Product Information (08.07.10)

Name of Kit: ImmunoComb® Poultry Mycoplasma (MG-MS-MM) Antibody Test Kit

Catalog No: 50PMT103/50PMT130

No of Tests: 30 (Standard Kit) / 300 (Lab-size Kit)

Intended Use: Mycoplasmas of poultry are an important cause of lost productivity in chicken and turkey flocks, worldwide. Many commercial flocks are raised free of one or more mycoplasmas; to ensure this status, intensive serological testing programs have been adopted to identify and eradicate infected breeder flocks.

The ImmunoComb® Kit is designed as a user-friendly, multiple-test kit for the determination of antibody levels against *Mycoplasma synoviae* (MS), *Mycoplasma gallisepticum* (MG) and **Mycoplasma meleagridis* (MM), simultaneously in the same sample. It can be used conveniently on a variety of sample materials: whole blood, serum, or egg yolk. This format is especially suitable for the serological monitoring of Mycoplasma-free flocks, and for the diagnosis of clinical or subclinical infection.

* Results obtained for MM are relevant for turkeys only and should be ignored when chickens are tested.

Diagnostic Method: The ImmunoComb® test is based on solid phase "dot"-ELISA technology. Antigen is applied to test 'spots' on the solid phase, which is a comb-shaped plastic card. The specimen to be tested is diluted in a buffer in the first well of a multi-chamber developing plate. The test spots on the Comb are

then incubated with the samples in the developing plate. Specific IgG antibodies from the specimen, 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 antigen spots. In the next step, the Comb is allowed to react with an anti-chicken IgG Alkaline Phosphatase conjugate, which will bind to antigen-antibody complexes at the test spots. After 2 more washes, the Comb is moved into 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 specimen.

Immunology: Serology is widely used to screen poultry and turkey flocks for the presence of MS, MG and MM infection. In infected birds, IgG antibody is present in serum, and in the yolk of eggs laid, from about 3 weeks post-infection, and persists indefinitely. Hence regular testing of an adequate number of samples from a Mycoplasma-free flock will give prompt warning of the entry of infection. In flocks with clinical signs of infection serology can be used to confirm the clinical diagnosis.

A large proportion of day-old progeny from infected chickens and turkeys have maternal antibody, which declines in the days after hatching, and so sampling from chicks/poults on the day of hatch can be used to detect vertical transmission from the parent flock. Adequate sample size is important, particularly if only a low level of infection is present in the parent flock.

Please note: In some countries vaccines against MG and MS are available. Serological responses to vaccination are variable depending on the type of vaccine used: in particular some live vaccines may cause little or no response to standard serological tests, and specialized testing is needed to detect vaccinal antibody. In cases where wild-type infection is present in a vaccinated flock the serological picture is further complicated. In situations where vaccines have been used, extreme care must be taken in the interpretation of serological results, taking into account the specific vaccine used, and the use of

additional testing methods may be necessary to ensure vaccine "take" or distinguish vaccination from infection.

Interpretation: The level of antibodies (i.e., antibody titer) is determined according to the intensity of the test color result. Thus, no or a light grey color indicates no (negative) or low level of antibodies. Higher levels of antibodies are indicated by darker color results. The color intensity is assigned a value from S0 (negative) up to S6.

Reading tip: If you are in doubt about which value to assign the color of a test result (e.g. between S3 and S4), always choose the lower value (S3 in this case).

Table 1. Interpretation of "S" Values

CombScale "S" Value	**Interpretation** MM Reading is relevant for turkeys only.
"S" = 0 to 1	Undetectable **levels of antibody to MS, MG or MM.**
"S" = 2 to 3	**Medium antibody level** -Retest 2 weeks later for seroconversion -A significant rise of antibody levels above "S"=2 indicates active infection.
"S" = 4 to 6	**High antibody level** -Infection in unvaccinated flock -Adequate vaccinal antibody in vaccinated uninfected flock.

