

Serological monitoring and interpretation of ELISA results

by Jose A. Linares, DVM, ACPV, technical director, Zoetis Global Poultry.

The poultry immune system reacts to many diseases by producing antibodies. This interaction between the host and pathogen gives us the opportunity to detect, quantify and/or monitor the immune status of birds using serology.

In poultry flocks, serological monitoring for field challenges or response to vaccination is particularly important for diseases for which protection is highly dependent on serum antibodies, such as infectious bursal disease, Newcastle disease, reovirus and avian encephalomyelitis. For certain diseases, such as mycoplasmosis and avian influenza, the detection of antibody in unvaccinated flocks is highly suggestive of field exposure.

Flock health monitoring through serology is used to correlate results with clinical signs and/or performance issues, detect breaches in biosecurity, recommend best practices (design vaccination strategies or evaluate vaccine administration) and to aggregate data. The analysis of aggregated serology results is useful to establish trends related to disease exposure or response to vaccination, detect subclinical disease, and to establish flock profiles or operational baselines.

ELISA (Enzyme-Linked Immuno-sorbent Assay) is a highly specific, sensitive and reproducible serological test. Many commercial pathogen-specific test kits are available. This testing platform can be semi- or fully automated, which is critical for high throughput testing, for example in response to a high consequence disease outbreak.

Table 1. A simple alternative to determine the number of samples required for 95% confidence of detecting disease in populations larger than 1,000 birds.

Minimum prevalence (%)	Interval	Number of samples
1	1 in 100	3 x 100 = 300
10	1 in 10	3 x 10 = 30
25	1 in 4	3 x 4 = 12

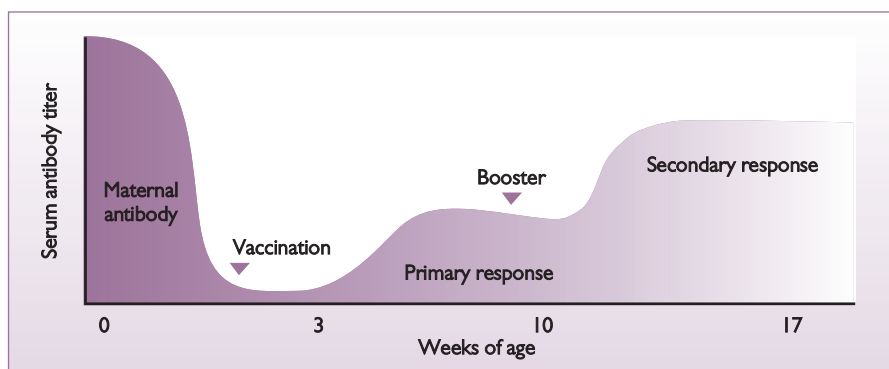


Fig. 1. Visual representation of the avian humoral (antibody) response (Dr C. Lamichhane, Zoetis).

The ELISA test can be used as a diagnostic and vaccination evaluation tool. The interpretation of ELISA results is enhanced by an understanding of the avian antibody response, experience gained by comparing results with previously obtained results, and relating ELISA results to flock performance.

Surveillance

Before looking at the interpretation of ELISA results, it is worth taking a closer look at the principles that underlie the use of serology to monitor flock health. In order to obtain meaningful results it is important to collect the correct number and type of samples.

First, serology requires good quality serum. Samples with heavy hemolysis and/or bacterial contamination should be avoided. Random sampling, collecting samples from multiple locations within the poultry house, averages out the effects of variation within the flock.

It is very important to provide flock information such as age, current vaccination program and clinical history to the laboratory in order to help with test selection for diagnosis and the interpretation of surveillance results.

The number of samples tested per flock is determined by statistical and epidemiological principles. The formula for determining the sample size necessary to detect presence or absence of a disease in a large population (>1000) is as follows:

$$\text{Sample size} = \frac{\log(1 - \text{level of confidence})}{\log(1 - \text{prevalence})}$$

Table 1 shows a simple alternative to determine the number of samples required for 95% confidence of detecting disease in populations larger than 1,000 birds.

The number of samples based on 1% prevalence (300 samples) is commonly used to help certify primary breeders as free from diseases such as pullorum and typhoid. The number of samples based on 10% prevalence (30 samples) is recommended during disease outbreaks and to monitor breeder flocks. The number of samples based on 25% prevalence (12 samples) is commonly used for routine surveillance in commercial flocks. There are two main types of disease surveillance: passive and active.

- Passive surveillance is conducted when the flock is sick or not performing as expected. As the name implies this is a passive mode of surveillance which is not used until there is a problem. Serology can be used in this case as a diagnostic tool by collecting serum at the onset of disease and 2-4 weeks later, and then comparing results.
- Active surveillance consists of scheduled monitoring of flocks, even 'healthy' flocks. Active surveillance by serology could be used to certify valuable breeding stock as free of certain diseases, or to detect subclinical challenges, as pathogens may leave their 'prints' behind in the form of

Continued on page 16

Continued from page 15

serum antibodies. Rather than testing only when a disease problem exists, it is best to monitor flocks at key points:

- Monitor maternal antibody levels, to time vaccinations.
- Prior to vaccinations or at the onset of clinical signs (acute sample).
- 2-4 weeks post-vaccination or post-onset of clinical signs (convalescent sample).

