Serology Interpretation—Real Science, Real Results

The ten principles to interpret and monitor with confidence
The ten principles of serology interpretation

Serology has been a fundamental bastion for poultry diagnostics and disease surveillance and best represents the most critical approach for diagnostic and disease monitoring when a thorough examination of all contributing factors are fully considered. These ten principles are designed to consider all the variables, track regional trends and evaluate relevant factors that support a comprehensive diagnosis you can trust. Because at IDEXX, we believe doubt is never an option.

1. **Use rapid, convenient, sensitive, specific, and economic serological methods.** There is no doubt that enzyme-linked immunosorbent assays (ELISA’s) represent a superior serological test. Hundreds or thousands of serum samples can be processed in a single day or week to detect and measure antibodies against a variety of poultry pathogens. Although other types of assays can also be sensitive, specific, and possibly less costly in the short term, they often require protracted procedures and intensive labor that can lead to costly expenses down the road.

2. **Create and organize an in-house database.** With the consolidation of the poultry industry, the spread of field operations into many geographical areas and the rapid dynamics of poultry production systems, it is critical to create systems that allow poultry health professionals to rapidly and objectively analyze their data to identify acute problems or trends in poultry disease.

An in-house database is essential to analyze serological data objectively. Under no circumstances should a poultry health professional rely exclusively on pre-established reference antibody titers that have been generated for regions, production systems, vaccination programs, and field exposure situations that may be different from the company’s data. For example, higher-than-normal antibody titers against infectious bronchitis virus (IBV) at processing age may point to these potential trending conflicts:

- The company is being challenged more than usual in the field.
- The vaccination program being used needs some adjustments.
- There may be other disease agents predisposing, co-participating or complicating the IBV challenge.

To create optimum standards for evaluation, we recommend using a system that automatically generates data that can be stored and manipulated electronically. This will improve accuracy and save time.
**Compare your database against your own region.**
An in-house serological database is the most important reference a poultry management program can generate and use routinely, but it is also wise to compare in-house data right where you live. By comparing your own vaccine-induced antibody titers against the rest of your regional industry, you’re likely to discover opportunities for improvement in terms of actual antibody titers and uniformity in the response to vaccination (typically read as coefficient of variation, or CV%). However, comparing your own database against data generated in other parts of the world can produce very different disease challenges in the field and is a less-effective, risky alternative.

**Consider seasonal changes in serological data.**
Even in the absence of infectious disease challenges in the field, antibody titers are generally not the same throughout the year. This is particularly true for latitudes that experience significant weather changes. Even regions that undergo only a “dry” season and a “wet” season may observe detectable variations in ELISA antibody titers between seasons. The winter and early spring months are typically associated with increased respiratory problems in the field, and thus antibody titers may be higher during such times of the year.

It is important to compare real-time data against in-house data generated during the same time of the year. This is why pre-established, industry-wide data generated for many regions of the world should not be used as a viable reference.

**Know the power and limitations of each laboratory assay.**
ELISA testing is used worldwide and it serves very important purposes. ELISA is an invaluable tool to look at trends in seroconversion against IBV, infectious bursal disease virus (IBDV), or avian reovirus (REO), to mention a few. Any significant variations in field challenge or vaccination approaches may be detected using ELISA. However, it should be known that ELISA cannot “type” the immune responses against specific strains or variants. For example, ELISA cannot determine whether a flock with IBV problems was challenged with Massachusetts, Arkansas, Connecticut, DEO72, etc., serotypes.

**Avoid risky single time-point evaluations.**
For clinical diagnostic situations, paired serum testing is critical. Paired serum involves testing of serum samples collected during the acute and convalescent periods of a disease. Many poultry health professionals have difficulty accepting this concept because they think if they wait for a second sampling during the convalescent period, then there would be nothing that can be done for the affected flock. In today’s massive production systems, it is critical to look for trends for the company in order to establish corrective actions for the whole company—and not for one individual flock.

Acute and convalescent sampling requires testing for antibodies immediately upon identification of the first clinical signs, and then 2–3 weeks after, to be able to identify seroconversion against one particular agent. Often times, it is not possible to hold the birds that long before they go to market, and thus we recommend a few birds remain in isolation for a few days before they are sampled again.

A simple isolation sampling step will produce valuable information that can save many losses to an affected company.
Comply with state, federal, and official regulations. Any methodologies required by official authorities must be used to monitor for disease. Many poultry companies have an internal testing protocol to maintain their health status, and an additional testing program to comply with local regulations and to satisfy requirements for exports into other countries.

Consider pathogen strains, variants, or serotypes circulating locally. It is a mistake to determine whether a certain antibody range is protective or non-protective, based on only the titer level or profile, without also considering the types of pathogen strains, variants, or serotypes circulating in the area. ELISA testing is generally a quantitative test but not a qualitative test in the sense that it cannot determine specifically against which strain, serotype of variant the antibodies are directed. Here are some typical examples:

- A titer of 3,000 may be protective against an IBDV Lukert strain, but it may not be protective against a DeIE strain of IBDV.
- A titer of 2,000 against IBV might represent a significant field challenge for some companies, while it may be routine for healthy flocks in other companies.
- A titer of 1,200 against REO may be interpreted as a field challenge by some, while the broilers may be perfectly fine.

This clearly illustrates the importance of identifying local trends of disease challenge, vaccine performance, vaccination crew performance, etc.—all dependent on a company’s internal procedures rather than challenges found in distant parts of the world.

Know your field situation and disease epidemiology when interpreting serology. Not all poultry producing areas have good communication between companies. Consider any available and reliable information when interpreting serology data because it may help to assess certain disease situations. For instance, if a company never experienced a challenge with AIV, does not know that there has been a diagnosis of AIV in the region and does not usually pay attention to NDV antibodies because NDV is usually not a problem in the area, it can easily overlook a trend of serconversion against NDV and may not consider testing for AIV.
Know how and when to interpret serology quantitatively and qualitatively. Any poultry health professional looks for “high” antibody titers as an indication of good seroconversion after vaccination, or as an indication of field exposure to a particular antigen. This represents a quantitative evaluation. It is also important to look for a uniform antibody response, which is usually reported as the coefficient of variation, or the CV. In poultry production, CVs are typically expressed as the CV%, which is simply the standard deviation divided by the mean, multiplied by 100, whether we are relating to antibody titers or body weight uniformity in a 20 week-old broiler breeder pullet flock. The CV is a measure of variation of antibodies within a group of serum samples. The lower the CV, the more uniform the antibody response. A low CV is typically associated with good vaccination procedures or with a recent antibody response after field exposure to a given pathogen. Thus, quantitatively, the higher the antibodies the better the response; the lower the CV the more uniform the response, regardless of the titer. In summary, an antibody response could be low quantitatively (low antibody titers) and uniform at the same time, which is not what we normally look for.

Because an ELISA titer or an ELISA titer range reflects simply a quantitative response, such titers should be used as follows: 1) as a reference for possible trends in seroconversion in a poultry company upon field challenges; 2) for identification of rapid seroconversion in paired acute and convalescent samples in a diagnostic situation; 3) for evaluations of vaccines and vaccine application procedures; or 4) to document the absence of antibodies against pathogens such as AIV, MG, or MS.

Standard data generated with a purely quantitative assay such as an ELISA in certain parts of the world should not be used to estimate levels of protection against specific pathogens without consideration of the local circumstances and the possibility of a field challenge with different variants, strains or serotypes; and without a working knowledge of the local disease epidemiology and poultry production systems.

For more information, about serology or to learn more about poultry health, please visit idexx.com/poultry.

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