

How can ELISA monitoring for titers improve your vaccination results?

by **Dr Bart van Leerdam, BioChek BV, Burg, Bracklaan 57, 2811 BP Reeuwijk, The Netherlands.**

The use of ELISA serology in poultry health management has been widely accepted as a useful tool to monitor the immune response following vaccination.

However, the veterinarian is often left with little or no practical guidelines for the interpretation of ELISA results following vaccinations.

Practical questions like “What level of titers and which coefficient of variation (CV) can I expect after vaccination?” and “Can I differentiate between good vaccination and poor vaccination using the ELISA?” and “What are protective titers after vaccination?” are often left unanswered.

This article highlights the use and interpretation of ELISA results after vaccination, and how serology can help in improving the effectiveness of vaccine application. Where possible, serological results from field case histories are discussed.

Justification of ELISA

Disease problems sometimes occur even in vaccinated birds. Is this due to the quality of the vaccine?

Maybe, but more often, vaccine breaks occur because of poor vaccine handling and/or poor vaccine application. Particularly, when dealing with live vaccination against respiratory diseases, like IBV and NDV, evaluating the success of vaccination is important.

This is because successful vaccination is not always imminent, as it is difficult to deliver an effective dose to 100% of the birds when using mass application techniques (drinking water and spray applications).

Furthermore, monitoring vaccination responses help to detect and diagnose vaccine failures, and will allow one to take corrective actions when vaccination has failed.

In this way, vaccination monitoring should be seen as a quality control of the performed vaccinations in the field.

This brings us to a very important point, when conducting ELISA monitoring; one has to be prepared to take proper action on results.

Without taking action on results, one cannot expect to improve, optimize and maintain the efficiency of vaccination programs.

Interpretation of results

To be able to successfully interpret ELISA results after serological monitoring of vaccinated flocks, one has to meet the following conditions:

- One must use external reference controls in the laboratory in order to give added assurance on the reproducibility and accuracy of results and allow for correct interpretation of results. Without reference controls one cannot know if abnormal titers, are the result of erroneous test procedures, or an actual reflection of the immune status of birds in the field.
- One must know what result to expect prior to testing (set baselines for successful vaccination and interpret results by comparing obtained results with these baselines).
- One must know what action to take if the results are not as expected.

The actual interpretation of vaccination results is usually done by evaluating the three main key components of an antibody response following vaccination, which are:

- Intensity of the response, as indicated by the mean titer. Do the birds develop titers levels in the expected range for the vaccine used? (=baseline titers).

These baseline titer values may vary according to the type of birds, age, vaccine type, vaccination program, etc. One should develop their own baselines for their vaccination programs and local conditions.

- Uniformity of response, as indicated by the %CV. Is the vaccine actually getting to the all the birds or not? Is the %CV within the required range or is there room for improvement?

The general guideline for %CV following vaccination is shown in Table 1.

Although these are general guidelines applicable to most live and inactivated vaccine applications, one should keep in mind that application with live vaccines against respiratory disease like IBV and NDV, generate in general variable titer responses,

The spread of respiratory live vaccines among flocks is often limited, and live respiratory vaccines also give a local immunity response, that can not be measured in ELISA.

So, when one vaccinates with live respiratory vaccines, the expected

%CV	Uniformity
Less than 40	Excellent
40-60	Good
Greater than 60	Need to improve

Table 1. The general guideline for CV% after vaccination.

CV for a good vaccination is 40-70%, rather than <40%. A CV of <30% after vaccination with H120 should be treated as suspect of challenge. However, when vaccinating with more immunogenic vaccines, like variant IBV strain 4/91, CV's below 45% are not uncommon.

In the case of breeders or layers, where a series of multiple live vaccinations are used to prime the birds before inactivated vaccination, complete seroconversion (100% positive birds) is a more important criterion for success than %CV alone.

