Flock Health Monitoring and the use of antibody or antigen detection

POULTRY
GEFLÜGEL
VOLAILLE
AVES DE CORRAL
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家禽

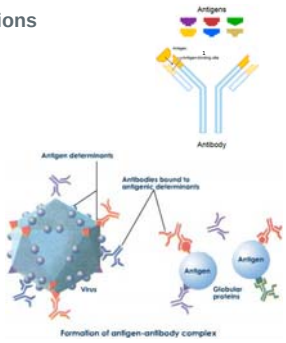
Learning objectives

- Understanding the origination of Antigens and Antibodies
- Timeline of Disease & Vaccination
- Understanding which tests detect each
- Interpretation of multiple test results in a flock

Understanding the origination of Antigens and Antibodies

Antigen and Antibody Definitions

- Antigen (immunogen)
  - Foreign molecule that stimulates antibody production (ANTIbody GENerator)
- Antibody
  - Produced in lymphoid tissue, and/or provided by the Hen In Egg materials
  - Adaptive immune response
  - Antibodies are produced in response to the invasion of foreign molecules (Antigens) in the body



Serology should be part of a comprehensive flock health program

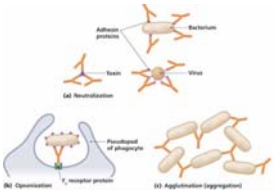
Prevention programs should include:

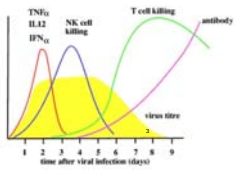
- Evaluation of production results
- Regular flock examinations
- Examination of culled and dead birds
- Periodic serological flock profiling

Timeline of Disease & Vaccination

Response to disease

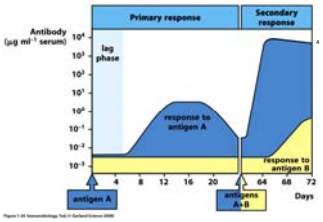
- Antibodies facilitate neutralization and removal of infections by phagocytic cells, or they prevent pathogen attachment.
- Timeline from infection to detection of antibodies is typically 7–10 days.





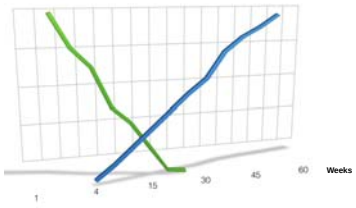
Immune response from exogenous stimulation

- Vaccination or infection stimulates primary immune response.
- If repeated vaccination or introduction of pathogen, secondary immune response is more rapid and robust.



Antibodies

- Maternal antibody levels will decrease after a few weeks
- Adaptive immunity will provide birds with their own antibody levels
- Vaccine reaction



Timing for Serologic Testing in Poultry

- It is important that serologic testing done on a routine schedule.
  - To monitor vaccination effectiveness and timing
  - To comply with local, national and international regulations
    - (Such as NPIP, export requirements, etc.)
  - To diagnose field infections
- Antibody levels typically lag behind acute infection and/or mortality associated with disease.
  - Acute and convalescent titers can help to diagnose disease.
  - Antibody levels may not have risen yet if testing is completed early in the disease state.
  - Increases in antibody levels (titers) typically happen several weeks after vaccination.
    - 3-5 weeks after live vaccines
    - 6-8 weeks after inactivated vaccines
- Sampling without regard to the timing of antibody response can be wasteful and misleading.

Examples of Timing for Serologic Testing

Poultry Type	Schedule Examples
Pullets	Monitor at 0-4 days to measure maternal antibody levels To evaluate immune response to vaccine: • Monitor 3-4 weeks after vaccination with live vaccine • Monitor 6 weeks after vaccination with inactive vaccine
Layers	Monitor at intervals of 4-8 weeks To evaluate immune response to vaccine: • Monitor 3-4 weeks after vaccination with live vaccine • Monitor 6 weeks after vaccination with inactive vaccine
Broilers	Monitor at 0-4 days to measure maternal antibody levels Monitor at 3-4 weeks, depending on vaccination program and field challenges Monitor before slaughter at about 6 weeks of age to evaluate vaccine response and field challenges

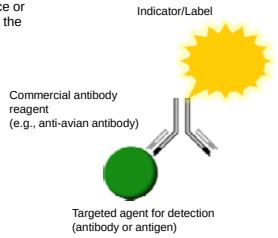
Avoid risky single-time-point evaluations.

- Paired testing for disease or vaccination events provides a context by which to evaluate your results.
- For diagnostic studies, acute and convalescent sampling is essential.
- Holding a few birds in isolation may be necessary if a flock is scheduled for slaughter before convalescent testing can be performed.

