Chapter Three: Poultry Serology

INTRODUCTION

SELECTION OF BIRDS

SENTINEL BIRD BLEEDING

BLOOD COLLECTION PROCEDURE

ADVANTAGES/DISADVANTAGES OF SEROLOGY

DISEASE MONITORING SCHEDULES

SEROLOGICAL RESULTS ANALYSIS

DATA ANALYSIS EXAMPLES

BASIC/MINIMUM DATA

QUALITY CONTROL

CONFIRMATION OF DISEASE OUTBREAKS

MONITORING FLOCKS OVER TIME

MONITORING RESPONSE TO VACCINATION PROGRAM
Poultry Serology

Introduction

Evaluating serum antibody titres for various poultry pathogens, has become an important flock management tool for poultry producers and poultry veterinarians alike. Determination of the presence, absence or level of specific antibodies to a disease entity may be determined through the use of certain serological tests eg ELISA tests, plate agglutination tests, haemagglutination tests and micro or tube agglutination tests.

The type of test used will depend on several factors including the test sensitivity/specificity, time to perform, cost etc.

Serological flock monitoring is currently used extensively in the poultry industry for the following purposes

- To measure response to, or success / failure of a vaccination program
- As a diagnostic tool in a disease outbreak investigation.
- As a diagnostic tool to detect diseases which are not part of any control program
  - To prove continued freedom from disease
  - Detect or confirm the presence of a new disease
- To check the immune status of a flock against a specific disease; either at a single point in time or over a period of time
- Evaluating the level of maternal antibody passed from parent birds onto chicks
- For date of vaccination prediction in various “Days to Vaccination” programs for Infectious Bursal Disease

Please Note: Considerations before blood collection

1. For what reason are the samples being collected (eg: routine, diagnostic, vaccine response etc)
2. Are you collecting samples at the right time for the application above?
3. Are you sampling a suitable number of birds?
4. Consult your Veterinarian about points 1-3 before submitting your bloods to the laboratory.
Sample Guide

Selection of birds for unbiased sampling

Birds on the Floor

- Sample size cut-off as regards sensitivity, specificity, reliability and reproducibility appears to be at 23 samples. Below 23 samples there is a marked decline in the reliability of the mean titre and vaccination date estimation. ELISA plates are manufactured with 12 rows of eight wells and therefore blood sample numbers in multiples of 8 are practically ideal for handling in the lab.
- Preferred sample group size = 24 birds (Figure 2).
Birds in Cages

- Number the cages eg: 1-100 on a cage floor plan.
- Use a random number table to draw cage numbers for the number of samples required (ideally 24 samples)
- One bird is bled per cage

Sentinel Bird Bleeding

This is used as a management tool to evaluate change of disease status in a particular flock and is most commonly applied in the monitoring and control of specific diseases such as *Mycoplasma gallisepticum*, avian influenza (AI) and salmonellosis, where live vaccines do not form part of the control program.

- This program needs to be very carefully designed in close consultation with your veterinarian.
- The sentinel birds **do not** receive vaccine for the particular disease/s being monitored.
- A small group of birds (eg 24) are randomly selected and individually identified with numbered wing bands.
- These individually identified birds (the sentinels) are then periodically bled according to a specific bleeding schedule to monitor their status for the specified disease/s.
**Blood Collection Procedure**

- Brachial vein under the wing (found at the first bend in the wing – “elbow”), is the most practical site for blood collection (Figure 4). Exception are day old chick’s, they are bled out at euthanasia from the jugular vein.
- The brachial vein is punctured with a needle (18 guage).
- A clean glass / plastic blood tube, without anti-coagulant, is placed at the stab site and blood allowed to run into the tube.

