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SEROLOGICAL SERVICE IN POULTRY INDUSTRY

By Dr Vincent TURBLIN, Deputy Regional Market Manager – Poultry - CEVA Animal Health Asia Pacific

In avian diagnostic medicine, the usual immediate challenge is making a definitive diagnosis for the presenting problem of morbidity or mortality. And if we deal with their occurrence in a more preventive or epidemiologic way, there are at least four main types of approach that can help in evaluating infectious diseases in poultry:

- ④ Recording and analysis of productivity data (mortality, growth rate, and egg production).
- ④ Recording and analysis of disease data (signs, lesions).
- ④ Measurement of serological response.
- ④ Isolation and identification of pathogenic agents.

If we are to obtain full benefit from the money invested in disease monitoring, it is important to understand clearly what the results mean. This, in turn, depends on the theoretical basis of the test system (what it measures and what it does not measure) and the frequency of its application. Indeed, we must keep in mind that a serological response may confirm that the birds have been exposed to the organism, but it is not necessarily the cause. It must be applied fairly regularly during the period of growth or if disease breaks out. And another misconception could be to think that "The more expensive the method used to evaluate infectious disease, the better it must be". In fact the opposite is usually true, because the more expensive the test, the fewer samples tested. All test systems have their weaknesses.

SAMPLE COLLECTION

Statistical basis of sampling

We use small samples to infer about the condition of a large flock. Statistical techniques are available to determine the number of samples necessary for a given purpose. It is the economic and practical considerations, which determine the number of samples taken.

First, sampling should generally be stratified. If, for example, we want to estimate the situation in a whole poultry farm, birds should be sampled in each of the houses, and not merely in the first division of the first house. On the other hand, biased sampling is sometimes necessary, and even desirable, if we are investigating a specific problem. If the problem is paralysis in breeding hens, we should not base our opinion only on examination of birds showing no symptoms.

The actual number of samples needed will vary according to the number of birds in the flocks. In practice 12-24 samples per air space is typical for most tests. Sixty samples per space is recommended for detection of unwanted pathogens (*Mycoplasma sp.*, *Salmonella enteritidis*).

Note that it is possible to run the tests on pooled samples, but this can reduce the sensitivity of the tests.

Containers for sample collection and transport.

Our basic target should be to obtain serum of good quality without bacterial growth or haemolysis, and in sufficient quantity to repeat a test if necessary.

Glass or plastic vials are the traditional containers for blood samples (5ml for glass or 1.5 ml for plastic vials). Immediately, the blood should be stoppered and if possible, laid horizontally to encourage the formation of a long thin clot.

Samples should be maintained at room temperature until the blood has clotted, then refrigerated until dispatched to the lab, or separate the serum into clean vials before shipping to the lab. One person at each site should be trained to take the samples required whenever necessary.

Yolk Preparation

Birds transfer a large amount of antibody into the egg yolk. For this reason, yolk extracts can be used as a substitute for serum samples for serological monitoring. A series of papers, related in *appendix*, has examined the relationship between serum and yolk antibody in some detail for Newcastle, Infectious Bronchitis, Gumboro virus as well as Adenovirus, Reovirus and Salmonella (enteritidis & typhimurium). All these studies confirmed that antibody titers in yolk are highly correlated with those in serum (correlation coefficients of 0.84 to 0.97). Nevertheless, because yolk is laid down in concentric rings for 7 days, the yolk should be thoroughly homogenized prior to sampling.

There are two methods most commonly used for yolk preparation:

- ❖ The first method is simply diluting yolk with PBS, and then the mixture is used for testing in place of serum.
- ❖ The second method involves the addition of chloroform to the mixture (usually 30-50%) to remove the lipids, but is quite costly and as such, less often used.

Table 1: Balance of advantages and inconvenients of Yolk serology.

ADVANTAGES	INCONVENIENTS
1) Ease of collecting sample	1) Sample extraction according to published methods can be laborious.
2) Samples “already packaged”	2) Extracts cannot be used for rapid plate agglutination tests
3) Eggs can be easily stored	3) Samples cannot usually be identified to individual bird
4) Eliminates bird handling and stress	4) Results are not always directly equivalent to serum

SERUM SUBMISSION

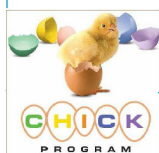
Consideration should be given to how and when samples will be transported to the lab.

Whenever possible, samples should be taken on a day which will allow their delivery to the lab within 24 hrs (avoiding weekends).

