# Use and economics of **ELISA in the prevention** of disease

he use of Enzyme-Linked Immuno Sorbent Assav (ELISA) testing in breeders is widely accepted. Basically, this serological test is used in different ways.

A lot of viral as well as bacterial pathogens will create an antibody response in chickens after challenge. By measuring the antibody response, ELISA can be a good method to come to the correct diagnosis of the disease challenge the birds are facing.

# Dr Luuk Stooker DVM and Dr Bart van Leerdam PhD. BioChek BV, The Netherlands. www.biochek.com

For certain types of birds and/or certain pathogens it is not as much the antibodies you want to detect, but the lack of presence of these antibodies. ELISA can be a very useful tool in screening and monitoring for absence of disease challenge. A good example is Mycoplasma gallisepticum or avian leucosis virus.

For a lot of pathogens birds are being vaccinated. Also vaccines will evoke a certain antibody response. 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

Age (weeks)	IBD	NDV	IBV	REO	MG	MS	SE/ST	AI	CAV	AE	EDS	FAV
0	1	1	1	1	1	1	$\checkmark$		1	1		1
6	1	1	1	1			1					
12	1	1	1	1			1		1	1	1	1
18	1	1	1	1	1	1	1	1	1	1	1	1
28	1	1	1	1	1	1	1	1		1		
40	1	1	1	1	1	1	1	1		1		
55	1	1	1	1	1	1	1	1		1		

Table 1. A comprehensive monitoring program.

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 you 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, you cannot expect to improve, optimise and maintain the efficiency of vaccination programs. Therefore you need to have in mind that building the right monitoring program for your type of operation is not the end of the process.

The next step in the process is interpretation. Although this is easy when monitoring for absence of disease, interpretation can be more difficult for example when evaluating vaccination responses.

Building your own baselines based on the vaccination program used and the local disease challenge is key. But often underestimated is that the right way of analysing and processing results as well as getting these results to the right people quick and accurate is at least as important. This can only be achieved, when the software built around the ELISA test is capable of doing this.

Setting up a good monitoring program for your operation depends on

parent stock.

Percent (%) 60

100

80

40

20

0

25

30

the type of bird you are designing it for, but local disease challenges as well as governmental and export regulations need to be taken into account too. And the financial benefit of your monitoring program has to outweigh the investment done.

The next examples shown are actual field cases where calculations have been made based upon market prices prevailing in that specific area at the moment.

# Long life flocks

As already mentioned above, monitoring schedules differ per operation. In the following examples costs were calculated based upon a very comprehensive monitoring program as described in Table 1.

Per sample moment, a total of 23 samples are taken. The total costs of monitoring per million broiler breeders based upon this schedule (average of 10,000 birds per house) is around €125,580.

### Example 1

Fig. 2. Avian encephalitis outbreak on a farm of 10,000 broiler breeder

Hatchability (%) — Egg production (%)

Fig. 1 shows a case of an infectious bronchitis outbreak in a flock of 6983 broiler breeders. As a routine, this producer froze samples taken at regular intervals during production to be able to test retrospectively when problems occurred.

The bars in this case indicate IBV mean titers and coefficient of variation (%CV). Either titers are within Continued on page 29

7000

6000

Fig. 1. IBV outbreak in a flock of 6,983 broiler breeders. Production losses of 8,203 female chicks and 4,116 eggs during a 60 week period.



### 5000 4000 .300 Fiters 2442 2000 530 1000 35 40 45 55 50 Age (weeks)

Continued from page 27 expected range for the vaccine program and this program is not providing full protection or titers are not within expected range after vaccination and vaccine take is suboptimal.

The titers and CVs give a clear picture of a poor priming of the live vaccine(s) used, followed by a strong inactivated response (24 weeks). Due to the poor priming, the titer drops in the consecutive weeks while CV increases, instead of persists with low CVs in case a good priming had occurred.

The titer only rises to high levels again after challenge with field virus (52 weeks).

The drop in egg production (4,116 lost hatching eggs) and drop in hatchability (8,203 lost chicks) account for a loss of €1,029 and €2,871 respectively. On top of this an increase in mortality rate of 2% (140 birds) occurred, which accounted for an extra loss of €1,081. This brings the total losses of this IBV outbreak to €4,963 or €0.71 per bird.

If monitoring for ELISA had been implemented and corrective actions in the form of, for example, a three times re-vaccination with live Massachusetts and variant strains taken place, the extra costs would have been €0.077 per bird (€0.014 for the serology and  $\bigcirc 0.063$  for the vaccinations). This represents a 9-fold return on investment.

### Example 2

Fig. 2 shows a case of an Avian Encephalitis (AE) outbreak on a farm of 10,000 broiler breeder parent stock.

Around 16 weeks no vaccine monitoring for AE was done. The lack of titer response at 24 and 29 weeks clearly indicates that vaccination for AE was poor. The titer rises to high levels after challenge with field virus during production.

The financial losses due to drop in egg production (23,000 lost hatching eggs) were €4,600. Further to that 35,000 hatching eggs needed to be destroyed and 22,000 day old chicks had to be culled, causing additional losses of €7,000 and €7,700 respectively. The biggest loss came from the culling of a total of 128,000 broilers that were already sold to customers, being €44,800. This brings the total losses of this AE outbreak to €64,100 or €6.41 per bird.

If vaccine monitoring with AE ELISA had been implemented and corrective actions in the form of revaccination with a live AE vaccine taken place, the extra costs would have been €0.016 per bird, thus saving €6.41 per bird.



BioChek ELISA plate.

This represents a 400-fold return on investment.

### Conclusion

These examples are clear cases of a good return on investment. In the field, not everything is so clear cut. Is there always a need for a comprehensive program? Things can go well for years without monitoring, so why make the investment?

First, when investing in a good vaccination program, why would you choose not to check if the vaccines applied give the response needed for a good protection? Often the costs of monitoring are just a fraction of the costs of the vaccine applied. Second, the investment in a very comprehensive program in long living flocks will be around 0.081 eurocents per hatching egg which is 0.37% of the cost price of one hatching egg (based upon cost prices calculated by LEI Wageningen Economic Research).

In summary, setting up a good monitoring system for ELISA along with taking actions based upon results, should give you a higher profitability in your breeder operation. The examples show a return of investment ranging from nine up to 400 times.