ImmunoComb

AVIAN CHLAMYDOPHILA PSITTACI ANTIBODY TEST KIT

INSTRUCTION MANUAL

Sufficient for 10/100 assays

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Instruction Cat. No: 63ACP711

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. 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.

III. 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 minutes or 120 minutes when using blood saturated paper disks.

IV. HOW DOES THE IMMUNOCOMB WORK?

- The ImmunoComb Kit contains 2 main components: a comb shaped plastic card, hereafter referred to as the Comb and a multi compartment developing plate.
- The Comb has 12 teeth. Each tooth will be developed in a corresponding column of wells in the developing plate. Samples (whole blood saturated paper disks, whole blood or serum) should be deposited into

separate wells in row A of the developing plate.

- Test spots of *C. psittaci* antigen are attached to the lowest spots on each tooth of the Comb. The top spot is the Internal Control, which indicate that the development is complete and valid. (See figure in section X).
- The first step of the test is to deposit samples (whole blood saturated paper disks, whole blood or serum) in a well in row A of the multi-compartment developing plate.
- 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 must include the Positive and Negative Controls supplied in the kit.**
- Next, the Comb is inserted into the well(s) with the sample(s) and transferred to the remaining wells (B-F) at timed intervals, according to the step by step instructions (see section VII). Specific IgG antibodies from the specimen, if present, bind to the antigen at the test spots and will be labeled in row C, which contains an enzyme labeled anti-parrot IgG antibody.
- At the end of the developing process, a purple-grey color results are developed in all Internal Control spots and in any positive sample tested spot.
- The intensity of the color result corresponds directly to the antibody level in the test specimen. Results are scored using the Positive Control tooth and CombScale (see section IX).

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. STEP BY STEP WITH IMMUNOCOMB

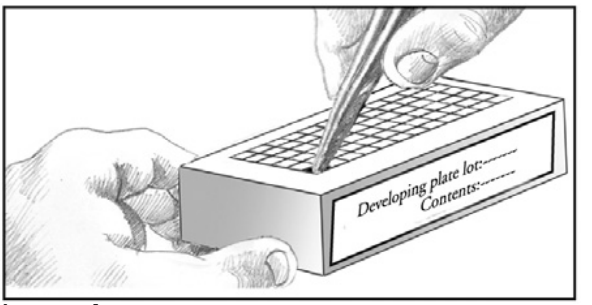
Before conducting the test, bring the developing plate to room temperature by removing all kit components from the kit carton and place them on the work bench for 60-120 minutes or incubate only the plate at 37°C/98.6°F for 25 minutes.

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

(1) Obtain sample from the bird (whole blood saturated paper disks, whole blood or serum). 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. (See Pipetting Technique section). Avoid spillage and cross-contamination of solutions.

***For whole blood only: If dispensing the sample with a fix pipette provided with kits catalogue number 50ACP401 , use the same tip to deposit twice 5µl into the same well in row A.**

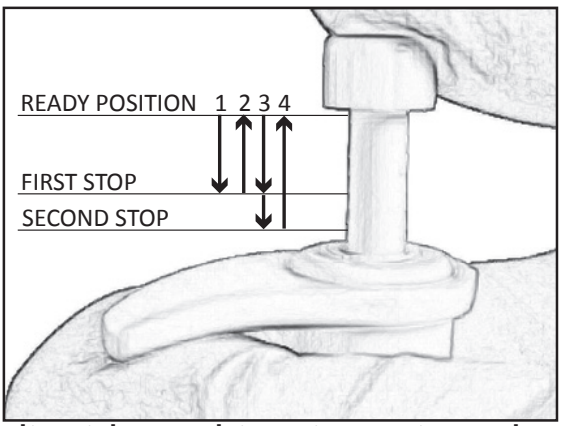
(4) Open the next 2 consecutive wells for control serum.
Take 5µl Positive Control serum (C+) and insert into well A next to the last sample. Mix the serum into the well.
Do the same with the Negative Control serum (C-) in the following well.

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.

Pipetting Technique

Forward Pippeting

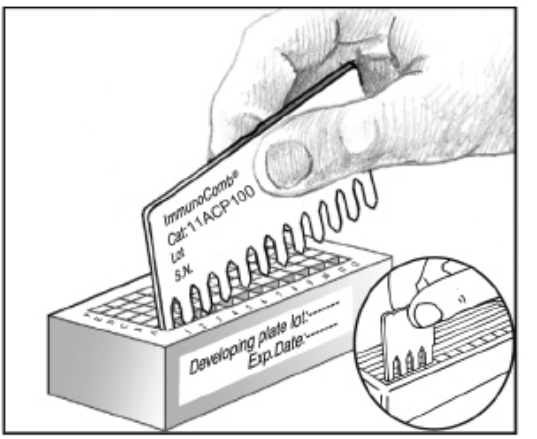


1- Press the operating button to the first stop.