Samples should be securely packed by group where they were collected (pen, sex, house, etc.) The group should be properly identified on the tubes or the bag.

Also important, clear information on the tests that are required for each sample should be indicated to the laboratory along with common information on flock health, vaccination program, etc.

For this purpose, always use the laboratory's submission forms (most laboratories will have a specific form for serology samples).



SEROLOGICAL ARCHIVE

Serum samples should be stored in a way that they can be retrieved easily. In case of storage for periods longer than 24 hours, the samples should be kept at -20°C. The size of vial must be adapted, both for convenience and minimizing air content (to avoid lyophilisation of water in the serum).

The storage period can last for:

1. One month for broilers: in case a repeat test is required, or other disease to check.
2. One year for breeders or layers is a reasonable period to retain samples for routine purposes.
3. More than one year: for epidemiology studies. Indeed, long term storage of the serum archive may provide useful information on disease patterns.



Serums bank can be very useful to analyze retroactively the problems encountered in the field. Especially in layers, since the titers reached by vaccination are already very high, seroconversion evolution can be demonstrated with the monitoring of antibody titers and CV% assessed at critical stages.

An index must be maintained to cross-reference the sample numbers according to bird types, company and farm of origin, and the date. Such a system can be set up in a manually recorded index book. However it is much more easily maintained and searched using a computer-based system.

SEROLOGICAL TESTS

Different serological test are available in laboratories:

- 🔍 Agglutination test
- 🔍 Agar gel precipitation
- 🔍 HI test
- 🔍 ELISA
- 🔍 Immunodot
- 🔍 Seroneutralization

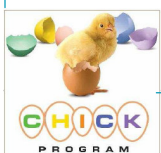


LABORATORY QUALITY CONTROL

It is reasonable to expect that results produced by a given lab for each assay are consistent over time, and that results produced by different laboratories from the same samples are comparable also. To achieve this, each assay must be validated to identify sources of variation that might occur. We must also keep in mind that there are non-assay sources of variation. These factors include how and when a sample is taken, manipulated, stored, transported, identified, and errors in report generation.

In validating an assay, the following areas need to be addressed:

- 🔍 **Specificity** : a highly specific assay will have a low tendency to show "false positive" reactions in birds exposed to a closely related pathogen.
- 🔍 **Sensitivity** (ability to detect low levels of specific Ab): a trade-off is that the higher the sensitivity of an assay, the lower is its specificity. In poultry production, it is common practice to apply a highly sensitive technique as a screening test, followed by more specific tests on repeat sampling of the positive flock.
- 🔍 **Accuracy** in quantifying purified amounts of the antibodies sought.
- 🔍 **Precision** is the ability of the assay to consistently reproduce a result from the same samples. It is expressed as %CV.



Much of the validation work required, are carried out by the manufacturer of test kits. A good first step in setting up a serological Quality Assurance (QA) system will be to request the manufacturer to provide data on within-plate and between-plate variations. The lab can then quickly validate the assay using known positive and negative sera. Once an assay has been validated, the continued satisfactory performance should be monitored by the use of a QA System.

The Quality Assurance System should include a definition of criteria for assay acceptability, along with means for identifying sources of variation and implementing corrective procedures:

- ④ One component of such a system might be to repeat an Inter-assay Precision test.
- ④ Alternatively, simply calculate the %CV for all assays of each standard control serum each month and plot the results over time. A reasonable target for %CV in routine testing is 10-15%.

The prime aim of in-house quality assessment is to confirm that the results generated are consistent over time. Inter-lab quality assurance schemes can be set up with other labs, in order to ensure uniformity of results. Accreditation schemes take this a step further, by independently checking procedures and standards, often with a periodic inspection, eg. the accreditation scheme for MG, MM and MS testing in most poultry exporting countries.

At every point in the validation and QA of a system, it is important for staff to be educated and motivated. It is equally important for those interpreting the results to understand the inherent variability of those, and to be able to express an opinion by comparing them with other test results. This may lead to a decision, which will take into account information from a variety of sources.

CLINICAL INTERPRETATION

Serology is most powerful and accurate when used in association with other sources of information:

- ④ Productivity data: mortality and culling, egg production, egg quality, hatchability, growth rate and uniformity, feed conversion ratios etc.
- ④ Condemnations data generated at slaughter
- ④ Clinical examination of affected flocks
- ④ Post-mortem examinations, backed up by appropriate microbiological and necropsy results.
- ④ Routine swabbing of barns after cleaning out and disinfecting, examining feed and raw materials, cull chicks etc.

