

ImmunoComb

AVIAN CHLAMYDOPHILA PSITTACI ANTIBODY TEST KIT

INSTRUCTION MANUAL

Sufficient for 10/100 tests

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I. INTENDED USE OF THE KIT

The Avian *Chlamydophila psittaci* test kit is designed for detection of avian whole blood/serum antibodies to *C. psittaci* (hereafter referred to as ACP). The kit can be used in facilities with limited capabilities for laboratory testing and assist in the diagnosis of clinical cases.

II. WHAT IS THE IMMUNOCOMB ASSAY?

The ImmunoComb test is a highly sensitive modified ELISA which can be described as an enzyme labeled “dot assay” that detects antibody levels in serum or whole blood. The ImmunoComb test kit contains all necessary reagents for developing the test. Results are obtained within 60-120 minutes.

III. GENERAL INFORMATION

Chlamydophila psittaci is a bacterium that infects many species of birds and can be transmitted to humans, causing an illness generally known as Chlamydiosis. This disease is also referred to as Psittacosis (“Parrot Fever”) in parrots and other psittacine birds and Ornithosis in chickens and turkeys. People who handle or are exposed to infected birds are at greater risk for contracting a Psittacosis Infection by *C. psittaci*, which is typically transmitted by the aerosol route. Detection of antibodies may help in diagnosis.

IV. 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* antigen is

attached.

- Samples (whole blood saturated paper disks, serum) should be deposited into separate wells in row A of the developing plate.

- Positive Control and Negative Control samples, supplied in the kit, should be applied in separate wells in Row A.

- Comb is inserted into sample wells so that antibodies from samples bind to the antigens on the Comb's teeth.

- Each plate may be used to test individual or any number of samples up to 10, by breaking off the desired number of teeth from the Comb and using the corresponding column of wells in the developing plate. Each run should include Positive Control and Negative Control wells/teeth.

- Non-bound antibodies are washed out in the second row.

- The next row contains an anti-parrots IgG antibody labeled with an enzyme. While Comb is immersed in this “conjugate”, the bound antibodies will be labeled.

- After another stage of wash, Comb is inserted into a row where the enzyme reaction takes place. This generates a color change, its intensity indicates the amount of antibodies present in each sample.

- Purple-grey color intensity obtained at the lower spots should be converted to the antibody levels by using the CombScale.

- Internal Control spots indicate that the development is complete and valid.

V. CLINICAL SIGNS

Infected birds may display a range of clinical signs from inapparent to severe illness primarily in respiratory tract. The sick bird appears 'unthrifty' and exhibits ocular-nasal discharge with or without diarrhea.

Chlamydophila organisms are shed in oral, ocular and respiratory secretions and in the feces. Infected but apparently healthy birds, as well as sick birds, are capable of shedding Chlamydophila. However, shedding may be intermittent, so a negative result from fecal or cloacal swab examination does not always rule out the possibility that a bird may be infected.

VI. DIAGNOSIS

A number of specific assays are currently used for diagnosing *C. psittaci* infection in birds. The tests are divided into 2 categories:

1. **Antigen detection in body secretions, feces and or cloacal swabs:** Methods include direct immunofluorescence, PCR and culture. Major limitations of these methods are false negatives, due to intermittent shedding of organisms and the requirement of specialized laboratory facilities and expertise in order to perform the tests.

2. **Evaluation of anti-Chlamydophila antibodies in the birds blood:** Techniques include complement fixation, elementary body agglutination and ELISA, which includes the ImmunoComb Antibody Test Kit. These serologic methods offer the advantage of being able to identify an infected bird that may not be shedding organisms. All methods except the ImmunoComb are performed by specialized laboratories.

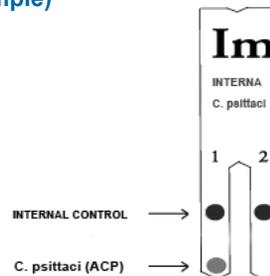
VII. STORAGE & HANDLING

1. Store the kit under normal refrigeration (2° – 8° C or 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.

VIII. 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 sample 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.

Fig. 1: Two teeth of a developed Comb (example)



IX. STEP BY STEP WITH THE IMMUNOCOMB

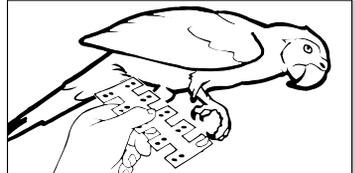
Before conducting the test, bring the developing plate to room temperature by removing all kit components from the kit carton and place them on the work bench for 60-120 minutes or incubate only the plate at 37°C/98.6°F for 25 minutes.

Perform assay at room temperature 20° – 25° C / 68° – 77° F.

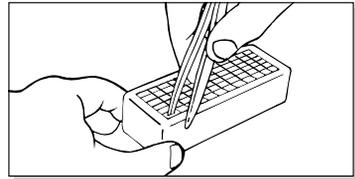
(1) Obtain blood from the bird.

When testing whole blood, collect sample in EDTA or heparin anticoagulant tube.

