AVIAN INFLUENZA TESTING AND DIAGNOSTICS
April 2015

This fact sheet provides definitions and a description of the tests used to diagnose avian influenza (AI) in U.S. bird populations.

SAMPLE COLLECTION

Testing for the presence of AI requires that samples be taken from live birds, dead birds or the environment birds inhabit. Samples are routinely collected from wild birds, domestic flocks, live bird markets and quarantined birds.

TESTING PROCESS

Initial AI screening tests are performed by one of more than 45 USDA approved laboratories in the National Animal Health Laboratory Network (NAHLN). In the case of wild bird samples, the U.S. Department of the Interior’s National Wildlife Health Center also performs initial screening tests. These labs will determine if AI virus is present and whether it is an H5 or H7 subtype. Because of the potential for H5 or H7 subtypes to mutate into highly pathogenic strains, those samples are forwarded to USDA’s National Veterinary Services Laboratories (NVSL) for confirmatory testing. NVSL then conducts additional screening tests and confirmatory tests, with research assistance from USDA’s Southeast Poultry Research Lab.

This USDA laboratory in Ames, Iowa, is the only AI reference laboratory in the United States recognized by the World Organization for Animal Health, known as the OIE. Although there is a network of laboratories across the nation approved to conduct AI screening tests, confirmatory testing in the United States is conducted only at NVSL.

STAGES OF USDA TESTING

I. Rapid screening tests

A series of AI rapid screening tests are performed that cannot differentiate between HPAI and LPAI viruses. Varieties of this test can screen for the presence of all strains of AI virus, specifically for H5 or H7 subtypes and the N1 subtype.

NVSL conducts the following rapid screening tests:

1) Matrix test – used to screen for AI viruses
2) H5 test – used to screen for H5 subtype
3) H7 test – used to screen for H7 subtype
4) N1 test – used to screen for N1 subtype
These results can be expected within 4-7 hours after receipt by NVSL.

II. Confirmatory tests

1. **Virus isolation test and H/N subtyping tests**
   Virus isolation is the gold standard test used to diagnose AI virus infections. The virus is isolated in embryos inside chicken eggs. A series of tests follow to specifically identify H and N subtypes of the AI virus. These tests cannot determine pathogenicity.

2. **Genetic sequencing test**
   This test involves identifying the genetic sequence of the virus grown and comparing it to known AI genetic sequences. These known sequences, such as that of the highly pathogenic H5N1 AI virus, are stored in databanks. If the genetic sequence matches that of a known highly pathogenic AI virus, the sample is considered to be highly pathogenic.

3. **Chicken pathogenicity test**
   This test involves the inoculation of 4- to 8-week old disease-free chickens and observation for signs of AI for 10 days. According to the USDA and OIE, highly pathogenic AI is defined as any AI virus that is lethal for 6 or more of 8 chickens (75% mortality).

Confirmatory AI test results can be expected within 5-10 days.

TERMINOLOGY

**Avian influenza (AI)**—the bird flu—is a virus that infects wild birds (such as ducks, gulls, and shorebirds) and domestic poultry (such as chickens, turkeys, ducks, and geese). There is flu for birds just as there is for humans and, as with people, some forms of the flu in birds are worse than others.

AI viruses are classified by a combination of two groups of proteins: the hemagglutinin or H proteins, of which there are 16 (H1-H16), and neuraminidase or N proteins, of which there are 9 (N1-N9).

**Pathogenicity**: the ability of the virus to produce disease. AI strains also are divided into two groups based upon the ability of the virus to produce disease: low pathogenic (LP) and highly pathogenic (HP).

**Low Pathogenic or “low path” avian influenza (LPAI)**: LPAI occurs naturally in wild birds and can spread to domestic birds. In most cases it causes no signs of infection or only minor symptoms in birds. These strains of the disease pose little significant threat to human health. These strains are common in the U.S. and around the world.

**Highly Pathogenic or “high path” avian influenza (HPAI)**: HPAI is often fatal in chickens and turkeys. HPAI spreads rapidly and has a high death rate in birds than LPAI.

**Genetic Sequence**: the process of determining the individual elements that make up a specific gene. This could be equated to finding the “blueprint” of the gene.