Understand which tests detect Antigen vs Antibody

Detecting antibody responses or evidence of disease agent

- Immunoassay—measures the presence or amount of an antibody or antigen, with the use of antibody and signaling reagent
- Clinical and postmortem examination
- Histopathology
- Serology
  - Ab ELISA
  - AGID
  - HI
- Antigen detection
  - PCR
  - Sequencing
  - IFA
  - Ag capture ELISA



ELISA

- ELISA detects antibodies after infection.
- Antibodies are found in serum, oral fluids, and GI tract (mucosal).
- Antibody type detected depends on test design and format:
  - Indirect ELISA—often IgG
  - Blocking ELISA—all antibody types (IgM, IgA, and IgG)
- The indirect ELISA provides a semi quantitative range of antibody levels when used routinely over time.

Benefits

- Low cost
- Commercial assays—high repeatability and validated test performance

Drawback

- Time to detection of seroconversion in acute infection diagnosis

PCR—nucleic acid detection

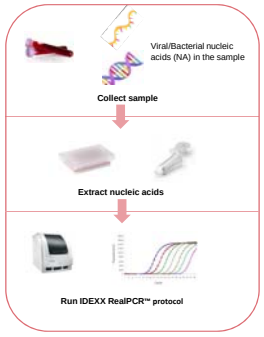
- Detects genetic material of pathogen
  - DNA or RNA
- Target sequence is amplified to enhance detection
- Reported as the number of cycles necessary for detection
  - Cycle threshold value (Ct-value)
  - Lower Ct = more initial target present

Benefit

- Highly sensitive and specific

Drawbacks:

- Extremely narrow target(s), may miss variants that are slightly different
- Requires continuous field sample monitoring to ensure adequate primers (i.e., RNA viruses)



Know the power and limitations of each laboratory assay

- ELISA is a valuable tool to look at trends in seroconversion against common diseases.
- ELISA cannot determine what strains of a disease are present.
- Data is only reliable when it is derived from good quality samples.

Light and dark hemolysis

Interpretation of multiple test results in a flock

Understanding test performance

- No diagnostic test is perfect.
- When calculating test performance, compare *known infection status* to the ability of the test to detect the status correctly.

		Known status +	Known status -
Test result	+	True Pos	False Pos
	-	False Neg	True Neg
		Se = TP/(TP+FN)	Sp = TN/(TN+FP)

Interpretation of simultaneous ELISA and PCR

ELISA	PCR	Interpretation
+	+	**Active viremia** (***circulation and infection***)
+	-	**Previous infection** (***exposed but no evidence of viremia at sampling***)
-	+	**Early infection**
-	-	**Negative** (***no circulation or infection***)

Differences between antibody and nucleic acid detection

Antibody

- Detects exposure and immune response to target agent
- Detects maternal antibodies, vaccination, pathogen exposure, or reinfection
- High repeatability
- Technically simple
- Cost-effective for continuous health monitoring over time

Antigen/Nucleic acid

- Real time detection of disease agent in the animal/herd
- High sensitivity to identical target agent
  - May have cross-reactions
  - May not detect new variants
- Technically demanding
- Costly

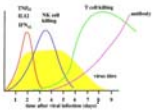
Antibody and PCR assays detect different targets and should not be used as confirmatory tests for each other.

Early in infection, the target may be present with limited antibody production, but as disease progresses, more antibodies will be present and with a low prevalence of the target agent.

Use of results

Antibodies

- Excellent screening tool
- Give comprehensive picture of disease status over time
- Early awareness of changes in disease circulation
- Useful for herd health management
  - Disease elimination
  - Vaccination timing
  - Identifying concurrent infections



Antigen/Nucleic acid

- Investigation for a specific agent
- Point-in-time information
  - Population sensitivity may be low if low prevalence and small sample size
  - Difficult to detect target agent late in infections
- Semiquantitative assessment of pathogen load
- Sample size and pooling influences ability to detect

Take-home points

- Antibody response is induced by presence of antigens/pathogens.
- Antigens and antibodies can be measured by several tests.
  - Understand test performance and know your goal.
- ELISA antibody tests are uniquely fitted for health/disease antibody surveillance.
  - With a population based sample type, infection dynamics of the population can be followed.
- Nucleic acid assays (PCRs) are sensitive for the target material, but sensitivity is dependent on:
  - Prevalence of disease agent in the population.
  - Sample size tested.
- Sensitivity is the ability for the test to detect a true positive as positive.
- Specificity is the ability for the test to detect a true negative as negative.
- The predictive value of any test is based on the prevalence of the disease or antibodies in the population.

Questions?

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