

ImmunoComb®

POULTRY *CHLAMYDOPHILA PSITTACI* (*CHLAMYDIA PSITTACI*) ANTIBODY TEST KIT

INSTRUCTION MANUAL SUFFICIENT FOR 30 ASSAYS

I. INTENDED USE

This kit is designed to determine poultry serum IgG antibody titers to *Chlamydomphila 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 the blood or serum. The ImmunoComb® provides results within 40 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 *C. psittaci* antigen is attached.
- Either saturate paper disks in bird's blood or take a serum specimen. Deposit sample into wells of the multi-compartment developing plate (wells in row A).
- Insert Comb into the sample wells (A) 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 and turkey IgG antibody labeled with an enzyme. Immerse the Comb in this "conjugate". The bound antibodies will be labeled.
- After 2 stages of wash, 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 to the anti-chlamydomphila immunoglobulin level.
- An internal control at the top spot indicates that the development is completed.

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 at 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 by 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. Use large amounts of water to flush kit solutions down sewage/drainage system.

V. READING 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 birds' immune response.
- The bottom spot on the ImmunoComb® tests for *Chlamydomphila 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.

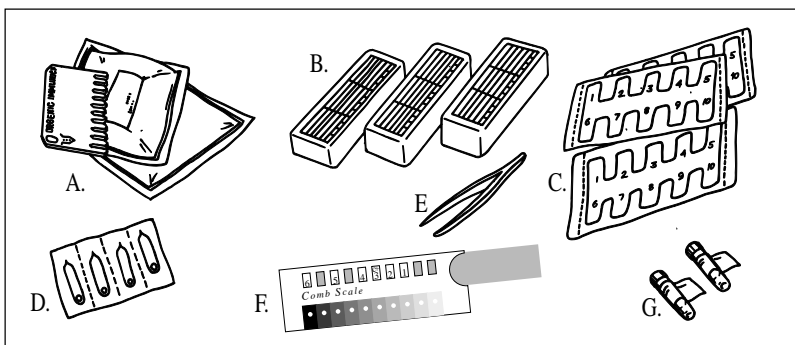
VI. INTERPRETATION OF RESULTS:

- Immune response may indicate clinical or asymptomatic contamination with *Chlamydomphila*, as well as residual antibodies during and after treatment. It is advisable to retest bird after treatment.
- Specimens with an higher color intensity than the negative control are considered positive.
- The negative control consists of non-immuned sera and should be read as zero (S=0).
- The positive control contains inactivated serum carrying anti-*Chlamydomphila* antibodies, and should be read as S=3 on the CombScale.

VI. BRIEF INSTRUCTIONS FOR USERS

- Gently turn over the developing plate to mix reagents.
- Insert 5ul of serum or a blood saturated disk in the specimen wells of the developing plate's compartment A.
- Insert 5ul of positive and negative controls for each test run. When using a disk, wait 60 minutes in order to allow for blood extraction.
- Insert Comb into compartment A's wells. Reinsert several times to prevent formation of air bubbles on the teeth. Incubate for 10 minutes at room temperature.
- Wash Comb by inserting into compartment B and incubate for 2 minutes.
- Insert Comb into compartment C, again reinserting several times to prevent formation of air bubbles on teeth. Incubate for 10 minutes at room temperature.
- Wash Comb by inserting into compartment D. Incubate for 2 minutes. Proceed to compartment E for another 2 minutes.
- Insert Comb into compartment F, again reinserting several times. Incubate at room temperature for 10 minutes. This stage determines Comb's color reaction.
- Stop the reaction by reinserting the Comb into compartment E. Incubate for 2 minutes. Withdraw Comb and dry.
- Read the results by using the CombScale as described in section V.

STEP-BY-STEP WITH ImmunoComb®



The ImmunoComb® kit includes: **A.** Three ImmunoComb® cards, each separately wrapped in an aluminum envelope; **B.** Three developing plates; **C.** Three specimen papers with pre-punched disks; **D.** Four blood lancets; **E.** One disposable tweezers; **F.** One calibrated CombScale color card; **G.** One tube of positive control serum and one tube of negative control serum; a user manual.