It has been shown that good priming has a profound beneficial impact on the persistency of titers after inactivated vaccination during production. One should check if 100% of the birds test positive.

- Persistency of response, as indicated by mean titer response over time. Do titers persist long enough over time? Is another vaccination needed to boost titers above minimum protective levels or to add local protection?

As already indicated, there is a clear relation between titers obtained after priming and the sta-

bility of titers during production period of breeders and layers after inactivated vaccination.

Good priming (positive and uniform titers) before applying the inactivated vaccine will lead to more stable and high titers during production.

Conversely, bad titers after priming (low non-uniform titers with high percentage of negatives) will lead to high titers at the beginning of the production and to low titers at mid- and end of lay.

In general, IBV titers have the tendency to be less stable during production, compared to titers of infectious bursal disease (IBD) and Newcastle disease (NDV).

The true reasons for this intrinsic instability are unknown, but it does stress the need for IBV titer monitoring at the critical points in order to determine if extra vaccinations are needed to boost declining titers.

Another factor determining success of inactivated vaccination is the level of antibodies at the moment of application. High antibody titers at the moment of application may interfere with the serological response of the inactivated vaccine.

This has been particularly noted when more immunogenic vaccines, like 4/91 are used shortly before inactivated vaccine in the priming program of layers and breeders.

An example of such a program would be when breeders are vaccinated during rearing with the standard live vaccines, like H120 and MA5, followed by a live vaccination with IBV 4/91 at 10-15 weeks, and then followed by the inactivated IBV vaccination at 18 weeks.

When using such a program a decline in antibody titers can be often observed when testing serum samples at 24 weeks, rather than a rise in antibody titer when compared to a vaccination program without the 4/91 vaccination.

In the BioChek system results could be as follows:

- Mean ELISA titer at 24 weeks of age.
- Program with 4/91 shortly before inactivated vaccine 2,000-5,000.
- Program with 4/91 eight weeks

Continued on page 9

Continued from page 7
before inactivated vaccine 6,000-16,000.

Possibly, this effect can be overcome by allowing sufficient interval between 4/9I vaccination and the application of inactivated vaccine.

An interval of at least eight weeks is advised.

Success or failure?

Indicators of successful vaccination are generally high, uniform and lasting titers that are within the expected range for the type of vaccine. These samples should be 100% positive.

Indicator of a poor vaccination result is generally the opposite: i.e., titers lower than expected, non-uniform, and non-persistent. These 'below the baseline' titers are usually associated with moderate to high % of negatives.

For some vaccinations, such as AE

and CAV, % seroconversion is the only meaningful indicator of success. For instance for AE, if >60-80% test positive after vaccination, vaccination is considered to be successful and re-vaccination is no longer required. The role of IBV monitoring at fixed intervals in layers and breeders is particularly useful for the early detection of failed vaccinations.

Immediate revaccination, after detection of vaccine failure, will consequently help in the prevention of production losses, due to bad handling and/or application errors.

Thus vaccination monitoring has a preventative nature, which is an important economic justification for the use of monitoring programs.

Protective titers or not?

Although the correlation between ELISA titers and protection in challenge trials has been demonstrated for some diseases like NDV and

IBD, one has to be very careful when it comes to making predictions on protective titers.

This is in the first place because the degree of protection depends on many variables such as, the vaccine strains used, the virulence of involved field challenge strains, type of bird, vaccination application method and schedule, and local variables such as temperature or feed quality.

A protective ELISA titer of 4000 for NDV for one farm, may not protect birds on the farm next door. One needs to obtain protective titer values, by testing flocks under their own local conditions.

Another reason why one has to be careful in correlating titers with degree of protection is, that for many bacteria (i.e. PM) and mycoplasmas (i.e. MG and MS), immunity is not antibody mediated and antibodies are produced as by-products.

Therefore, titers will not directly

indicate immune status. Also titers of some viruses, i.e. fowl pox and ILT, do not reflect immune status. However, ELISA titers of these diseases can be still very useful to monitor success of vaccination.

