PERFORMANCE EVALUATION OF FIVE DETECTION TESTS FOR AVIAN INFLUENZA ANTIGEN WITH VARIOUS AVIAN SAMPLES

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The Food and Agriculture Organisation, World Organisation for Animal Health and World Health Organisation have identified the need for early detection, transparent reporting and early introduction of control strategies as key components in the fight against the H5N1 infection. Countries through much of the outbreak region are considered as less developed and there are limited veterinary laboratory facilities available to support early detection of suspected outbreaks. Rapid detection tests that are simple, reliable and relatively affordable could be used in these countries to allow expedient H5N1 screening. This study reports on the ability of five commercial or in-house influenza antigen detection tests to detect infection in known H5N1 virus culture positive swab samples.

Various new generation antigen detection tests for influenza A and H5 antigen specific tests in poultry have been developed. Some of these are rapid immunoassays that require no special equipment and can be performed readily in the field. Others such as enzyme-linked immunosorbent assays (ELISA) could be used to test large numbers of surveillance samples in district laboratories with minimal equipment and at lower cost in comparison with molecular diagnostic techniques or virus culture. This study evaluated five influenza antigen detection tests (rapid influenza A virus chromatographic or plate immunoassay tests) by using avian influenza H5N1 virus positive swab samples to estimate their diagnostic sensitivity.

The advantages of rapid immunoassays include their ease of use particularly under field conditions with results available quickly after commencing the test (15 to 20 min for Tests 1 and 2 and 30 min for Test 5). However, as seen in this study, test sensitivities were not high and negative results may need to be interpreted with some caution. Antigen detection ELISAs like Tests 3 and 4 can be conducted in simple laboratories as bench top procedures but they require a moderate level of facilities, technical expertise and access to a spectrophotometer to read the results. They take several hours to complete but have the advantage of being able to test large numbers of specimens in semi-automated systems relatively inexpensively. An additional advantage of Tests 3 and 5 in the context of H5N1 surveillance is that they are H5 avian influenza specific compared with Tests 1, 2 and 4 and could be used to raise alert levels for H5N1 disease outbreaks earlier.

For diagnostic and surveillance testing, especially for individual birds, in diseases such as H5N1 HPAI, test sensitivity needs to be as high as possible for control programs to succeed. Virus culture is still the principal and gold standard diagnostic test for diagnosis of avian influenza and is required for confirmation of outbreaks. However, this testing requires sophisticated facilities, equipment, a high level of biosecurity, and is an expensive and a relatively slow procedure. An alternative procedure for rapid detection of avian influenza viruses is by the use of RT-PCR or NASBA for specific influenza virus RNA detection. These procedures have shown a relatively high sensitivity for the detection of influenza RNA. For comparative purposes, the results of influenza RNA detection tests (NASBA, RRT-PCR or conventional RT-PCR), that had been conducted at the time of submission for selected samples in the evaluation, were included in Table 1 and clearly demonstrate a higher detection rate for RNA detection tests than influenza antigen detection tests.

Although influenza antigen detection tests have relatively low sensitivity for individual birds, flock sensitivity is improved by testing multiple affected birds from an outbreak, they are relatively inexpensive compared to virus culture or PCR tests and have the advantage of being able to be used in the field or at small district laboratories closer to where outbreaks of avian influenza may occur. Rapid influenza antigen detection tests can be considered as a flock-screening tool to facilitate rapid investigative and control interventions.

The antigen detection tests, including the rapid chromatographic immunoassays, the Dot-ELISA and the antigen detection ELISAs are tests that could be used for preliminary flock investigations for the diagnosis of H5N1 outbreaks. These tests could serve a valuable role in testing cloacal or tracheal swabs from sick or dead birds in the field or small district laboratories in remote locations to raise the alert level with disease control authorities if positive reactions occur. However, the relatively low sensitivity of the tests as individual bird tests means that they should only be used on optimal clinical specimens from diseased birds, testing affected birds on a flock basis and testing samples as close to the onset of disease as possible before viral titres diminish. It should also be kept in mind that their sensitivity for waterfowl and wild birds is lower than for chickens and that they are not appropriate for testing surveillance samples from healthy birds. These tests should only be considered as screening tests to be followed up by confirmatory tests such as RT-PCR techniques or viral culture wherever possible but they could assist in facilitating rapid investigations and control interventions.

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