ImmunoComb®

Poultry *Chlamydophila psittaci* Antibody Test Kit

INSTRUCTION MANUAL
SUFFICIENT FOR 300 TESTS

I. INTENDED USE

This kit is designed to determine poultry serum IgG antibody titers to *Chlamydophila psittaci* (previously known as *Chlamydia psittaci*).

II. WHAT IS THE IMMUNOCOMB® ASSAY?

The ImmunoComb® is a self-contained portable kit. A sensitive test which detects antibody levels in whole blood, serum or egg yolk. 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 *Chlamydophila psittaci* antigens are attached.
- Use chicken or turkey serum, plasma or egg yolk specimen. Deposit sample into cells of the multi-compartment developing plate.
- Insert Comb into sample cells 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-chicken 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 bottom spot color intensity to the anti-Chlamydophila-immunoglobulin level.
- An internal control at the top spot of the Comb indicates that the development is completed.

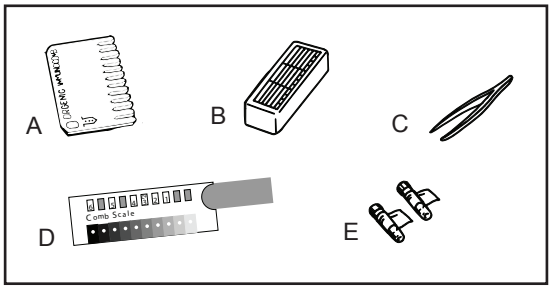
IV. READING & INTERPRETING THE RESULTS

- The CombScale results should be compared only within the same species or to follow changes in immune response.
- Use the enclosed CombScale to compare titers with the positive control. When retesting a bird, compare the S-values to determine changes in the bird's immune response.
- The bottom spot on the Comb tests for *Chlamydophila psittaci*. The top spot is the internal control.
- In order to determine its titer, compare the specimen's color intensity with that of the positive control (C+) included in the kit.
- Immune response may indicate clinical or asymptomatic contamination with Chlamydophila, as well as residual antibodies during and after treatment. It is advisable to retest bird after treatment.
- Specimens with higher color intensity than the negative control are considered positive.
- The negative control consists of non-immune sera and should be read as zero (S=0).
- The positive control contains inactivated serum carrying anti-Chlamydophila antibodies, and should be read as S=3 on the CombScale.

V. HANDLING & STORAGE

- Store the kit under normal refrigeration: 2º - 8º C (36º - 46º F). **Do not freeze the kit.**
- Before conducting the test, all kit elements and specimens must be at room temperature – preferably for 60 – 120 minutes (or 22 minutes at 37º C or 98.6º F). **Perform assay at room temperature of 20º - 25º C (68º - 77º F).**
- Avoid spillage and cross-contamination of solutions.
- Mix reagents by inverting developing plate several times prior to use.
- Do not mix reagents from different kits or from different compartments of the same kit.
- Do not touch teeth of ImmunoComb® card.
- When using developing plate, pierce cover of each compartment by strictly following test procedure instructions. **Do not rip off or remove cover of entire developing plate all at once.**
- The ImmunoComb® kit contains inactivated biological material. Kit must be handled and disposed of in accordance with accepted sanitary requirements.

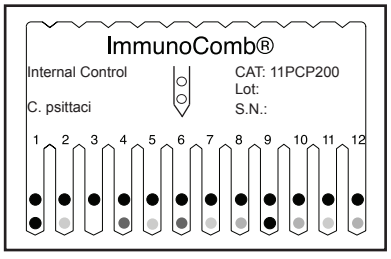
VI. THE IMMUNOCOMB® KIT INCLUDES:



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

Note: A pipette or capillary tubes are needed. The capillary tubes are available at Biogal or through your supplier: 40 capillary tubes & 1 piston, CAT. NO. 10000140.

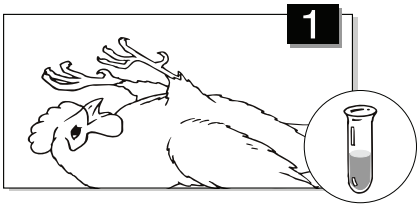
An Example of a Developed Comb for *Chlamydophila psitacci* Antibodies



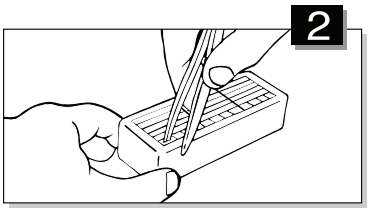
VII. STEP-BY-STEP DEVELOPMENT PROCESS

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

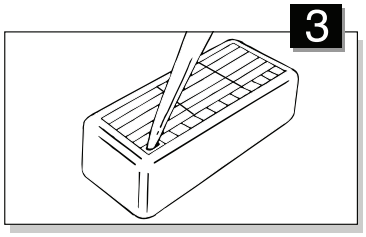
When using serum specimen(s)



Use either 5µl serum sample (preferable), or 10µl whole blood sample. Use either pipette or a capillary tube.