2- Dip the tip attached to the pipette into the sample to a depth of about 1 cm and slowly release the operating button. Wait for a while, then withdraw it from the liquid touching it against the edge of the reservoir to remove excess liquid adhering to the the outer surface of the tip.

3- Dispense the sample into a well in row A by gently pressing the operating button to the first stop. After a second, press the operating button to the second stop. This will empty the tip completely. Remove the pipette from the well.

4- Release the operating button to the ready position.

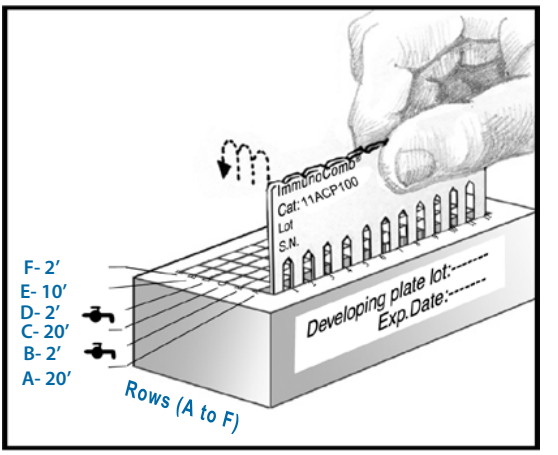


(5) Remove the Comb from its protective envelope. **Do not touch the teeth of ImmunoComb card.** For testing less than 12 samples, cut or break the Comb by folding in allocated notches for the number of tests required.

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.**

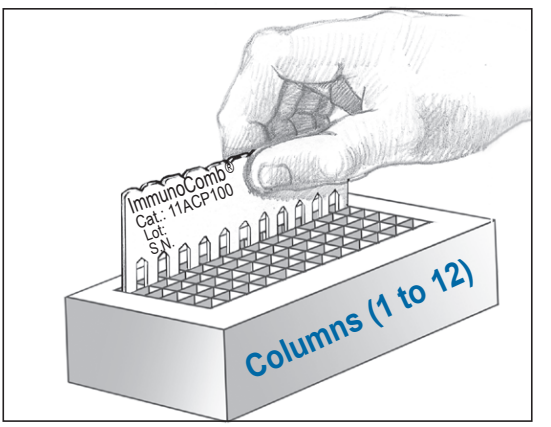
Avoid scratching the front active side of the Comb by leaning it to the back while mixing.
Gently shake off excess liquid from Comb teeth onto a tissue before moving it to the next row.



- 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.

VIII. READING AND INTERPRETING THE IgG ANTIBODY RESULTS

■ The lower spot on the Comb is the *Chlamydophila psittaci* spot.

■ *C. psittaci* IgG level is determined by comparing each specimen's color intensity to the Positive Control (C+) color intensity.

■ 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 IX).

■ Results with identical or higher color intensity than the Positive Control are scored ≥S3, which indicates high antibodies titer. Results scored as S2 are considered positive as well. Results scored <S2 and >S1 are considered inconclusive.

■ Different species of birds present different sensitivity in this test. A low positive result may not be significat 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.

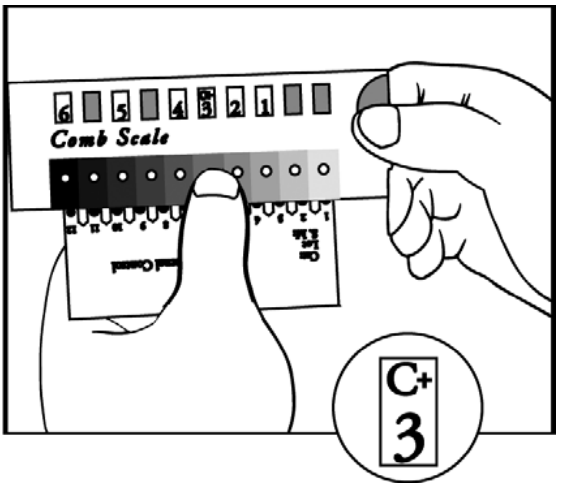
■ The Negative Control consists of non-immune sera and should be read as zero (S=0). Specimens with no color or only a trace of purple-grey are scored as S0 or S1 and are considered negative.

■ The dry Comb may be kept as record.

IX. READING RESULTS WITH THE COMBSCALE

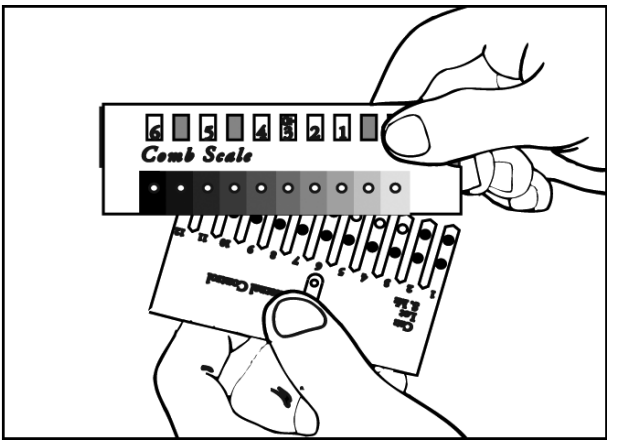
The CombScale S value is the number that appears in the yellow window corresponding to the color tone, when Positive Control color is calibrated to S3.

