

Getting the Most Out of Your Diagnostic Lab

Saad Gharaibeh

**Minnesota Poultry Testing Laboratory-Veterinary Diagnostic Lab, University of Minnesota, Willmar,
MN 56201**

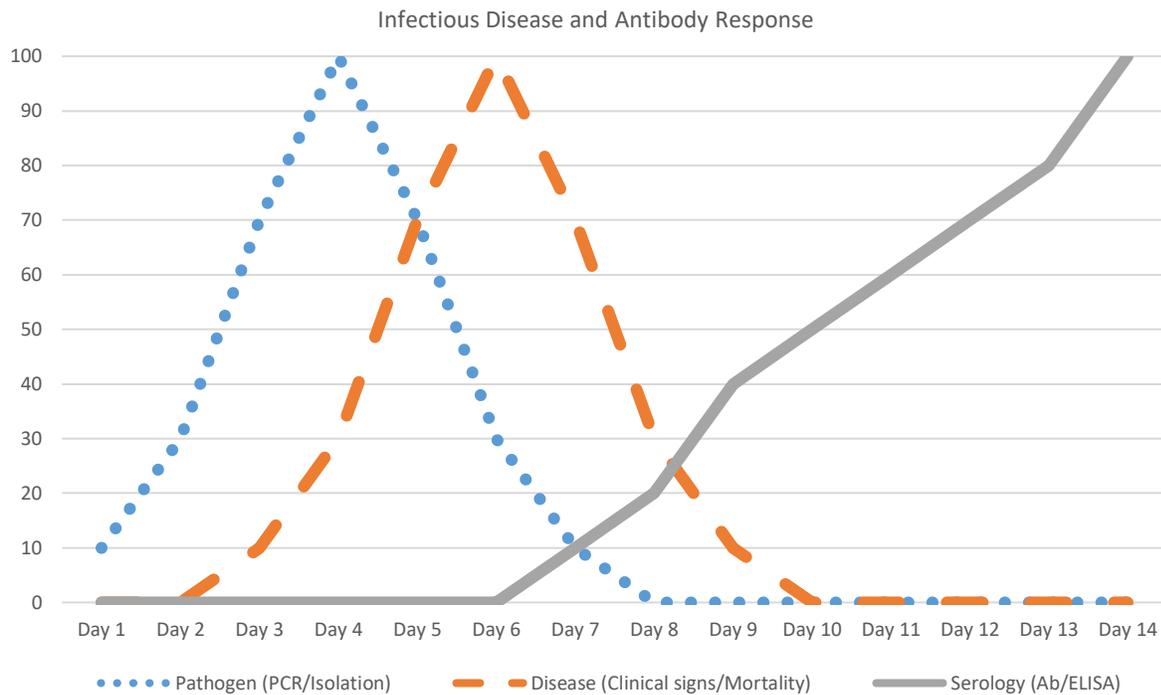
Severe diseases such as highly pathogenic avian influenza and velogenic Newcastle disease are still a concern for poultry producers and government agencies alike. However, poultry producers deal more with subclinical diseases that affect their production numbers such as common respiratory disease. Companies are also concerned and try to improve their management practices that affect poultry health and welfare such ventilation, and water quality.

Tissue injury maybe classified into degeneration, inflammation, or neoplasia. Nutritional deficiencies and toxicosis with certain harmful substances usually cause degeneration of tissues. Infectious agents (virus, bacteria, fungus, and parasites) usually cause inflammation. Pathological changes in poultry organs examined during necropsy procedure followed by tissue microscopic examination (histopathological) will direct the pathologist to what is the existing disease process. Poultry veterinarian can then formulate a differential list of possible causes for this disease process. Further laboratory testing for infectious agents will usually limit the list of possible causes to one specific cause.

There is no one-laboratory test that will substitute all other tests. Every test has its strengths and limitations. There are general categories of diagnostic tests. Tests that detect the presence of the agent or part of it in tissues include PCR, virus isolation, bacterial culture, fecal flotation, intestinal scraping, histopathology, electron microscopy, immunohistochemistry, in situ hybridization, direct immunofluorescent, and antigen-capture ELISA. Tests that detect the presence of antibodies (immunoglobulins) in blood/serum include ELISA, plate/tube agglutination, agar gel immunodiffusion, hemagglutination inhibition, compliment fixation, virus neutralization, and indirect immunofluorescent tests. It is very important to understand the strengths of each test and its limitation and to realize that it is one of the tools in the box of the diagnostic lab.

An example of a common infectious viral disease such as Newcastle disease (ND) will be used to explain the course of disease and which diagnostic test might be suitable at different stages to diagnose this disease. Most infectious disease will have a similar scenario. To illustrate these stages we will use the attached (figure 1). It explains a flock timeline after exposure to an infectious agent, expression of clinical signs, and production of antibodies (serological response).

Figure 1. Susceptible flock status when exposed to an infectious agent.



Most if not all avian species are susceptible to ND infection. However, the infectious dose and disease outcome depends on many factors including the species infected and the infective virus strain.

Birds infected with ND shed the virus in feces and oropharyngeal secretions. Susceptible birds are infected by inhaling or ingesting contaminated material. Replication of the virus occurs in target organs including respiratory, digestive, and occasionally reproductive tract. Virus shedding starts shortly after infection and clinical signs and lesions of disease start to develop. Usually there is an overlap between the presence of the virus (shedding) and the initial clinical signs. At this stage, agent detection tests (such as PCR) can be used and maybe positive depending on their sensitivity.