Slit open the protective aluminum covering of **compartment (well) A** with the tweezers.



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

When using egg yolk specimen(s)

Separate the entire egg yolk and wash gently with tap water. Withdraw 1 ml yolk from the outer layer and transfer to a test tube; add 1 ml isotonic saline solution (0.85% NaCl) and mix thoroughly. Deposit 10µl of each diluted yolk specimen in the respective wells. Mix by withdrawing and expelling with the pipette several times. Proceed to the next step immediately.

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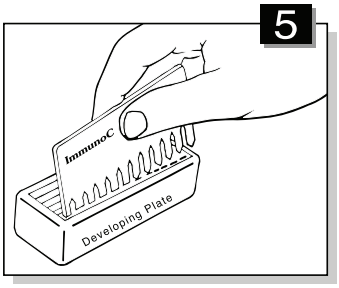
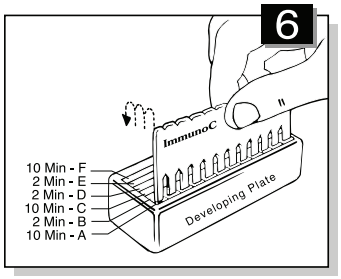
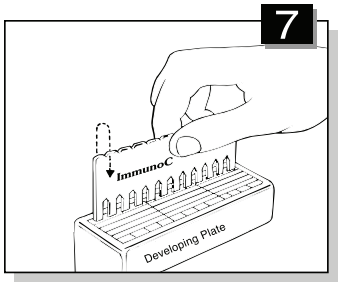
Open the next 2 consecutive wells for control serum.
Take 5µl positive control serum (C+) and insert into well A next to the last sample. Mix the serum in the well. **Do the same with the negative control serum (C-) in the following well.**



Remove one Comb from its protective wrapping and insert (printed side facing you) into well of **Row A**. Gently move Comb up and down several times, then incubate in wells of **Row A** for **10 minutes**.

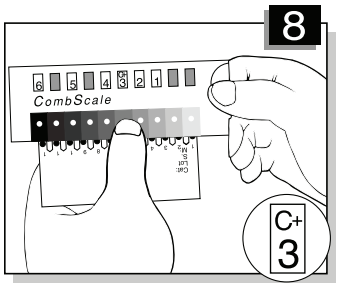


Pierce the cover of wells in **Row B** with the tweezers. Follow the same procedure for remaining rows at the end of each incubation period. Gently shake off excess liquid onto a tissue. Insert Comb in wells of **Row B** and incubate for **2 minutes**, shake off and transfer the Comb to **Row C** and incubate for **10 minutes**. Place the Comb in **Row D** for **2 minutes**, **Row E** for **2 minutes**, and **Row F** for **10 minutes**, allowing the color reaction process to develop.

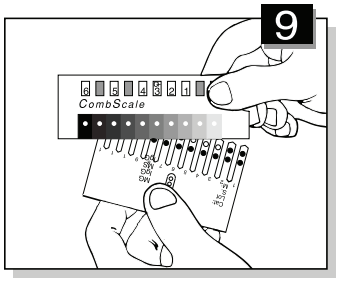


After the Comb has completed the cycle for **Row F**, transfer it back to **Row E**. Incubate in **Row E** for **2 minutes** to fix color.

VIII. 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 color resulting from the positive control (C+) sample to the color scale by sliding the yellow ruler until the "C+" mark appears in the window corresponding to the color. Finally, hold the slide in this position during reading, **separately read each antigen-spot.**



B. Read each of the spots separately: Choose the most suitable color and read the titer in the yellow windows.

<u>REMEMBER:</u> A DIFFERENCE OF ONE COLOR LEVEL WILL NOT AFFECT THE DIAGNOSIS!

Another way to read the results is by using the CombScan 2007. 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 2007 assists labs in reading ImmunoComb® results and conserving the data, and is supplied free of charge upon request.