

Avian Influenza Diagnostics and Testing

Avian influenza (AI), commonly known as “bird flu,” is caused by a virus that can infect domesticated and wild birds, including chickens, turkeys, pheasants, quail, ducks, geese, and guinea fowl. AI virus strains are classified into two groups by pathogenicity, or the ability of the virus to produce disease, in domestic chickens. Highly pathogenic (HPAI) virus strains are extremely infectious, often fatal to domestic poultry, and can spread rapidly from flock-to-flock. Low pathogenic (LPAI) virus strains occur naturally in wild birds without causing illness, but some are capable of mutating into HPAI viruses.

The U.S. Department of Agriculture’s (USDA) Animal and Plant Health Inspection Service (APHIS) works closely with States and the poultry industry to prevent AI from becoming established in the U.S. poultry population.

Sample Collection

Wild migratory birds, domestic flocks, live bird markets, and import quarantine facilities are routinely sampled and tested to detect AI viruses. Samples are taken from live birds, dead birds, or the environment that they inhabit. In live domestic birds, samples are taken by swabbing the throat. A fecal swab sample can be taken from wild birds. Samples are placed into sealed tubes and taken to USDA-approved laboratories for AI screening tests, or sent directly to USDA’s National Veterinary Services Laboratories (NVSL) for screening and confirmatory tests.

Testing Facilities

Many of the AI screening tests, including those done on samples from wild birds and live bird markets, are conducted by one of more than 50 USDA-approved laboratories that are part of the National Animal Health Laboratory Network (NAHLN). The NAHLN is a cooperative effort between APHIS, USDA’s Cooperative State Research, Education and Extension Service (CSREES), and the American Association of Veterinary Laboratory Diagnosticians. NAHLN laboratories conduct nationwide surveillance testing for early detection of animal diseases and have the resources to test large numbers of samples during a disease outbreak. The U.S. Department of the Interior’s National Wildlife Health Center is a NAHLN laboratory that

performs initial AI screening tests on some of the wild bird samples collected around the United States.

Screening tests conducted at these laboratories detect the possible presence of an AI virus and whether it is an H5 or H7 subtype. The H5 and H7 subtypes have the potential to mutate into highly pathogenic strains. If a screening test is positive for an H5 or H7 subtype, NAHLN laboratories forward samples to the NVSL in Ames, IA, for additional screening and confirmatory tests.

National Poultry Improvement Plan H5/H7 LPAI Monitored Program

The National Poultry Improvement Plan (NPIP) is a voluntary program administered cooperatively by USDA, States, and the poultry industry. The program’s H5/H7 LPAI Monitored Program requires the testing of broilers, commercial layers, and meat-type turkeys. Screening tests are performed in 135 NPIP-authorized laboratories throughout the country.

APHIS operates secure data reporting systems that incorporate NPIP testing data in national AI surveillance. This information helps to assure international partners that the U.S. AI surveillance system is working to verify the safety of our poultry exports.

Stages of Testing

Screening tests conducted by the NVSL and NAHLN laboratories are polymerase chain reaction (PCR) tests. The PCR tests run at these laboratories include the: matrix test, which screens for AI viruses; H5 test, which screens for the H5 subtype; and H7 test, which screens for the H7 subtype. The NVSL can also run an N1 test, which screens for the N1 subtype. PCR testing can determine H5, H7, and N1 subtypes within 24 hours, but cannot differentiate between HPAI and LPAI viruses.

The NVSL conducts confirmatory testing after the screening tests, which may include virus isolation, H/N subtyping, genetic sequencing, and chicken pathogenicity tests. Results of confirmatory tests are generally available within 5 to 14 days.

Virus Isolation vs. Serology

Virus isolation tests detect viruses present in a sample. Positive virus isolation is an accurate method of confirming the presence of a viable virus, that is, a virus that could infect other birds. Serology may be used to detect the presence of antibodies to AI viruses in a bird’s serum, or blood plasma. The presence of antibodies indicates that the bird was infected by an

AI virus at some point in the recent past. Birds with antibodies to an AI virus, however, may not currently be infected with that virus.

If laboratories identify H5 or H7 AI subtypes in a domestic flock only through the detection of antibodies (positive serology), the standards set by the World Organization for Animal Health (OIE) require further investigation of the situation. If no other evidence of an infection is found, then the OIE does not classify the finding as a “notifiable” AI infection. While detection of the same H5 or H7 antibodies in multiple flocks would be reported to the OIE, virus isolation and PCR tests are the standard for determining if a bird or flock is currently infected with an AI virus.

Virus isolation detects AI viruses by inoculating sample material into the embryos of chicken eggs and incubating them to allow any virus present to “grow,” while a series of tests are used to identify H and N subtypes of the virus. These tests cannot determine pathogenicity. Genetic sequencing tests identify the genetic sequence of a detected virus and compare it to known AI virus sequences. If the sequence is similar to that of a known HPAI virus, the sample is categorized as highly pathogenic. Chicken pathogenicity tests are conducted by inoculating 4- to 8-week old disease-free chickens with a virus isolate and then observing the birds for signs of AI for 10 days. The OIE defines HPAI as any AI virus that is lethal for 6 or more of the 8 chickens, or a 75 percent or greater mortality rate.

Response to AI Detection

Although response actions depend upon the subtype and pathogenicity of the AI infection, APHIS works cooperatively with States and the poultry industry to ensure proper disease response while minimizing economic hardship to the producer. The National HPAI Response Plan and individual State’s LPAI Initial State Response and Containment Plans, which are part of the NPIP LPAI H5/H7 Monitored Program, outline specific actions.

If an HPAI infection is found in a poultry flock, all surviving birds are depopulated to prevent the spread of the virus. If laboratory testing detects LPAI H5 or H7 viruses, the infection may be controlled through depopulation or controlled marketing, depending on the Initial State Response and Containment Plan. Controlled marketing allows poultry that are infected with or exposed to H5/H7 LPAI to move to market on a limited basis, according to the process laid out by a State’s response and containment plan. Under controlled marketing, infected or exposed poultry may not be transported until 21 days after the acute phase of the infection, and poultry must test negative for H5/H7

LPAI viruses within 7 days prior to slaughter. Testing reduces the risk of spreading H5 or H7 LPAI strains and maintains the safety of the controlled marketing process, while allowing producers to recover value from infected poultry.

APHIS cooperates with State partners to conduct surveillance in the area surrounding AI-infected birds and enforces movement controls to reduce the risk of spreading the virus. APHIS reports all findings of HPAI, as well as findings of H5 or H7 AI viruses in commercial poultry, to the OIE.

For More Information about Avian Influenza

USDA efforts to protect against and respond to bird flu: www.usda.gov/birdflu

Report Sick Farm Birds: If your farm birds are sick or dying, call USDA’s Veterinary Services toll free at 1-866-536-7593, or your State Veterinarian or local extension agent.

Report Dead Wild Birds: Dead wild birds can be reported to State or Federal wildlife agencies. Information on how to make contact with wildlife officials in your State is available at www.usda.gov/birdflu.

Safe Food Preparation: USDA Meat and Poultry Hotline—1-888-MPHotline (1-888-674-6854), TTY: 1-800-256-7072 (available in English and Spanish). Online answers are provided at www.fsis.usda.gov by clicking on “Ask Karen.”

Current List of Countries/Areas Affected with HPAI H5N1: www.usda.gov/birdflu

U.S. Government efforts to protect human health: www.pandemicflu.gov

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