

OIE/FAO guidelines for correct application and interpretation of diagnostic results for the diagnosis of avian influenza (AI) on serum samples

Introduction

Serological diagnosis of AI should be performed in 2 steps, unless the subtype circulating in a given country is already known. The first step aims at the detection of antibodies to any AI virus. The second step, to be performed on samples that are positive to step 1 identifies the viral subtype causing infection. OIE certified reagents should be used for diagnostic purposes, and protocols described in the *OIE Manual* should be used.

Step 1: detection of antibodies to the group antigen (Type A). These tests are able to detect antibodies to the group antigen of influenza A viruses. The antigen-antibody reaction is against the nucleoprotein (NP) or Matrix (M) proteins of AI viruses. These antigens are present in all influenza A viruses regardless of the H or N subtype. Positivity to these tests indicates that the birds has encountered an influenza A virus but **no information** on the AI subtype which has caused seroconversion can be deduced. The AI subtype used for the production of the antigen contained in the test is not an indication of the virus that has caused the positive result.

AGID: Agar gel immunodiffusion test. This is a simple and reliable test in chicken and turkey sera. It is very specific but is of limited sensitivity, for this reason it must be used as a diagnostic tool on a flock basis. It can be performed in any laboratory with basic equipment. It is completely unreliable in waterfowl as these birds do not produce precipitating antibodies. It has not been fully validated in other avian species.

ELISA: Enzyme linked immuno-sorbant assay. This is a test that requires more advanced laboratory equipment that must include a spectrophotometer. It is a highly sensitive test, but it lacks in specificity. In case of indirect ELISA tests, care must be taken in ensuring that the secondary antibody in the test (anti-species) is directed against the species under examination. Competitive ELISA tests have the advantage that serum of any species can be examined. The manufacturer's instructions should be followed and assumptions on test reactivity for species other than those mentioned in the kit's specifications should be avoided.

NOTE: serological positivity to type A in waterfowl (wild and domestic) is a normal finding.

Step 2: detection of subtype-specific antibodies (H subtype). This test is to be used in birds that are known to be infected with AI (either following a positive serologic test against the group (type A antigen), or as a result of clinical history). The test is used to identify the haemagglutinin subtype of the virus causing the seropositivity. **The haemagglutination inhibition (HI)** test is to be used for this purpose. In order to avoid wastage of diagnostic reagents it is recommended that serological positivity to Notifiable Avian Influenza (NAI) viruses is immediately excluded or confirmed. Initial testing should be performed using H5 and H7 subtype antigens. At least two antigens of the same H subtype but with different neuraminidase subtypes (eg. H5N1 and H5N9 and H7N1 and H7N3) should be used in the initial approach to diagnosis. A sample is considered positive if it causes inhibition of the haemagglutinating activity of 4 HA units at a titre of at least 1:16 (2^4).

Low degree cross-reactivity with other H subtypes may be observed due to homology with the neuraminidase antigen. This cross-reactivity is generally not higher than 1:16 (2^4) and disappears with another antigen with different neuraminidase.

For example:

A serum sample is positive to H9N2 at a titre of 1:256 (2^8). If tested with H5N2 antigen a positive inhibition result may be observed at 1:8 (2^3). When tested with an H5N9 antigen the sample will be negative.

In case of serological positivity to NAI

In case of first detection of antibodies to viruses of the H5 or H7 subtype in a given country, this result should be confirmed by an OIE reference laboratory. Further investigations aiming at the isolation of the virus should be promptly initiated.