Product Information (30.10.07)

Name of Kit: **ImmunoComb® Avian *Chlamydophila psittaci* Antibody Test Kit**

Catalog No: 50ACP201 / 50 ACP210

No of Tests: 12 (Standard Kit) /120 (Lab Kit)

Intended Use: This kit is designed to determine IgG antibody titers to *Chlamydophila psittaci* (previously known as *Chlamydia psittaci*) in avian blood (or serum).

Diagnostic Method: The ImmunoComb® test is based on solid phase "dot"-ELISA technology. Antigens are applied to test 'spots' on the solid phase, which is a comb-shaped plastic card. (The Comb has 12 teeth-sufficient for 12 test samples.)

The samples to be tested are mixed with diluent in the first row of wells of a multi-chamber developing plate. The test spots on the Comb are then incubated with the sample in the developing plate. Specific IgG antibodies from the samples, if present, bind to the antigens at the test spots.

The Comb is then transferred to a well, where unbound antibodies are washed from the antigens spots. In the next step, the Comb is allowed to react with an anti-avian IgG Alkaline Phosphates conjugate, which will bind to antigen-antibody complexes at the test spots. After 2 more washes, the Comb is moved to the last well, where a color result develops via an enzymatic reaction. The intensity of the color result of test spots corresponds directly to the antibody level in the test sample.

Review of disease: *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 where infection is relatively common 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.

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.

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 antibody in the birds blood:** Techniques include complement fixation, elementary body agglutination and the 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 method except the ImmunoComb® are performed by specialized laboratories.

Main Applications:

- Assisting the clinical diagnosis of *Chlamydophila psittaci* infection in individual birds.

- Identifying latently infected birds, in order to control bird-to-bird transmission and prevent potential human infection.

- Performing serological follow-up and evaluation of the success of antibiotic treatment in sick birds.*

* **NOTE:** It is recommended to save serum or blood on a pre-punched disk when testing sick birds the first (acute) time. This sample can be retested as a reference, with the 2nd follow-up (convalescent) sample. Serum can be stored frozen. When blood sample is obtained on filter paper disks (provided in the kit), one disk might be used for the current test, while the second disk may be refrigerated (sealed in a plastic bag) for up to several months.

Technical Tip for Collecting Blood from a Bird: When collecting blood, use caution to avoid sample cross contamination and/or infection to birds. Between birds you should wash hands, sanitize clippers with alcohol, and avoid touching the pre-punched disks on the filter paper where the blood is to be placed. Label the tooth of the filter paper (with the 2 pre-punched disks) with the bird's ID and species beforehand. This is important so that you can match the results to the proper birds.

With the bird under control, examine the toenail to insure that it is clean. A swab of alcohol can be used to clean the area if necessary. Use a clean pair of nail clippers to clip the toenail just enough to nick the vein and produce blood flow. This should be approximately 2/3 of the distance from the root of the toenail (See illustration below).

Remove the first bead of blood with a swab or absorbent cotton ball, then carefully place the pre-punched disks to the blood flow and absorb a few drops of blood. When blood flow is slow, one should squeeze gently on the toe with a pumping action. It is necessary to completely fill both disks on both sides of the filter paper. The usual amount of blood needed is 1-3 drops.

Once the sample has been collected, set the filter paper aside to dry on a clean surface. Check that the bird is no longer bleeding before returning it to its cage. To stop further bleeding, apply a coagulant such as Kwik Stop to the area. Cornstarch or flour may also be applied as alternatives.

Allow the disks to dry completely by leaving them at room temperature for 30-60 minutes. Once the disk is completely dry, it may be stored in a plastic bag or used for testing.

In order to perform the ImmunoComb® Avian Antibody Test for *Chlamydophila psittaci* place the dry disk at the bottom of the well in row A of the developing plate one hour before beginning the test.

Table 1. Interpretation of Results

Color	CombScale Value	Result	Interpretation
White	0	Negative	No antibodies to *C. psittaci.* Retest in 7-10 days in acutely ill birds.
Trace of Gray	>0 - 1	Suspicious	Variable, according to type of bird (see reference chart).
Light Gray	>1 - 2	Low Positive	Low antibody titer to *C. psittaci* (see reference chart).
Medium Gray	3 - 4	Positive	Moderate level of antibodies to *C. psittaci.*
Dark Gray	5 - 6	High Positive	High antibody titer to *C. psittaci.*

Variability of Antibody Response, by the ImmunoComb®, to *C. psittaci* in Different Avian Species

Reference Chart

	Very Sensitive (1)	Sensitive	Less Sensitive (2)
Psittacines	African Grey Macaw (Ara) Timneh Conure Amazone Cockatoo Roselia	Lovebird Parakeet Princess Lorikeet Cockatail Lory	
Other Birds	Turkey Peacock Pheasant Guinea Fowl	Ostrich Quail Myneh Owl (Uhu) Black Kite Volture Toucan	Pigeon Pelican Swan Eagle Starling

1. Suspicious or low positive results may **not** be significant in these birds (i.e., 'false positives').
2. Suspicious or low positive results most probably **are** significant in these birds.

References:

Bendheim, U., Wodowski, I., Ordonez, M. & Naveh, A. (1994). Entwicklung eines ELISA kit fur die antikorperbestimmung bei psittaziden. (The development of an ELISA-kit for antibody determination in birds including poultry and psittacines.) Proceedings of *Deutsch Veterinarmedizinische Gesellschaft, Munchen, Germany.*

Lublin, A., Leiderman, E., Mechani, S., Malkinson, M. & Weisman, Y. (1997). Influence of ambient temperature on shedding of *Chlamydia psittaci* in pigeons. *The 4th Conference of the European Committee of the Association of Avian Veterinarians,* ***May****, London, England.*

Phalen, D. N. (2001). The use of serologic assays in avian medicine. *Seminars in Avian & Exotic Pet Medicine*, **10 (2)**, 77-89*.*

Phalen, D. N., Hofls, M., Dahlhausen, B. & Styles, D. (1999). Diagnosis of *Chlamydia psittaci* infections in cocktiels and columbiformes. *Proceedings: Birds and all that Jazz. 20th Annual Conference and Expo,* ***September****, New Orleans, Louisiana, USA.*