When using a paper disk, carefully cut one of the bird's toe nail. Take a specimen paper and saturate a pre-punched disk with the blood on **both sides** of the disk.



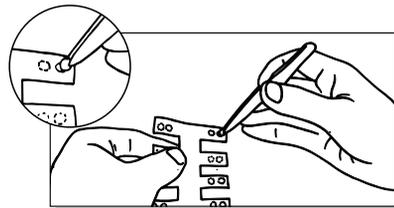
(2) Mix reagents by gently shaking the developing plate several times prior to use. Use the tweezers to pierce the protective aluminum cover of row A. One well for each sample/specimen.



(3) Deposit a sample into a well in row A.

When using a paper disk: Punch out a disk saturated with blood. The blood saturated disk may be dry.

Insert the disk into a well of row A. Immerse it totally in the liquid reagent. Proceed with the other samples into the next wells.



Incubate disk in well A for 60 minutes at room temperature to allow antibody extraction.

When using serum/whole blood samples: For testing serum use **5µl**.

For testing whole blood use **10µl***.

Raise and lower pipette plunger several times to achieve mixing.

Avoid spillage and cross-contamination of solutions

Proceed to the next step immediately.

***For whole blood only: If dispensing the sample with a fix pipette provided in this kit, use the same tip to deposit twice 5µl into the same well in row A.**

(4) 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 into the well. **Do the same with the Negative Control serum (C-) in the following well.**

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.

(5) 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 20 minutes. **Mix as described above.****

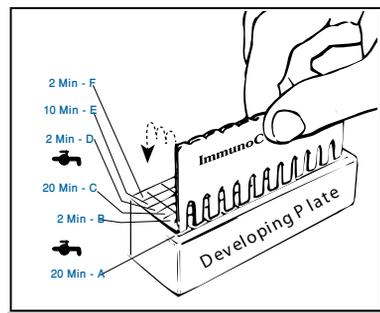
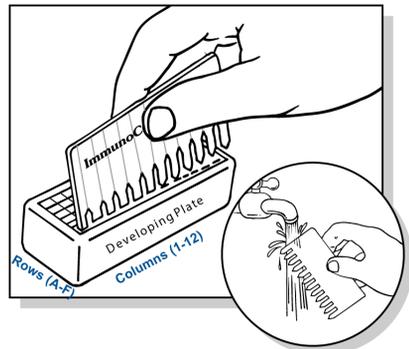
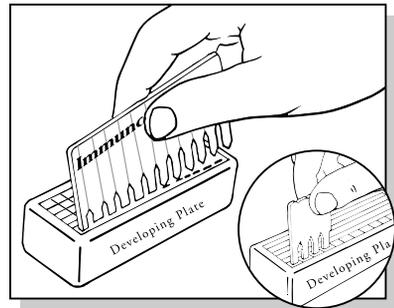
■ Use tweezers to pierce the foil of the next well(s) in row B. **Wash Comb under cool tap water** and insert it into **row B** for 2 minutes. Mix as described above.**

■ Pierce the foil of the next well(s) in row C. Shake off excess liquid and insert Comb for 20 minutes. Mix as described above.**

■ Pierce the foil of the next well(s) in row D. **Wash Comb under cool tap water** and insert it into **row D** 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 10 minutes. Mix as described above.**

■ Pierce the foil of the next well(s) in row F. Upon completion of color development in row **E**, move Comb to **row F** for **2 minutes** for color fixation. Take Comb out, shake off excess liquid and let it dry for 5 minutes before reading the results.



X. READING AND INTERPRETING THE IgG ANTIBODY RESULTS

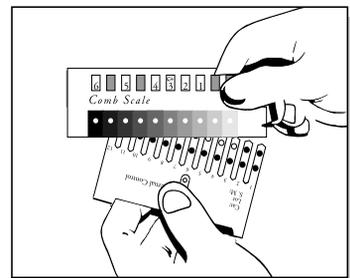
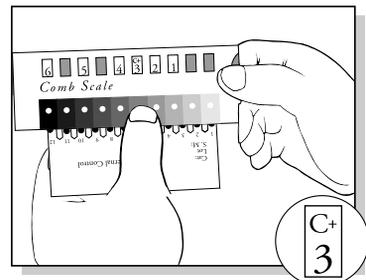
1. While reading this section refer to the example of a developed Comb and the table on page 7.
2. The lower spot on the Comb is the *Chlamydophila psittaci* spot.
3. Evaluate the results of each spot separately.
4. *C. psittaci* IgG level is determined by comparing each specimen's color intensity to the Positive Control (C+) color intensity.
5. The intensity of purple-grey color accepted on the test spots is scored on a scale of 1 to 6 by comparing any test color result to the Positive Control color result, which is scored as 3 and to a color scale (CombScale - see section XI).
6. Results with identical or higher color intensity than the Positive Control are scored $\geq S3$, which indicates high antibodies titer. Results scored as S2 are considered positive as well. Results scored <2 and >1 are considered inconclusive.
7. Different species of birds present different sensitivity in this test. A low positive result may not be significant in more sensitive bird species and vice versa. For detailed information about the species and their degree of sensitivity, please refer to our website (www.biogal.co.il) for product information.
8. The Negative Control consists of non-immune sera and should be read as zero (S=0). Specimens with no color or only a trace of purple-grey are scored as S0 or S1 and are considered negative.
9. In section XII you'll find example of a developed Comb and its results interpretation.
10. The dry Comb may be kept as record.