The immune response

An understanding of the antibody response in birds can also help in the interpretation of serology results. Maternal antibodies, those

passed from the hen to the progeny via the yolk sac, disappear between three and four weeks of age.

The primary antibody response occurs at first exposure to an infectious agent or vaccine (primer). Serum antibodies can be detected within 7-10 days after vaccination or initial infection and usually reach a peak level between 14-21 days.

The antibody level usually declines and disappears within a few weeks after the antibody level peaks because the infectious agent was neutralised.

The second time a bird is exposed to an infectious agent or is revaccinated (booster), the immune response is usually faster and antibody levels are greater compared to the

primary response. This secondary antibody response is associated with memory cells, which quickly recognise the infectious agent and rapidly produce a higher immune response. A secondary antibody response usually declines more slowly over time.

ELISA tests

There are two types of ELISA tests, indirect and competitive. In the indirect ELISA colour development is proportional to the amount of antibodies. In the competitive ELISA colour development is inversely correlated to the amount of antibodies. The indirect ELISA test is widely used today as it is simple, inexpensive and quantitative.

Scheduled serological monitoring via ELISA allows users to establish baselines. For example, a basic broiler operation monitoring program could include collecting serum samples at 1-3 days of age to measure maternal antibody, followed by sampling at market age to measure the response to vaccination and also monitor for field infections.

Baselines can be created by aggregating antibody response results from multiple flocks with a similar vaccination program. These baselines help establish what is 'normal' for flocks in a farm, poultry operation or geographical area.

Deviations from the expected results could indicate potential problems, such as disease challenges, issues with vaccination procedures or dysfunction of the bird's immune system (immunosuppression).

Meaningful interpretation of ELISA results requires accurate flock information such as age and current vaccination program, and results must be assessed in the context of the health and performance of the whole flock. For example, routine surveillance could detect higher than expected antibody titer values to a particular pathogen for which a flock is vaccinated, but neither clinical signs nor a negative impact in flock performance observed.

Such a case would indicate that the vaccination program is working. The bird's immune system recognised the pathogen, mounted a specific antibody response, which in turn minimised the clinical effects of the infection.

In practice, the antibody response to priming live vaccines results in a low mean titer, low maximum titers and may have some negative sera.

Table 2. Guidance for interpreting CV values.

Result (%)	Interpretation
<30	Excellent
30-50	Good
51-80	Fair
>90	Poor

The response to the booster vaccine results in an increase mean titer, higher maximum titers and an increase in the uniformity of titer values [a lower Coefficient of Variation (CV)]. A lower CV means a more uniform response to challenge or vaccination.

Table 2 provides a guidance for interpreting CV values.

Possible causes of a high CV value are:

- Not all birds were vaccinated.
- In young birds, source breeder flocks of different ages have dissimilar antibody titers.
- Early exposure to an infectious agent.
- The serum samples were taken too soon after applying the last vaccine.

CV is just one of the parameters that a typical ELISA report may include. Indirect ELISA reports provide the following data:

- SP (Sample/Positive) ratio. Colour change is measured by an optical density (OD) reader. The SP ratio compares the OD of the test sample to the OD of a positive reference sample.
- Titer value. Calculated by a logarithmic transformation of SP values resulting in a scale used to compare one sample to another.
- The Arithmetic Mean Titer and Geometric Mean Titer. Values used to compare one flock to another flock. The Geometric Mean Titer allows better interpretation in a population with a higher variation in titer values.
- CV (Coefficient of Variation). The uniformity of titer values in a population.
- Titer groups. Groups or ranges of titers used as an aid to differentiate titer values. Titer groups simplify the graphical representation of titer values, referred to as a histogram.

Summary

Given all the variables affecting antibody response to vaccination or infection, it is highly recommended for poultry operations to establish serological flock profiles and baselines. Flock profiles are based on antibody responses from one flock at different ages.

Baselines aggregate antibody response results from multiple flocks with a similar vaccination program. Baselines help establish what is 'normal' for flocks in a farm, poultry operation or geographical area.

The good news today is that even once a baseline is established, poultry diagnosticians are not confined to using specific kits. In some cases ELISA kit manufacturers have developed tools that are able to assist in the mapping of historical baseline data from one product to another to allow easy conversion. ELISA results should be interpreted in the context of the flock health status and performance. The true value of serological surveillance is realised by the analysis of aggregated data to help optimise and protect operational results. ■

Sample	OD	SP ratio	Titer	Titer group	Result
1	0.700	1.000	4111	5	+
2	0.800	1.167	4926	6	+
3	0.900	1.333	5760	7	+
4	1.000	1.500	6613	8	+
5	1.400	2.167	10175	10	+
6	1.200	1.833	8366	9	+
7	0.900	1.333	5760	7	+
8	0.800	1.167	4926	6	+
9	1.200	1.833	8366	9	+
10	0.900	1.333	5760	7	+

Fig. 2. Infectious bursal disease (IBD) ELISA results.