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 **Positive Control tooth**. Slide the yellow ruler until the C+ mark appears in the window above that color you just found.

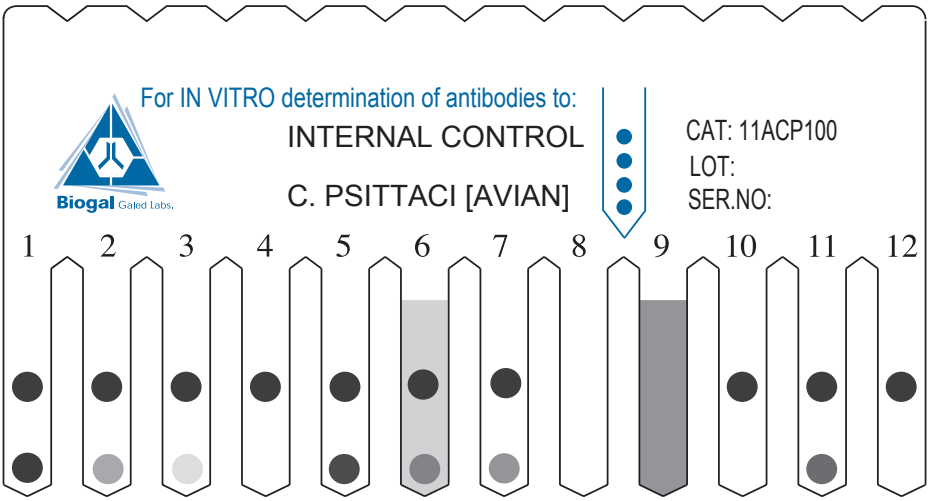


Hold the ruler in this position during the entire reading. This step actually calibrates the C+ to S3, which is the "cut-off" point to which test spots will be compared.

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.



X. EXAMPLE OF A DEVELOPED COMB

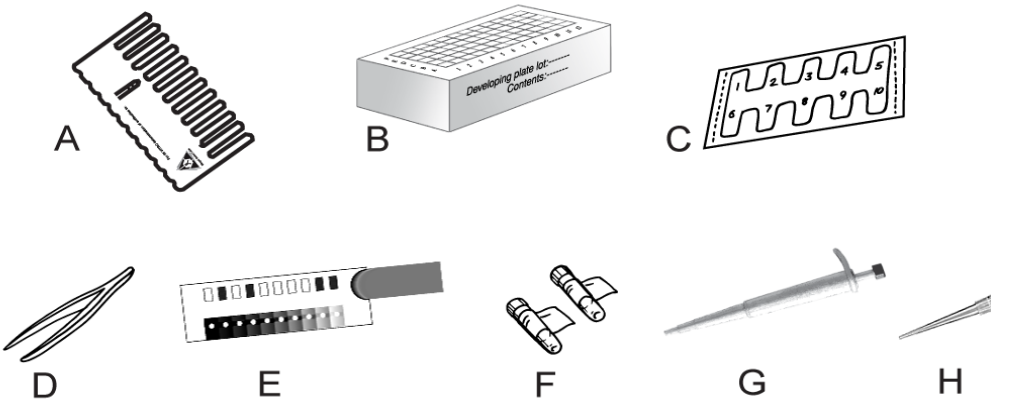


Tooth Nº	Results	
1,5	**S>3**	High positive reaction to *C. psittaci*
2,7	**S≥2**	Positive reaction for *C. psittaci*
3	**S1-2**	Inconclusive - Considered suspicious
4,10	**S0**	Negative reaction to *C. psittaci*
6		Positive reaction with high background
8	*	No internal control Development failed
9	*	High background color - interferes with reading.
11	**S3**	Positive control
12	**S0**	Negative control

*** Repeat Test**

XI. KIT CONTENTS

Components	10 Test Kit (50ACP401)	10 Test Kit (50ACP501)	100 Test Kit (50ACP310)
A. ImmunoComb card (wrapped in aluminum foil)	1	1	10
B. Developing plate	1	1	10
C. Specimen paper with prepunched disks	1	1	10
D. Disposable tweezers	1	1	1
E. Calibrated CombScale color card	1	1	1
F. Positive and Negative Control serum tubes	1 of each	1 of each	1 of each
G. Junior fix pipette 5µl	1	-	-
H. 10 µl universal grad tip	15	-	-
Instruction manual	1	1	1



XII. STORAGE & HANDLING

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

XIII. 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 and plasma samples 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.

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.

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