![Figure 4](image)

- Following collection, allow samples to stand for 1 hour at room temperature to form a clot (Figure 5)
- Clot is removed after >1 hour preferably by centrifugation and the serum poured off into separate clean tubes (Figure 6), or by gently removing the clot with a wire.
- Serological assays are performed in the laboratory on the separated serum (Figure 6)
Blood tubes are individually marked with the identification of the bird /flock / site. Mark the tube NOT the tops, if tops dislodge the ID of the tubes come into question (Figure 7).

Bloods from the same flock/house can be placed in their respective bags and each bag clearly marked. (Figure 7 and 8).

Samples may be stored at 4°C overnight in a fridge, do not freeze whole blood or serum prior to submitting to the laboratory, as this may lead to haemolysis and freeze thaw damage which can affect certain assays (eg: serum PAT)

Serum or un-spun clotted blood should then be transported at 4°C to the laboratory.

Serum samples can be frozen prior to laboratory analysis if Plate Agglutination Tests are not being performed on them (Figure 8).

**Advantages and Disadvantages of Serology**

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Several tests can be run on the same sample</td>
<td>Historical test detecting previous disease / vaccination</td>
</tr>
<tr>
<td>Rapid tests with quick turnaround time</td>
<td>Difficult to differentiate vaccine response from field infection</td>
</tr>
<tr>
<td>Good flock screening procedure</td>
<td>Does not indicate time of infection</td>
</tr>
<tr>
<td>Can be specific (Disease present/absent; Vaccine response present/absent)</td>
<td>It is a group test eg: IBV it does not distinguish serotypes / strains.</td>
</tr>
<tr>
<td>Can provide a guideline (average flock titre level; % flock infected; estimation when flock infected)</td>
<td>Unclotted whole blood does not keep well, requires careful handling</td>
</tr>
<tr>
<td></td>
<td>Need good quality, fresh, unhaemolysed, unfrozen serum.</td>
</tr>
</tbody>
</table>
Disease Monitoring Schedule for Commercial Poultry

Broiler Breeders
- Newcastle Disease (NDV)
- Infectious Bronchitis Virus (IBV)
- Infectious Bursal Disease (IBD)
- Avian encephalomyelitis (AE)
- Egg Drop Syndrome (EDS)
- Chicken Anaemia Virus (CAV)
- Avian Influenza (AI)
- Avian Pneumovirus (APV)
- REO virus
- Salmonella
- Mycoplasma gallinarum/synoviae (MG/MS).

Layers
- Newcastle Disease (NDV)
- Infectious Bronchitis Virus (IBV)
- Avian encephalomyelitis (AE)
- Egg Drop Syndrome (EDS)
- Avian Influenza (AI)
- Salmonella
- Mycoplasma gallinarum/synoviae (MG/MS).

Broilers
- Newcastle Disease (NDV)
- Infectious Bronchitis Virus (IBV)
- Infectious Bursal Disease (IBD)
- Salmonella
- Mycoplasma gallinarum/synoviae (MG/MS).

Number of blood samples to collect?

- Sample size depends on:
  - Size of the population
  - The likely prevalence of the disease
  - The required reliability level of results
- Sample size can be chosen such that if the result is negative it can be concluded that it is very unlikely the flock has the disease.
- The higher the prevalence of the disease in a flock the smaller the sample size to find a positive.
- Use a Statistical Table as a guide for the number of samples to take at say the 95% confidence level.
Sample size for detecting at least one positive if the disease is present at the specified level.

<table>
<thead>
<tr>
<th>Population size</th>
<th>50%</th>
<th>25%</th>
<th>15%</th>
<th>10%</th>
<th>5%</th>
<th>1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>5</td>
<td>11</td>
<td>19</td>
<td>28</td>
<td>56</td>
<td>225</td>
</tr>
<tr>
<td>1 000</td>
<td>5</td>
<td>11</td>
<td>19</td>
<td>29</td>
<td>58</td>
<td>258</td>
</tr>
<tr>
<td>2 000</td>
<td>5</td>
<td>11</td>
<td>19</td>
<td>29</td>
<td>58</td>
<td>275</td>
</tr>
<tr>
<td>5 000</td>
<td>5</td>
<td>11</td>
<td>19</td>
<td>29</td>
<td>59</td>
<td>290</td>
</tr>
<tr>
<td>10 000</td>
<td>5</td>
<td>11</td>
<td>19</td>
<td>29</td>
<td>59</td>
<td>299</td>
</tr>
</tbody>
</table>

Eg if the expected prevalence of disease is 10% in a flock of 5 000 then 29 samples are needed to be 95% confident of detecting at least one positive sample.