Sample size and frequency of sampling

Important: For the diagnosis of flock infection, it is essential that sample size is adequate, and that sampling is representative. Birds from all parts or rooms of the house, and from all sources (e.g. male and female breeders) must be sampled. The lower the incidence of infection, the larger the sample size needed to be able to detect it.

Applications:

1. For the periodical surveillance of Mycoplasma-free flocks.

2. As an aid in the diagnosis of MS or MG– related diseases, and in turkeys, also in the diagnosis of MM.

3. For the serological screening of hatching eggs, or day-old chicks or poults, for MS, MG (and MM in turkeys).

4. For the evaluation of antibody levels in vaccinated flocks, interpreting the results in the light of the specific vaccine used.

Other Diagnostic Methods:

Other serological methods:

(a). Serum Plate Agglutination (SPA) can be used as a simple, sensitive screening test for MG and MS, but false positives are more likely to occur. The ImmunoComb® test is a useful confirmatory test where the SPA test is used for screening. Data from a comparison between SPA and ImmunoComb® are given in Table 2, overleaf.

(b). Haemagglutination Inhibition (HI) is sometimes used as a confirmatory test, but is time-consuming and may lack sensitivity.

(c). Other ELISA methods are available similar to the ImmunoComb® test but lack the convenience and simplicity of the ImmunoComb® method. Some specialized tests have been developed for antibodies to MG and MS vaccine strains.

(d) Direct detection of mycoplasmal DNA (such as PCR), or isolation of mycoplasma, can be used for final confirmation of infection.

Table 2. A Comparative Study:

The ImmunoComb® (IC) and Serum Plate Agglutination (SPA) for *Mycoplasma gallisepticum* and *Mycoplasma synoviea* in Poultry

A study performed by the Afula regional Poultry Health Station, Afula, Israel, 1987.

		SPA TEST[1]	SPA TEST[1]	IC[2]	IC[2]
FLOCK	**TYPE**	**MG**	**MS**	**MG**	**MS**
1	Layers	0/25	0/25	0/25	1/25
2	-#-	0/26	4/26	0/26	5/26
3	-#-	0/21	0/21	0/21	0/21
4	-#-	0/18	0/18	0/18	0/18
5	Breeders	20/20	0/20	20/20	0/20
6	-#-	20/20	0/20	19/20	0/20
7	Turkeys	0/20	3/20	0/20	2/20
8	-#-	0/20	0/20	0/20	0/20
9	-#-	19/19	0/19	14/19	0/19
10	-#-	1/11	0/11	2/11	0/11

1. No. of positive / No. tested
2. No. of positive / No. tested; positives are specimens resulting in an ImmunoComb® reading equivalent to S2 or above.

References:

Chhabra, P. C. & Goel, M.C. (1981). Immunological response of chickens to *Mycoplasma gallisepticum* infection. *Avian Diseases,* **25**: 279-293.

De Wit, Sjaak J.J. (2002). Blood testing as a diagnostic tool. *International Poultry Production,* **12** (1): 18-20.

Ewing, M. L., Lauerman, L. H., Kleven, S. H. & Brown, M. B. (1996). Evaluation of diagnostic procedures to detect *Mycoplasma synoviae* in commercial multiplier-breeder farms and commercial hatcheries in Florida. *Avian Diseases,* **40:** 798-906.

Kempf, I. & Gesbert, F. (1998). Comparison of serological tests for detection of *Mycoplasma gallisepticum* antibodies in eggs and chicks hatched from experimentally infected hens. *Vet. Microbiol.,* **60:** 207-13.

Ley, D. H. (2003). *Mycoplasma gallisepticum* infection. Kleven, S. H.: *Mycoplasma synoviae* infection. Chin, R. P., et al.: *Mycoplasma meleagridis* infection. In Chapter 22 of *Diseases of Poultry,* 11th Edition, ed. Saif, et al.

Nicolet, J. (1996). Scientific and Technical Review: Animal Mycoplasmoses and Control. *International Office of Epizootics*, **Vol. 15 (4)**.

(PI PMT 08/07/10)