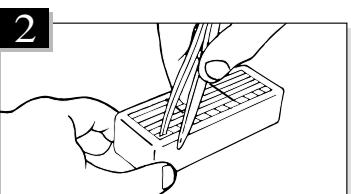
A pipette or capillary tubes are also needed. The capillary tubes are available at Biogal, or through your supplier:
40 capillary tubes + 1 piston, cat. no. 10000140.

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

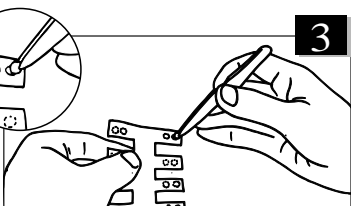
When using a blood paper disk



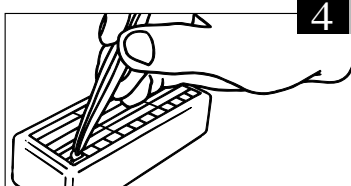
When using a paper disk, pierce one of the chick's veins. Take a specimen paper and saturate a pre-punched disk with the blood.



Slit open the protective aluminum covering of compartment A with the tweezers.



When using a paper disk punch out a disk saturated with blood.

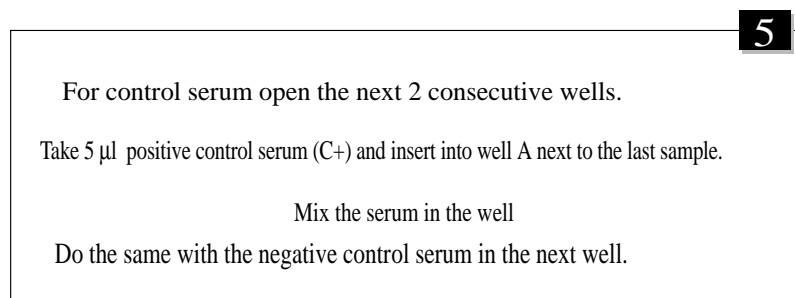


Insert the disk into well #1 of compartment A. Dip it well into the liquid. Proceed with the other samples.

Wait 60 minutes for extraction of antibodies.

For egg yolk specimens:

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 10 µl 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.



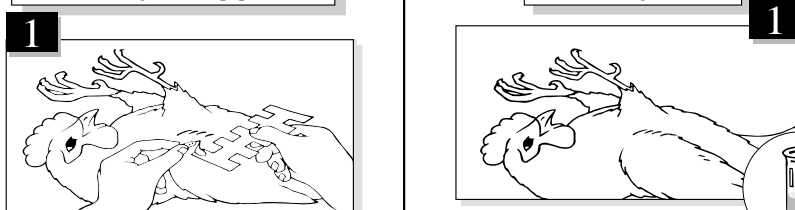
For control serum open the next 2 consecutive wells.

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

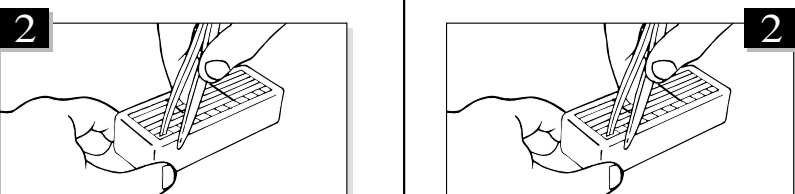
Mix the serum in the well

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

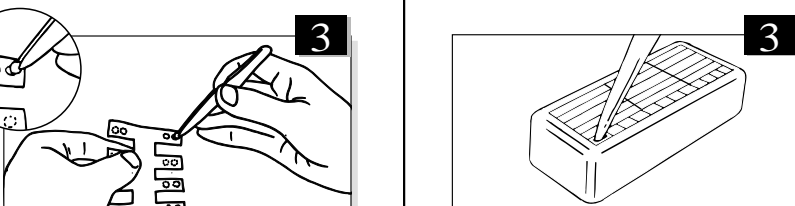
When using a serum



When using a serum sample use a 5 µl. pipette or a capillary tube.