**Suggested Bleeding Schedules for Commercial Poultry**

- Broilers bleed schedule:
  
  - 0 to 3 days for maternal antibody, 31-35days, >39days.

- Broiler breeders bleed schedule:
  
  - pullets (4-7weeks, 10-12weeks, 17-20weeks)
  - Breeder layers - every 6 to 8 weeks from about 26 weeks of age.

- Commercial layers bleed schedule
  
  - pullets (4-7weeks, 8-12weeks, 13-20weeks)
  - layers (21-30weeks, >31weeks)

Please note that bleeding schedules will depend on the farm situation and the ages of birds from which to bleed will depend on the vaccination programme being used.

The disease entities to be tested and the type of tests to be used should be discussed with your veterinarian.
Types of serological tests which may be performed

- ELISA
- Serum Plate Agglutination (SPA)
- Haemagglutination Inhibition (HI)
- Micro agglutination
- Tube agglutination
- Agar Gell Immunodiffusion (AGP or AGID)
Serological results analysis

In depth analysis of Elisa antibody titres, frequently utilizing custom-made computer software packages and forms an integral component of modern commercial flock health management (Figure 9). Practical, meaningful and relevant “on farm” use of the antibody titre results obtained in the diagnostic laboratory are facilitated by these software programs using well defined, statistically valid, guideline reference titre ranges. Such manipulation of the serological results achieved enable more scientifically sound decisions to be taken, as regards what actions need to be implemented on the farm, based on the results achieved.

Integration of the antibody titre results achieved into various software applications enable the veterinarian and commercial producer to more critically analyse their results to obtain the maximum benefit for their client / enterprise.
Data Analysis Examples

Basic or Minimal Data

- Use to distinguish between positive and negative status. The example above is from a flock where a negative Avian Influenza (AI) status has been confirmed (Figure 10).

Quality Control

- Making use of various parameters calculated by the software program (arithmetic mean titres, geometric mean titres, min/max titres, coefficient of variance) the user can quality control the individual and sample group data, which indicates the significance and quality of the results obtained (Figure 11).
- Such quality control allows for continued monitoring of your sample collection procedures, effect of sample number and statistical significance of the result.
Confirmation of Disease Outbreaks

- Integrated data analysis by the software program (arithmetic mean titres, geometric mean titres, min/max titres, coefficient of variance) and comparison to expected normal ranges for same age birds, allows for investigations into potential disease outbreaks.
- When this serology data is used in conjunction with other post mortem and laboratory findings, disease outbreaks can be confirmed.
- The above example is of an investigation into an AI disease outbreak. The first 2 bar graphs are from flocks exposed to field strain AI while the last 2 bar graphs are from age matched negative flocks (Figure 12).

Monitoring of flocks over time

- Software packages store data allowing for comparison of various sample submissions from the same farm / flock over time
- Changes in disease status can then be continually monitored. The example above demonstrates how this particular flocks AI status has changed from negative to positive over time (Figure 13).
Monitoring Response to Vaccination Programs

- Can monitor multiple flocks at a single point in time. For example the group of graphs above left demonstrate an analysis of four houses containing 16 week old breeders that had been vaccinated for Newcastle Disease Virus. All 4 houses are showing uniform, low % CV, good NDV vaccine response titres (Figure 14).
- Can monitor the vaccine response in a single flock over time. In the example above (Figure 15) a typical vaccine decay response curve for NDV is demonstrated in a single flock over time.

Further Reading