

ImmunoComb®

OVINE TOXOPLASMA & CHLAMYDOPHILA (CHLAMYDIA) ANTIBODY TEST KIT

INSTRUCTION MANUAL SUFFICIENT FOR 300 ASSAYS

I. INTENDED USE

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

II. WHAT IS THE ImmunoComb® ASSAY?

- The ImmunoComb® is a self-contained portable kit. A sensitive test which detects antibody levels in sheep or goat serum. The ImmunoComb® provides results in 38 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 specimen and deposit into sample wells 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.
- Non bound antibodies are washed out in the second compartment.
- The next compartment contains an anti-sheep 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 into the anti-toxoplasma immunoglobulin level.
- An internal control, the top spot, indicates that the development is completed.
- The ImmunoComb® may be divided into three separate sections. Each segment processes 4 teeth.

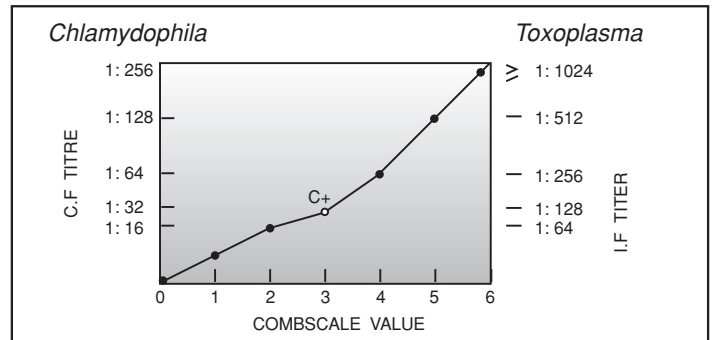
IV. HANDLING & STORAGE

1. Store the kit under normal refrigeration: 2° - 8° C (36° - 46° F). **Do not freeze the kit.**
2. Before conducting the test, maintain all kit elements and specimens 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. It is recommended to incinerate kit after use.

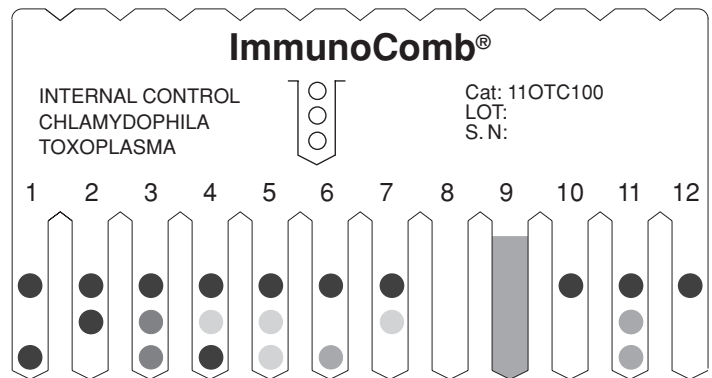
V. READING AND INTERPRETING THE RESULTS

- To determine the IgG titer of *Toxoplasma* or *Chlamydomphila* specimens, compare the color intensity of the Comb's appropriate teeth with the color spot series on the enclosed CombScale (see illustrations 7 & 8 for details).
- The bottom spot on the ImmunoComb® tests for *Toxoplasma*. The second spot tests for *Chlamydomphila*. Evaluate the results of each disease separately.
- Compare the specimen's color intensity with that of the positive control (C+) included in the kit, in order to determine its titer.
- The positive control (C+) for *Chlamydomphila* is calibrated to a 1:32 titer (C.F.).
- The positive control (C+) for *Toxoplasma* is calibrated to a 1:128 titer (I.F.).
- Specimens with an identical or higher color intensity than the positive control are considered positive.
- The negative control consists of non-immune sera and should be read as S=0-1.
- Specimens with a color intensity lower than the positive control are considered negative or non-immuned.
- When a test color is darker than S6, it may indicate either an acute or a recent infection.
- To evaluate the titer, use the CombScale provided in the kit and determine the titer using Fig. A, as reference. The procedure is detailed in section VI.

Fig. A. Relationship between the CombScale's value ("S") to *Chlamydomphila* C.F. and *Toxoplasma* I.F. titers.

