

Avian Influenza Diagnostic and Surveillance Testing Procedures

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In light of the current Highly Pathogenic Avian Influenza (HPAI) outbreak in North America, many questions have been asked about the differences between the types of tests used to diagnose this disease.

There are several tests for Avian Influenza detection and the interpretation of the test results is different.

What Happens When a Bird Is Infected with AI Virus?

Let's look at an individual bird that has been infected by an Avian Influenza virus. The virus will circulate in the animal's tissues and will be shed by the bird for several days. How long it will shed virus depends on the species of bird and the virulence of the virus. Low pathogenicity viruses (LPAI's) will probably be shed longer and many do not kill the host. High pathogenicity viruses (HPAI's) cause much more damage and usually kill the host. With the current HPAI circulating in North America, domestic ducks appear adapted to the virus and shed for longer periods but do not die of the disease. Poultry species, however, will shed large amounts of virus and often die 4-6 days post infection. Large amounts of virus can be found also in the fresh dead carcasses.

If the animal does not die but survives beyond one week or more, the bird's immune system has time to produce antibodies (substances that fight the virus) and these antibodies can be detected in the serum or egg yolk. Serum or egg yolk antibodies are present in the live bird for weeks or months. The actual virus by this time is usually gone.

The above scenario suggests that the best time to do a virus detection test is soon after infection until right at death or up to 2 weeks after exposure to the agent. The virus load, however, steadily declines with time until it is cleared.

The best time to detect antibodies in the bird is after the second week and for several weeks or sometimes months afterwards.

Serological Tests:

These are serum or egg yolk tests that check for the presence of Avian Influenza antibodies. Antibodies are substances produced by the birds in response to exposure to the viruses. They

are generally detected 10 days and thereafter in the serum or egg yolk of the birds. The serum antibodies do not differentiate which AI viruses the birds were exposed to, but detect antibodies from all the group A Influenza viruses.

When a serological test is POSITIVE, it means that sometime in the PAST, the bird had been exposed to the Avian Influenza virus. The birds often are no longer shedding actual virus, as the antibodies produced help the animals rid themselves of the infection. However, this does not mean the entire flock has cleared the virus or that the virus is gone from the environment.

Two serological tests approved for monitoring for Avian Influenza exposure by the National Poultry Improvement Plan are the Agar Gel Immunodiffusion test (AGID) and the Avian Influenza Enzyme Linked Immunosorbent Assay (ELISA). Both tests are SCREENING TESTS ONLY!! Finding a positive AGID or ELISA result on serum samples is merely a cue that further confirmatory testing is needed. Occasional false reactions (both positive and negative) have been known to occur with any screening test. The advantage of the AGID and ELISA tests is that the results may be available in 24 hours (AGID test) or within a few hours (ELISA tests). They are also very inexpensive to run.

It is important to note that serological tests are not approved for waterfowl!! Virus isolation is required for waterfowl.

Virus Detection Tests:

Virus isolation in chicken embryos is considered the “gold standard” for identification of Avian Influenza viruses. Oropharyngeal and/or cloacal swabs are used for this purpose in the live animal. In order for this test to detect virus, the bird needs to be actively shedding the virus. The advantage of this test is its high sensitivity and specificity. The disadvantage of this test is that not all laboratories in the US are equipped to run the test and it is more expensive.

Antigen detection ELISA test kits are available for rapid bird-side testing for Avian Influenza. Oropharyngeal swabs are collected from birds, mixed with specific fluid reagents in a test tube and then a test strip is inserted in the tubes. Results are available in 15 minutes as positive or negative. The tests are fairly specific but not as sensitive as some tests. Large amounts of virus are needed in the sample for the test to turn positive. This means there is a fairly narrow window of time when the test will detect AI virus (within the first week of infection generally). A positive result is considered significant, but false negatives are possible if the amount of virus shed is very low. The advantage is the convenience of bird-side testing. The disadvantage is the expense. This test is also considered a screening tool. If a positive result is detected, more confirmatory tests will be needed.

Molecular tests (rRt-PCR tests): The polymerase chain reaction (PCR) assay is a rapid and sensitive method for detecting the genetic material of influenza viruses. These tests can identify if the virus is present in the sampled material and also the sub-types of the virus (H5, H7). They do not differentiate whether the virus is highly pathogenic or low pathogenic. For that, live chicken testing or genetic sequencing are necessary. The advantage of these tests is their quick turn-around time (one day or less) and high sensitivity. The disadvantage is the expensive

equipment needed, and highly trained technicians. Only certain laboratories (NAHLN or National Animal Health Laboratory Network) are approved to run this test and standardized training, methods and equipment are needed.

So what test should you pick to screen your birds for Avian Influenza? That depends on many variables.

Are you part of the National Poultry Improvement Plan AI Clean or AI monitored program? You should contact your state Department of Agriculture, Bureau of Animal Health and talk to the National Poultry Improvement Plan coordinator to learn more about requirements and tests available in your state. Most states also have an Avian Influenza Emergency Response Plan that talks about testing requirements in case of outbreaks. The response plan may also differ according to LPAI and HPAI situations.

Not every state has every test available through their laboratories. The testing methods may differ for upland gamebirds and waterfowl, and many states only allow certified poultry testers or state employees to sample the animals. There are certainly differences in test sensitivity, specificity, turn-around time and expense. Any “suspicious” screening test must be reported to the state agency and will likely trigger further investigation, quarantine and confirmatory testing by the National Veterinary Services Laboratory (NVSL) in Ames, IA. Only NVSL is approved to say whether a flock or premise is truly infected with an AI virus and do further testing to characterize the virus.

You can also contact the National Poultry Improvement Plan office for more information on AI program requirements for upland gamebirds and waterfowl using the link below.

www.poultryimprovement.org

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