

V. Product performances

1. Evaluation studies done for the product

Evaluation of Bioline Influenza A in Clinical Specimen

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Introduction

Avian influenza is caused by influenza virus, in Orthomyxoviridae Family, contains RNA genome. This virus can be divided into three types: A, B and C per matrix protein (M) (Swayne and Halvorson, 2003). Only Influenza Virus Type A is communicable and highly pathogenic avian influenza virus (HPAI). The virus can be further classified into subtype per two envelope proteins: 15 varieties of haemagglutinin (H) and 9 varieties of neuraminidase (N) (Webster, 1997). Most of HPAI Type A that causes disease in chicken are H5 and H7. In Thailand, the first epidemic was reported in February 2004, from chicken farm, and it has been reported until now. The epidemic covered more than 30 provinces all over the country (www.oie.int). Many kinds of avian are susceptible to Influenza virus infection. Symptoms may be varied due to virus subtype and kind of infected animal (Perkin and Swayne, 2003). Avian Influenza not only caused a great damage to avian farm industry, but also caused panic in public health due to human being susceptibility to this avian flu subtype. Infected people has respiratory problem and can be fatal. Current laboratory diagnostic method is based on embryonated egg inoculation, followed by heamagglutination and subtyping with standard serum (OIE, 2000, Swayne *et al.* 1998). The whole process is time consuming, high cost, and requires classified laboratory and experienced technicians. Then the conventional method may not convenient for screening at field site. At present, a test kit has been developed named Bioline Influenza A Antigen Test, for primary diagnosis with ease and quick result. The kit is intended for Influenza Type A Ag detection. From in-house preliminary study, the performance was acceptable. So, the objective of this study was to evaluate the performance of Bioline Influenza A Antigen in comparison to other commercial kits to support further development for better performance.

Objectives

To evaluate the performance of test kit in comparison of other commercial kits.

Materials and Methods

1. Twenty-four specimens were performed in the evaluation. The clinical samples can be divide 2 groups. Group I were 11 samples which influenza A infection (H5N1, H3N2), included parainfluenza type 1 and type 3, adenovirus and mixed infection (adenovirus/parainfluenza type 2, adenovirus/parainfluenza type 3, parainfluenza type 1/measle virus) These samples were performed other commercial kit such as Directigen Flu A, QuickVue, Binax Flu A and Bioline Influenza A and compared with virus isolation. Group II were 13 negative samples which could not be detected respiratory infection.
2. Methods of test
 - A. **Procedure for direct swab sample, nasopharyn-geal, throat or nasal swab**
 - 1) Add provided assay diluent into test tube until marked line (1 ml).
 - 2) Immerse swab into the test tube containing assay diluent.
 - 3) Mix the swab until the sample has been dissolved into the diluent, remove as much liquid from the swab as possible.
 - 4) Leave the test tube until the large particles have settled down to the bottom of the tube.
 - 5) Remove the test device from the foil pouch, and place it on a flat and dry surface.
 - 6) Using the disposable dropper provided, dispense eight drops of the supernatant from extracted sample into the sample well (S), avoiding adding bubbles.
 - 7) Interpret test results at 10 minutes.
 - B. **Procedure for nasopharyngeal aspirate or nasal wash or allantoic fluid.**
 - 1) Dispense three drops of specimen into a provided test tube with provided disposable dropper.
 - 2) Add six drops of the assay diluent from dropper bottle into the tube contained specimen, mix well by pipetting up and down.
 - 3) Dispense eight drops of the mixture into the sample well (S) with the same dropper, avoid adding bubbles.
 - 4) Interpret test results at 10 minutes.
3. Interpretation of the test
 - 1) **Negative result (no antigen detected) :**

The presence of only one purple band (C band) within the result window, no purple band appears in the test region (T band) indicating influenza A antigen was not detectable in the specimen.
 - 2) **Positive result (antigen present) :**

The presence of two purple bands (T and C) within the result window, no matter which band appears first indicates influenza A antigen was detectable in the specimen.

3) Invalid Result

If the purple color band is not visible within the result window after performing the test, the result is considered invalid. The directions may not have been followed correctly or the test may have deteriorated. It is recommended that the specimen be re-tested.

Result

The Biline Influenza A test and other commercial kit could be detected influenza A antigen in allantoic fluid of H3N2 (#D39) and H5N1 (#D1) as showed in Table 1. Comparison Biline Influenza A with IFA and PCR, the result showed positive with Biline Influenza A test as same as IFA and PCR.

We had performed specificity which using other respiratory infection such as parainfluenza, adenovirus, mixed infection and group II (negative sample). The result was found non-cross-reactivity by Biline Influenza A test (Table 2).

Table 1: The results of influenza A antigen detection in clinical samples and virus isolation.

Assay	Clinical samples		Virus Isolation	
	#D39	#D1	#D39	#D1
	(H3N2)	(H5N1)	(H3N2)	(H5N1)
IFA (infected cell)	Pos	Pos	Pos	Pos
Directigen Flu A	Pos	NA*	Pos	Pos
QuickVue	NA*	NA*	Pos	Pos
Binax Flu A	NA*	NA*	Pos	Pos
Biline Influenza A	Pos	NA*	Pos	Pos

*NA = not available, no specimen enough to test.

Table 2: Specificity of Bioline Influenza A in clinical samples.

Result by IFA and/or culture	No. test	RT-PCR (H1,H3,H5) Clinical samples	Bioline Influenza A Clinical samples	
			Neg	Pos
Parainfluenza 1	3	Neg	3	0
Parainfluenza 3	1	Neg	1	0
Adenovirus	2	Neg	2	0
Mixed infection*	3	Neg	3	0
Neg for all viruses	13	Neg	13	0
Overall	22	22	22	0

*Adenovirus/parainfluenza type 2, adenovirus, parainfluenza type 3, parainfluenza virus1/measles

Conclusion:

The study shown that Bioline Influenza A Antigen test kit could also detect influenza A infections in human as same as the other commercial kits. No evidence that cross-reactivity with other infected viruses in respiratory system. These results indicate that Bioline Influenza A is rapid and easy tests that can be used by laboratories in outpatient settings to detect influenza A viruses both human and animal within 10 minutes. In addition, Bioline Influenza A can be used to screening influenza A antigen in culture fluid from virus isolation.

Reference :

OIE. 2000. Highly pathogenic avian influenza In: Manual of standards for diagnostic tests and vaccines, 4th ed.

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