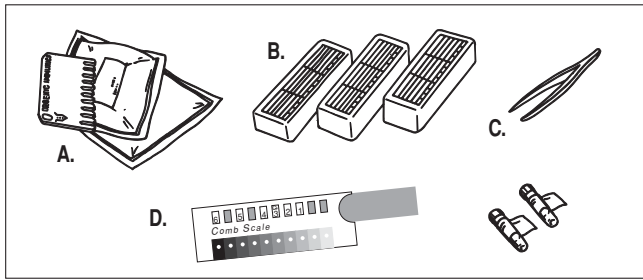


Example of a developed Comb

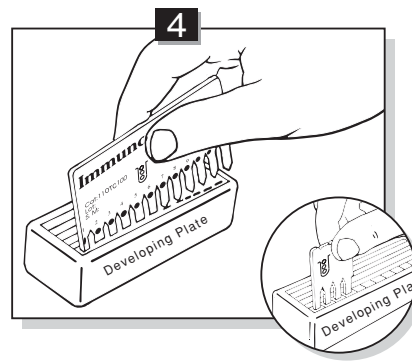


| TOOTH No. | RESULT & REMARKS |
|-----------|--|
| 1 | High positive reaction for Toxo., Negative for Chlamy. |
| 2 | High positive reaction for Chlamy., Negative for Toxo. |
| 3 | Positive reaction for Chlamy., and for Toxo. |
| 4 | Low reaction for Chlamy., Positive for Toxo. |
| 5 | Low reaction for Chlamy., and for Toxo. |
| 6 | Positive reaction for Toxo., Negative for Chlamy. |
| 7 | Low reaction for Chlamy., Negative for Toxo. |
| 8 | No internal control - developmant failed. |
| 9 | High back ground color - invalid test. |
| 10 | Negative reaction for Chlamy., and for Toxo. |
| 11 | Positive control. |
| 12 | Negative control. |

The ImmunoComb® kit includes:

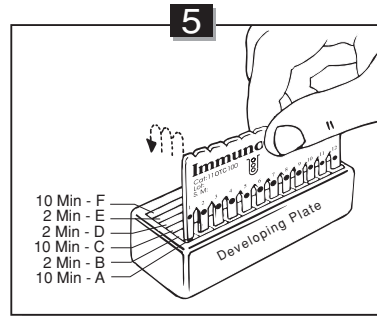


A. 30 ImmunoComb® cards, each separately wrapped in an aluminum envelope; **B.** 30 developing plates; **C.** One disposable tweezers; **D.** One calibrated CombScale color card; **E.** One tube of positive control serum and one tube of negative control serum; a user manual.

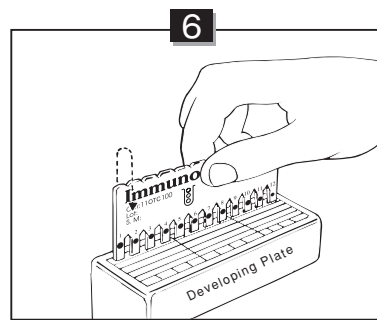


Remove one ImmunoComb® from its protective wrapping and insert (printed side facing you) in compartments of **Row A**. Gently move Comb up and down several times, then let incubate in **Row A's** compartments for **10 minutes**.

When using 1/3 or 2/3 of a Comb, break the Comb by folding back on notch 4 or 9 respectively. Keep the rest in its original sleeve for further use.



Pierce the cover of the appropriate section of compartment **B** with the tweezers. Follow same procedure for the remaining rows at end of each incubation period. Gently shake off excess liquid onto a tissue. Insert Comb in **row B** compartment and let incubate for **2 minutes**, shake-off and transfer Comb to **Row C** and incubate for **10 minutes**. Similarly, the Comb 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.

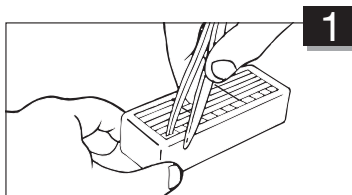


After the Comb has completed the cycle for **Row F**, transfer it back to **Row E**. Incubate in **Row E** for **2 minutes** to fix color.

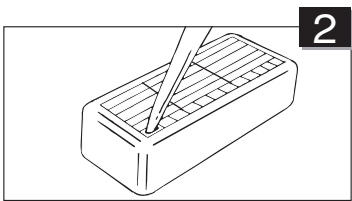
**AIR DRY AND
READ RESULTS**

Step by step with the ImmunoComb®

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



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



Using a pipette, dispense 5µl sample into each well. Mix by depressing the plunger a number of times.

3

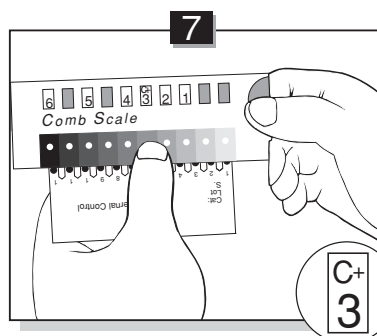
For control serum open the next 2 consecutive

Take 5 µl positive control serum (C+) and insert into well A next to the last sample.

Mix the serum in the well.

Do the same with the negative control serum in the next well.

VI. READING RESULTS WITH THE COMBSCALE



A. Adjust scale with positive control:

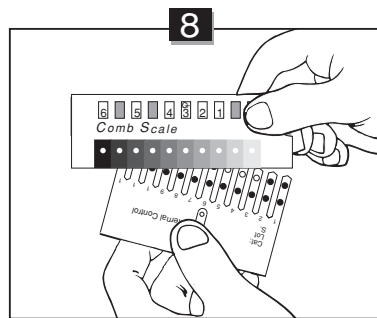
When the Comb is completely dry align it with the calibrated color CombScale. Compare the color resulting from the positive control (C+) sample to the color scale: slide the yellow ruler until the "C+" mark appears in the window corresponding to the color.

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 !!!



Another way to read the results is by using the CombScan 2000. This is a software program that utilizes a computer and twain compatible scanner. When a comb is placed on the scanner, the program translates the color results into numerical values. The CombScan 2000 assists labs in reading ImmunoComb® results and conserving the data, and is supplied free of charge upon request.