

Product Information

Name of Kit: ImmunoComb® Poultry IBD-ND-IB
Antibody Test Kit

Catalog No: 50PTV203/50PTV230

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

Intended Use: The Kit is designed to determine serum, blood, or egg-yolk antibody titers against Infectious Bursal Disease, Newcastle Disease and Infectious Bronchitis Virus, simultaneously in the same sample. This trivalent format is especially useful for monitoring the antibody response of flocks after vaccination, and for periodical serological surveillance of flocks.

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 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 samples 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-chicken IgG Alkaline Phosphatase 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.

Immunology: Serology can provide the veterinarian with information about the presence/absence and the quantity of antibody present in birds. Presence of antibody could be due to several different reasons:

- *Passively acquired maternal antibody (in chicks).
- *Active humoral immunity following vaccination.
- *Active humoral immunity after infection.

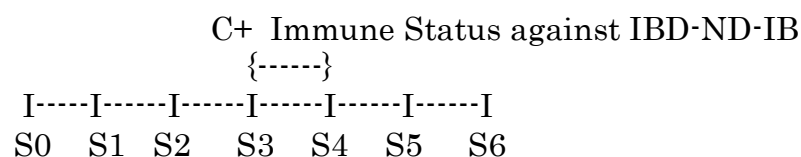
In a group of birds, the antibody level may vary between individuals: **flock profiling** by testing a random representative sample of birds will give an indication of the degree of variation.

Periodic surveillance during flock life will show changes in flock titer in response to the vaccinations given, and assist in monitoring vaccination efficacy and decision-making as to the timing of vaccinations. If a rise in antibody correlates with the appearance of clinical signs of disease, a presumptive clinical diagnosis can be made.

ImmunoComb® results have been shown to correlate well with other serological methods, including Serum Neutralisation (SN) and ELISA for Infectious Bursal Disease, and Hemagglutination-Inhibition (HI) for Newcastle Disease and Infectious Bronchitis. It is not necessary in general use to convert the Immunocomb score into units of the other tests: the Immunocomb values are easily understood and especially convenient for comparison of results, over a period of time or between different flocks.

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. In vaccinated flocks, values of S3 and above indicate adequate levels of humoral immunity.

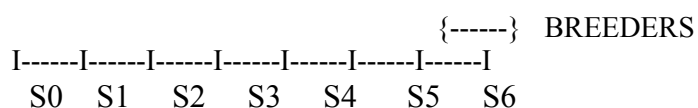
Fig. (1)



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).

Recommended Values for Breeders: In order to assure transmission of maternal antibodies to their eggs, values in breeding hens between S5 and S6 are considered optimum.

Fig.(2)



Recommended Values for Chicks: Upon hatching, chicks should then have antibody titers of S4-S5, due to the passive transfer of maternal antibodies via the yolk sac to the embryo. In the days after hatching this passive antibody declines steadily, until vaccination or exposure to disease, after which titers rise due to active immunity within 2-3 weeks.

In many cases the presence of maternal antibody, while protecting the young chick from disease, also interferes with vaccination by preventing activation of the immune system (e.g., Infectious Bursal Disease). Here the timing of vaccination is crucial, the optimum time being as soon as maternal antibody has fallen low enough to allow the chick to respond to the vaccine. Early vaccination may result in failure to immunize the birds, while late vaccination leaves a “window” of time in which the flock is unprotected.

In practice the maternal antibody level differs between breeding flocks, and hence we suggest close monitoring of young chicks, from the first day of life, to ensure that antibody levels do not fall below a minimum of S3, and to optimize timing of vaccinations.

Interpretation of Flock Profile: When the ImmunoComb® results of individual birds are plotted as a Histogram, or bar graph (CombScore sheet), the results depict the degree of uniformity of antibody levels in the birds tested. This is referred to as a “Flock Profile”.

Important: Flock profiles should be based on a minimum of 30 birds per flock randomly selected (flock size 5,000 to 10,000 birds, with a common source and vaccination history). The two examples below illustrate why the flock profile as a whole, and not just the mean CombScore, need to be considered.

Example A of Flock Profile: See Fig.3.

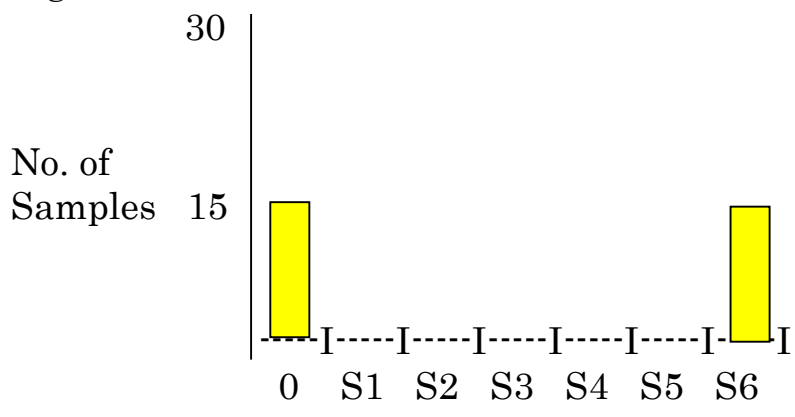
Of 30 birds tested: 15 birds had antibody level S6
 15 birds had antibody level S0 (negative)