In diagnosing a disease, planning and implementing a control strategy is often necessary to achieve the cooperation of many different people. The key to cost-efficient use of serology lies in the planning of sample collection and choice of assays appropriate to each specific problem or objective. The samples made available and the anamnestic information on flocks will often have a considerable bearing on the interpretation of the serological results.

There are 2 main strategies of serological sampling:

- ④ Routine sampling is done at periodic intervals with a view to detecting a serologic response to infection or vaccination.
- ④ Targeted sampling is done in order to investigate a specific disease incident using paired samples (at 12-21 days interval e.g., seroconversion time).

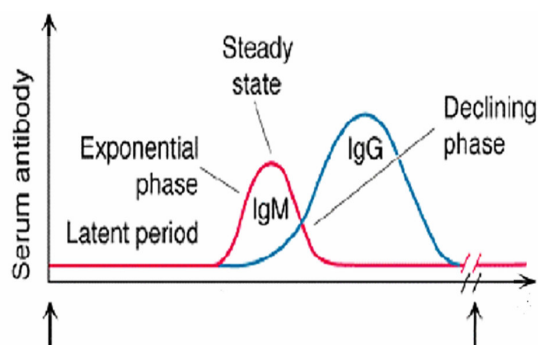
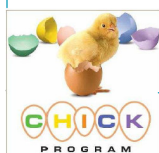


Figure 1: Schematic evolution of Humoral Response after stimulation (seroconversion).



LIMITATIONS OF SEROLOGICAL TESTS

Serological tests depend on the detection and/or quantification of antibodies against infectious agents. However, most serological tests have the following limitations:

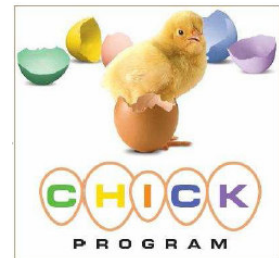
- 🔗 Birds remain negative for at least 4 days after infection. This period may vary according to the test used and may be very much longer when latent infection occurs.
- 🔗 Most tests only evaluate circulating antibodies, and take no account of mucosal antibody or cell-mediated immunity.
- 🔗 Without repeated sampling, it is impossible to say whether the antibody level is rising, stable, or falling.
- 🔗 No distinction of antibodies from field strains or vaccinal strains in most of the cases, unless a DIVA method is possible for the vaccine used.
- 🔗 Antibodies to antigenically related agents may cause confusion due to cross reactions.
- 🔗 We can add to this list, the risk of false positive and false negative reactions, which is inherent to any test system actually.

Nevertheless, careful and repeated applications of the simple techniques including validation and verification are capable of overcoming most, if not all, of the above limitations. However we cannot expect that serology will give us the right answers if we ask our labs the wrong questions and wrong tests.

SOLVING PROBLEMS

A routine monitoring program will sooner or later show flocks with very poor response in terms of antibody after vaccination. In considering what to do about these flocks, it should be remembered that the response of the birds to vaccination depends on three main factors:

- 🔗 Factors related to the application of the vaccine, such as the skills of the vaccinators, number of vaccines applied on one occasion, the route of application and sometimes interfering factors such as poor water quality.
- 🔗 Factors related to the vaccine, such as antigen concentration, virulence of vaccine strain, nature and quality of adjuvants.
- 🔗 Factors relating to the physiology and the immune status of birds, immunosuppression by IBDV, MDV, CIAV, Adenovirus, mycotoxins, stress and other diseases.



CONCLUSION:

Avian serology consists of a combination of classical tests methods, such as the agar-gel precipitin (AGP) test, the plate agglutination test, the virus-neutralization (VN) test, the hemagglutination-inhibition (HI) and the Enzyme-Linked ImmunoSorbent Assay (ELISA). Most of the tests are relatively easy to perform, but quality control is essential.

Nonetheless, even though serology is a wonderful tool both for diagnostic and epidemiologic purposes, we shouldn't forget it has to be used as part of a whole diagnostic process. Serological results have to be considered with other datas, among which, the isolation of the agent still has an importance in the investigative process. Isolates can especially be important in assessing virulence and pathogenesis of strains involved.... hence the growing implementation of molecular methods (PCR, sequencing, etc.) for identifying and classifying the pathogens isolated in samples.



APPENDIX

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