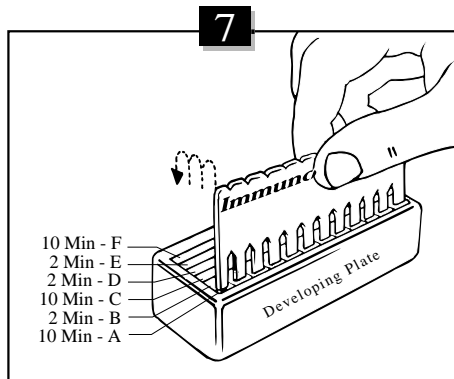
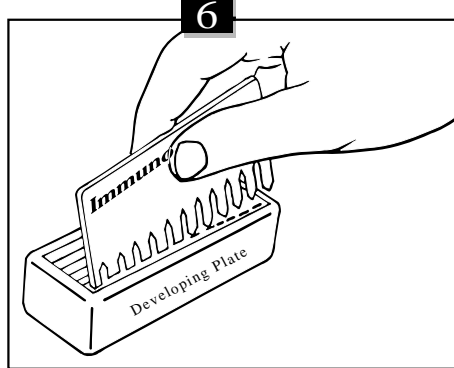
Slit open the protective aluminum covering of compartment A with the tweezers.



Dispense 5 µl 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.

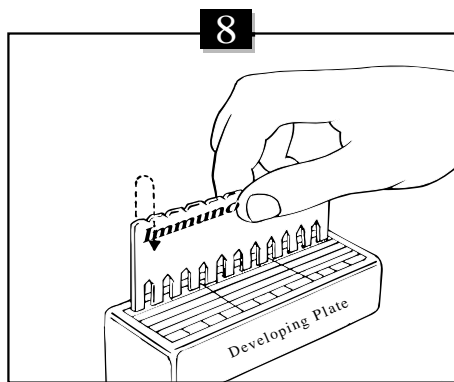
Proceed to step 5.



Remove one Comb from its protective wrapping and insert (print 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**.

Pierce the cover 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.

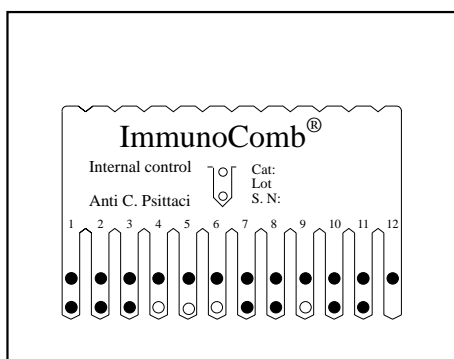
Insert Comb in **Row B's** compartment and let incubate for **2 minutes**, shake-off and transfer Comb to **Row C** and incubate for **10 minutes**. Similarly, the Comb is placed in **Row D** for **2 minutes**, **Row E** for **2 minutes**, and **Row F** for **10 minutes**, allowing the color reaction process to develop.



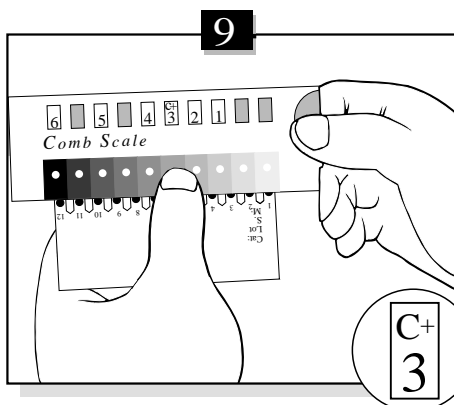
After the Comb has completed the cycle for Row F, transfer it back to Row E. Incubate in Row E for 2 minutes to fix color.

**AIR DRY
AND READ
RESULTS**

Developed ImmunoComb®
for *C. psittaci* Antibodies



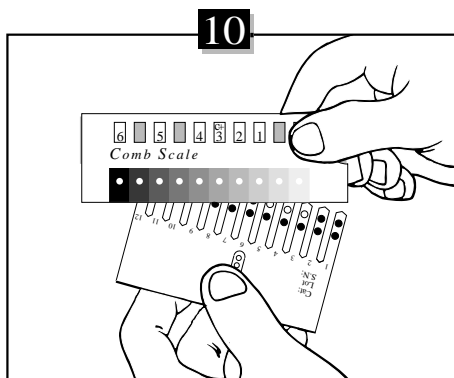
READING RESULTS WITH THE CombScale



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 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 THE DIAGNOSIS !!!