunoComb[®] is placed in Row D for **2 minutes**, Row E for **2 minutes**, and Row F for **10 minutes**, allowing the color reaction process to develop.



After the ImmunoComb[®] has completed the cycle for Row F, transfer it back to Row E. Incubate in Row E for **2 minutes** to fix color.

AIR DRY AND READ RESULTS.

VI. READING RESULTS WITH THE COMBSCALE



A. Match "C+" with positive Control:

When the Comb is completely dry align it with the calibrated color CombScale. Compare the color resulting from the positive reference to the color scale by sliding the yellow ruler until the "C+" mark appears in the window corresponding to the color. Separately calibrate each antigen-spot.

FINALLY, HOLD THE SLIDE IN THIS POSITION DURING READING.

B. Read each of the spots separately:

Choose the most suitable color and read the titer in the yellow windows.

REMEMBER: A DIFFERENCE OF ONE COLOR LEVEL WILL NOT AFFECT DIAGNOSIS !!!