Calculation of CombScore for flock:
 15 birds x S6 = 90
 15 birds x S0 = 0
 90 Total Score

30 birds tested

$$\text{CombScore for flock} = \frac{\text{Total score}}{\text{No. of birds tested}} = \frac{90}{30} = 3$$

Fig.(3):



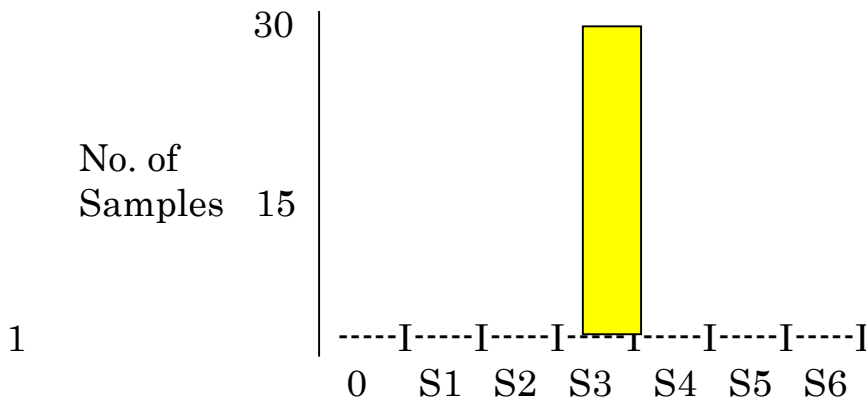
Conclusions: The average CombScore, S3, suggests that the flock has adequate antibody for protection against disease. However, the flock-profile histogram clearly shows that only half of the birds have antibody. This picture could result from faults in the vaccination program, or from an outbreak of disease.

Example B of Flock Profile: See Fig.4.

Of 30 birds tested, all had antibody level S3.

$$\text{CombScore} = \frac{\text{Total Score}}{\text{No. of birds tested}} = \frac{30 \times S3}{30} = \frac{90}{30} = 3$$

Fig.(4):



Conclusions: The average CombScore is the same as in example A, but the flock profile shows that the antibody levels of the birds are uniform. This indicates that the vast majority of the flock has a reasonable level of protection.

The above examples depict extreme situations: in real life most flock profiles are somewhere in between these two extremes. Adequate samplings, and careful examination of the flock profile, are essential in understanding the immune status of the flock.

Main Applications:

1. To evaluate vaccine efficacy. Monitoring 2-4 weeks after vaccination to check level and uniformity of response is of economic value, especially in breeders.
2. To calculate appropriate time of vaccination. Monitoring the level and decline of maternal antibody in chicks allows appropriate timing of vaccinations, such as Infectious Bursal Disease, which may be interfered with by maternal antibody. (See “Interpretation”). In breeders, declining vaccinal titers as flocks age may indicate the need for revaccination to ensure optimal protection for offspring.

3. To screen flocks periodically. Comparing vaccination programs and diagnosing flock diseases fast and correctly.

Routine testing of all breeder flocks at regular intervals allows the breeding company to build up a useful “bank” of data, and thus variations from the “norm” due to exposure to field virus, or to a fault in the vaccination procedure, can be easily identified.

Results obtained must always be interpreted in the light of the flock’s vaccination program, location of farm, and the presence of significant clinical signs. Comparisons should **not** be made between different kits, supplied by different manufacturers, as there will be numerical discrepancies between the results, though the general conclusions correspond.

Other Diagnostic Methods:

- (i) Other serological methods: HI, ELISA, SN: for confirmation of antibody levels.
- (ii) Virology, or direct detection of viral DNA (such as PCR), or viral antigens (such as immunodiffusion), to confirm the diagnosis of disease.

References:

Brown, J., Thayer, S.G., and Fletcher, O.J. (1988). A method to rapidly convert Newcastle disease virus and infectious bronchitis virus ImmunoComb scores into conventional hemagglutination-inhibition titers. *Avian Diseases*, **32**, 517-518.

De Herdt, P., Ducatelle, A.R., Uyttebroek, A.E., Sneepe, A., & Torbeyns, R. (2001). Infectious bronchitis serology in broilers and broiler breeders: correlations between antibody titers and performance in vaccinated flocks. *Avian Diseases*, **45**, 612-619.

Naqi, S.A., Marquez, B., Sahin, N. (1983). Maternal antibody and its effect on infectious bursal disease immunization. *Avian Diseases*, **27**, 623-631.

Thayer, S. G., Nersessian, B. N., Rivetz, B. & Fletcher, O. J. (1987). Comparison of serological tests for antibodies against Newcastle Disease Virus and Infectious Bronchitis Virus using the ImmunoComb® solid-phase immunoassay, a commercial enzyme-linked immunosorbent assay, and the hemagglutination-inhibition assay. *Avian Diseases*, **31**, 459-463.

(PI PTV 13/7/04)