The time between exposure to ND virus (NDV) and the development of clinical signs varies depending on the host species, the immune status of the host, the virulence of the virus, the dose of the virus, and the route of exposure. The average incubation period is 5-6 days. Susceptible chickens infected with a virulent strain usually show signs of disease in 2 days and die by the 4th day. On the other hand, it takes 1 week for well-vaccinated layers to start showing decrease in egg production and this might be the only sign.

As soon as 6 hours after infection, bird splenic-cells start producing interferon and interleukin (mediators of inflammation) to activate macrophage (one of the white blood cells) defense against the virus and it takes 1 day to start the cell mediated immunity (CMI) response. However, regular diagnostic laboratories do not have the means to measure interferon or CMI.

First IgM (initial antibody) maybe detected 4 days after exposure. IgG is detected 7 days after exposure. Commercial ELISA kits measure IgG not IgM; however, it is possible to measure IgM by other serological tests such hemagglutination inhibition (HI). These antibodies can be detected by serological tests and be positive at this time depending on their sensitivity. It is important also that we deal with poultry as a flock and not as an individual animal and that is why it is recommended to examine around 30 serum samples from a flock to detect different levels of response in individuals within the same flock.

Immunosuppressive diseases and conditions will affect all of these processes timing and level of response. Immunosuppressive disease such as hemorrhagic enteritis in turkeys and infectious bursal disease in chickens may affect the immunity the flock without showing clinical signs of infection (subclinical disease).

So for an early ND infection (first few days of clinical signs), a single serological test at this time is of limited value. It is better to attempt agent detection by PCR or virus isolation. Samples to be collected for virus isolation and PCR are oropharyngeal swabs in viral transport medium. If the disease has started 3-4 weeks ago, virus detection by PCR and virus isolation maybe of limited value at this time. However, if you do serology for NDV, you will find titers at this time. How would you interpret if these titers were due to this recent infection or due to prior vaccination? Proper interpretation of these results requires building your baseline data and experience from previous flocks tested at similar age with similar vaccination history.

Serological monitoring will establish a baseline data of antibody titers of what is expected under certain field condition using a specific vaccination program. A deviation from this baseline value should be easily noticed and possible causes could be identified or suspected. Also, acute and convalescent (paired) serum samples are very helpful if no vaccination is being used around this time. There are some discrepancies between different serological kits and between different laboratories. The use of a single laboratory with the same testing procedure helps minimize these differences.

The challenge is to identify the most significant flock problem and not to get distracted by individual bird problems. Although the submission may include cull birds which usually have certain problems not reflecting the real situation of the flock. The poultry veterinarian who performs necropsies in the diagnostic lab is not always familiar with the farm management conditions and usually does not have the flock production numbers.

The following information is of value to the diagnostic laboratory investigating the disease problem in a poultry flock:

1. Flock information: age, breed, housing system, presence of other flocks in the farm,
2. History: When did the disease started, previous diseases in the flock, vaccination history, treatment history.
3. Disease signs: Respiratory signs, diarrhea, retarded weight gain, increased condemnations at the plant, drop in egg production, ...
4. Management conditions: wet litter problem, too hot barn, dusty barn,

There is a set of tissues that are suitable for detecting different infectious agents. Consult with your poultry veterinarian or the diagnostic lab to determine the best tissues to submit. For an acute respiratory disease, different respiratory tissues are used differently for best detection results. Tracheas

will be a good sample to attempt detecting respiratory viruses and certain bacterial such as *Mycoplasma* sp. and *Bordetella* sp in turkeys. However, nasal turbinates are considered a better sample for avian metapneumovirus and infectious coryza (*Avibacterium paragallinarum*) in chickens. Lungs are a better sample for culturing fungi, *Pasteurella multocida* (fowl cholera), and *Ornithobacterium rhinotracheale* (ORT). Airsacs are a better sample to culture *E. coli* for bacterial susceptibility testing. However, if you are suspecting a previous respiratory infection and want to attempt virus isolation of infectious bronchitis or influenza virus, your chances of isolating the virus from cecal tonsil is better than trachea or respiratory tissue in this case. A laboratory Manual for The Isolation, Identification, and Characterization of Avian Pathogens book has detailed information on specific samples appropriate for each disease with an appendix as a quick reference diagnostic chart for viral diseases.

- Collet, S. (2016). Principles of Disease Prevention, Diagnosis, and Control Introduction. In: Swayne, D., Glisson, J., McDougald, L., Nolan, L., Suarez, D., and Nair, V. (Eds). Diseases of Poultry, 13th ed., Wiley-Blackwell Publication.
- Miller, P., and Koch, G. (2016). Newcastle Disease. In: Swayne, D., Glisson, J., McDougald, L., Nolan, L., Suarez, D., and Nair, V. (Eds). Diseases of Poultry, 13th ed., Wiley-Blackwell Publication.
- Williams, S., Dufour-Zavala, L., Jackwood, M., Lee, M., Lupiani, B., Reed, W., Spackman, E., and Woolcock., P. (Eds). (2016). A laboratory Manual for The Isolation, Identification, and Characterization of Avian Pathogens. OmniPress, Inc., Madison, WI.