XI. READING RESULTS WITH THE COMBSCALE

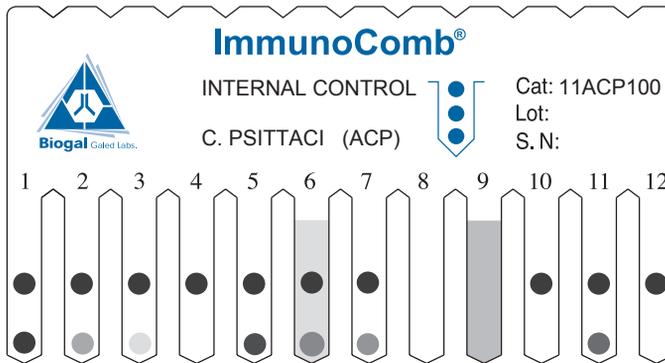
When the Comb is completely dry, align it with the calibrated color CombScale provided in the kit. Find the tone of purple-grey on the CombScale that most closely matches the tone of purple-grey on the Comb that most closely matches the **Positive Control spot**. Slide the yellow ruler until the C+ mark 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+ color 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.



XII. EXAMPLE OF A DEVELOPED COMB



TOOTH No.	RESULTS	REMARKS AND SCORES ("S")
1, 5	High positive reaction for <i>C. psittaci</i>	S >3
2, 7	Positive reaction for <i>C. psittaci</i>	S ≥ 2
3	Low reaction for <i>C. psittaci</i>	S1-2 Inconclusive - Considered suspicious
4,10	Negative reaction for <i>C. psittaci</i>	Negative S<1
6	High background with positive reaction	ACP positive
8	No internal control-Development failed	Invalid test
9	High background color	Invalid Test
11	Positive control	S 3
12	Negative control	S 0

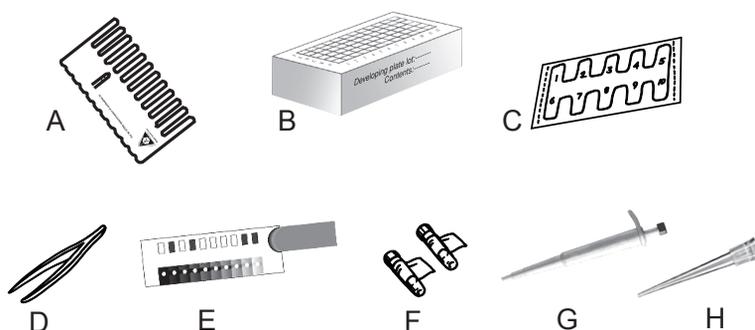
Another way to read the results is by using the CombScan. This is a software program that utilizes a computer and a twain compatible scanner. When a Comb is placed on the scanner, the program translates the color results into numerical values. The CombScan assists labs in reading ImmunoComb results and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

As with all diagnostics tests, a definitive clinical diagnosis should not be based entirely on the serological results, but should only be made by the veterinarian after all clinical and laboratory findings have been evaluated.

For further assistance please contact your local Distributor, or Biogal Gated Laboratories directly by e-mail: info@biogal.co.il or by tel: 972-4-9898605 / fax: 972-4-9898690.

XIII. KIT CONTENTS

Components	10 Test Kit (50ACP301)	100 Test Kit (50ACP310)
A. ImmunoComb card (wrapped in aluminum foil)	1	10
B. Developing plate	1	10
C. Specimen paper with prepunched disks	1	10
D. Disposable tweezers	1	1
E. Calibrated CombScale color card	1	1
F. Positive and Negative Control serum tubes	1 of each	1 of each
G. Junior fix pipette 5µl	1	-
H. 10 µl universal grad tip	15	-
Instruction manual	1	1



XIV. REFERENCES

- Bendheim et al. (1994). Proceedings of Deutsch Veterinarmedizinische Gesellschaft, Munchen, Germany.
- Lublin et al. (1997). The 4th Conference of the European Committee of the Association of Avian Veterinarians, May, London, England.
- Morales et al. (2007). Proceedings of the 12th International Conference of the Association of Institutions for Tropical Veterinary Medicine (AITVM), Montpellier, France, 20-22 August, 2007. Does control of animal infectious risks offer a new international perspective?
- Phalen et al. (1999). Proceedings: Birds and all that Jazz. 20th Annual Conference and Expo, September, New Orleans, Louisiana, USA.
- Phalen D. N. (2001). Seminars in Avian & Exotic *Pet Medicine*, **10** (2), 77-89.