

TESTS FOR DIAGNOSIS OF AVIAN INFLUENZA

Tests of AI Exposure (Blood)

- **AGID** – The Agar gel immunodiffusion test uses positive control antigen obtained from the National Veterinary Services Laboratory (NVSL) in Ames, Iowa. When placed in an agar well along with serum samples from birds, this blood test can detect the presence of antibodies to avian influenza in birds, indicating that they have been exposed to the virus. A precipitation line is formed between the well containing the serum (if antibody is present) and the antigen. The test does not differentiate between the types of influenza viruses or whether it is a recent or past exposure.

Tests of the Presence of AI Infection (Swabs of secretions or feces)

- **Directigen Flu A test®** is an enzyme immunoassay test for the rapid detection of influenza A viral antigen from specimens. Usually cloacal and tracheal swabs are selected as bird samples, although lung tissue and oviduct contents can be used. The test does yield some false positives when other proteins interfere. A positive test usually indicates the presence of influenza virus in those tissues or samples and is hence followed up with viral isolation as a confirmation. The Directigen® is a human test adopted in some situations for avian work.
- **Synbiotics FLU DETECT™** – Synbiotics Antigen Capture Test Strip is an invitro, rapid immunochromatographic assay designed to aid in the qualitative detection of Influenza type A virus in tracheal and cloacal swabs from symptomatic birds or flocks. The FLU DETECT™ Test Strip is designed to detect all 15 subtypes of Influenza type A virus.
- **rRT-PCR** – Real Time, Reverse Transcriptase -Polymerase Chain Reaction, is a rapid molecular test, that can be used for diagnosis of group A influenza viruses. The test detects presence of viral ribonucleic acid utilizing matrix (MA) gene specific primers and a fluorescent probe. The assay also helps in subtyping positive Group A influenza viruses into H5/H7/nonH5/H7 subtypes with use of specific haemagglutinin (HA) gene primers and probes. The test has higher sensitivity for detecting AI in tracheal swab samples than cloacal or fecal swab samples. Overall sensitivity and specificity of the test compared to virus isolation, as determined by National Veterinary Services Laboratory, was found to be 88.2% and 99.5%, respectively. However, this test sensitivity was found to be much higher (95.1%) when using the test on a positive premise.
- **VI** – Virus isolation uses 9 to 11 day-old embryonated chicken eggs to which centrifuged or filtered samples of cloacal or tracheal swabs or similar samples from respiratory or intestinal tissues are inoculated. Death in the embryos after 48 hours often indicates the presence of live virus. The allantoic fluid harvested from the embryo (which would then contain the virus) is reacted with chicken red blood cells as an additional confirmation, and a panel of antisera is prepared to identify the various subtypes of influenza virus.