Understanding Elisa results

The use of ELISA serology in poultry health management has been widely accepted for many diseases, including Infectious Bronchitis Virus (IBV). It is a useful tool to monitor the immune response following vaccination, and to diagnose the disease. However, this is only effective if the data obtained is well understood.

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In literature it is very difficult to find practical guidelines for the interpretation of (IBV) Elisa results. Practical questions like "What level of Titers and which Coefficient of Variation (CV) can I expect after IBV vaccination?" and "Can I differentiate between vaccination and field challenge using the IBV Elisa?" are often left unanswered. This article highlights the use and interpretation of IBV Elisa results after live and inactivated vaccination, and how serology can help in the identification of Infectious Bronchitis Virus (IBV) challenges in the field.

Justification of IBV monitoring

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, such as IBV, 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) for live IBV vaccinations.

Monitoring vaccination responses helps 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 you have to be prepared to take proper action on results. Without taking action on results, you cannot expect to improve, optimise and maintain the efficiency of vaccination programmes.

Interpretation of Elisa results

To be able to successfully interpret Elisa results after serological monitoring of vaccinated flocks, the following conditions must be met:

1. External reference controls must be used in the laboratory in order to assure the reproducibility and accuracy of results, and to 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.
2. Baselines should be established prior to running the test so that you know what to expect for results before testing. This allows for easy interpretation of results, using the comparison to the baseline to easily judge the success of your vaccination programme.
3. You 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 key components of an antibody response following vaccination. These are:

1. **Intensity of the response**, as indicated by the Mean Titer. Do the birds develop titer levels in the expected range (the baseline titers) for the vaccine used? These baseline titer values may vary according to the type of birds, age, vaccine type, vaccination programme, etc. One should develop baselines for vaccination programmes and local conditions. An example of baselines for IBV vaccinations for broilers is given in *Table 1*. This table shows that baselines (mean titer response) can vary according to the vaccine strains used. The use of relatively mild H120 vaccines will give a significantly lower titer response compared to the response obtained from more immunogenic strains, such as IBV variant 4/91.

2. **Uniformity of response**, as indicated by the %CV. Is the vaccine actually getting to the all the birds? Is the %CV within the required range or is there room for improvement?

The general guideline for %CV following vaccination is:

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

for effective IBV vaccination

Table 1 - BioChek vaccination baseline broilers

Titer values may vary according to age and type of bird, vaccine type, vaccination programme, and other factors such as placement programmes. You may find different results under different circumstances.

TEST	VACCINE TYPE	MEAN TITER RANGE AT PROCESSING (35D- 40D)	SUSPECT TITER INFECTION
IBV	live, 1x (H120)	300 - 1 500	> 3 000
	live, 1x (MA5, IB Primer)	1 000 - 2 000	> 4 000
	live, 2x (H120)	1 000 - 2 000	> 4 000
	live, 2x (MA5, IB Primer)	1 000 - 4 000	> 6 000
	live, 2x (H120 + 4/91 / CR88)	3 000 - 6 000	> 9 000

These guidelines are based on our experience and information from clients.
BioChek does not accept any responsibility for the results using these guidelines.

Although these are general guidelines applicable to most live and inactivated vaccine applications, you should keep in mind that application with live vaccines against respiratory disease like IBV generally creates variable titer responses. The horizontal spread of respiratory IBV from vaccines among flocks can also be limited, and live IBV vaccines can also give a local immune response that cannot be measured with an Elisa test. So, when one vaccinates with live vaccines such as H120, the expected CV for a good vaccination is 40-70%. 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 and beneficial impact on the persistency of titers after inactivated vaccination during production. One should check if 100% of the birds test positive.

3. **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 stability 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 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 programme of layers and breeders. An example of such a programme 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 programme 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 programme without the 4/91 vaccination. In the BioChek system results could be as follows:

Mean Elisa titer at 24 weeks of age
Programme with 4/91 -short before inactivated vaccine: 2000–5000
Programme with 4/91 -8 weeks before inactivated vaccine: 6000–16000

Possibly, this effect can be overcome by allowing a sufficient interval between 4/91 vaccination and the application of inactivated vaccine. An interval of at least eight weeks is advised.

Vaccination success or failure

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

A poor vaccination generally presents the opposite result: titers that are lower than expected, non-uniform, and non-persistent. These "below the baseline" titers are usually associated with moderate to high percentage of negatives. 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 programmes.

When IBV field challenges do occur, monitoring can also help by providing early diagnosis of the disease, limiting production losses. Ways to monitor to help diagnose IBV field challenge, as well as serological results from field case histories, are discussed in the second part of this article, which will appear in the next issue of World Poultry. ■