Conclusions

Poor administration and/or vaccination techniques are the most common cause of vaccine failure in poultry. Results have demonstrated that ELISA monitoring is useful for finding out if a vaccine has been correctly applied or not.

If results are poor, it allows you to re-evaluate your vaccination procedures to find out what went wrong and take corrective action.

In this way, regular vaccination monitoring should improve the effectiveness of vaccine application and, in turn, improve disease control and economic performance of poultry flocks. ■

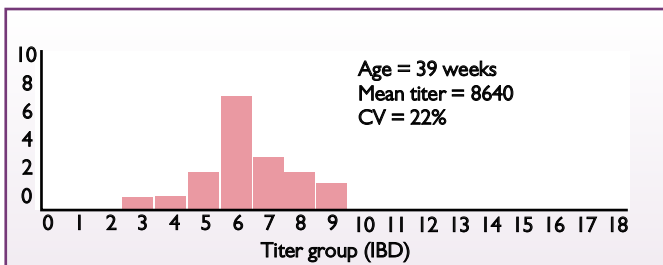
Case history: Inactivated vaccination of broiler breeders

Broiler breeder flocks were vaccinated at 18 weeks with inactivated ND+IB+IBD vaccine. ELISA results at 39 weeks revealed some major differences between flocks. Throughout most of the year, the ELISA results were good, until during the summer holiday when the results began to show suddenly very poor and non-uniform titers.

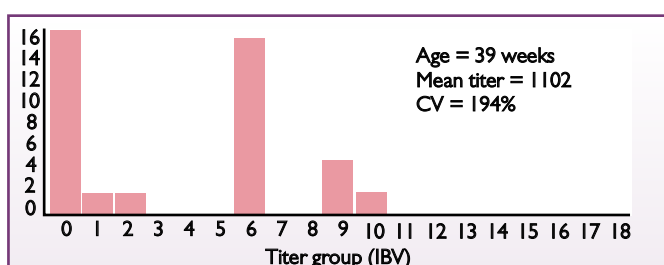
Further investigation revealed that during the summer holiday the regular vaccination crew was replaced by a temporary vaccination crew, which explained the poor quality of vaccination during summer holidays. The flocks vaccinated by the regular vaccination crew had high and uniform (CV < 40%) titers, whereas other flocks vaccinated by a temporary holiday crew, revealed very poor and non-uniform (CV > 65%) titers. The results are shown below.

Good vaccination: Normal vaccination crew

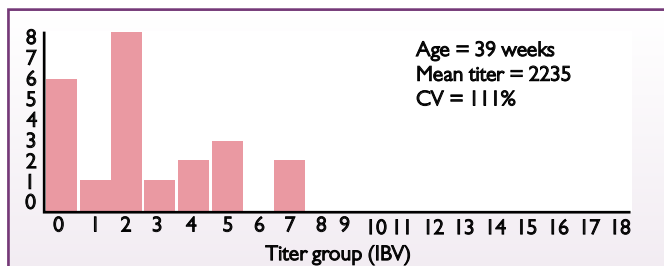
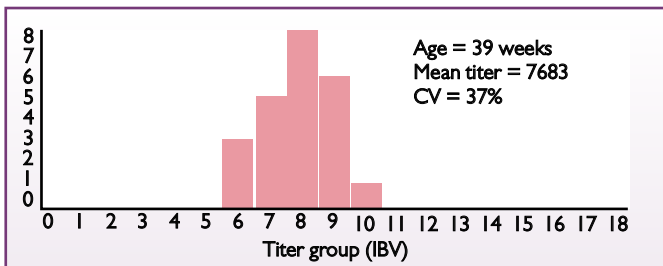
Infectious bursal disease



Poor vaccination: Holiday replacement crew



Infectious bronchitis virus



Newcastle disease virus

