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RESEARCH ARTICLE **(Open Access)**

Evaluation of the Binax NOW Flu A+B Enzyme Immunochromatographic Assay in comparison with Real-Time PCR during the Pandemic of Influenza 2009

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Abstract:

The Binax NOW Flu A+B enzyme immunochromatographic assay was compared to Real-Time PCR assay for 542 specimen from nasal-wash or nasopharyngeal swab collected during the pandemic of 2009. The overall sensitivity, specificity, positive predictive value, and negative predictive value of the assay were 44.6%, 95.8%, 73.5%, and 86.9%, respectively. The assay sensitivity shows mixed values decreasing significantly in infants and children age, which is linked with the quality and the way sample is collected.

Keywords: Imunochromatografic assay, Real-Time PCR, neuraminidase, influenza A and B.

1. Introduction

With the appearance of the pandemic A/H1N12009 and the advent of antiviral agents that target the neuraminidase enzyme, the ability to diagnose and discriminate between influenza A and B viruses has become more important. Numerous enzyme immunoassays (EIA) are a support to the technicians but often suffer lack of sensitivity, although specificity is often high (1,2,4, 6–15). Testing with the molecular methods like the Real-Time PCR have demonstrated increased sensitivity and can assess specimen quality but are not conducive to rapid testing in a physician's office or small hospital laboratory (3). Viral culture provides excellent virus recovery but usually not in the time frame that can impact treatment with antiviral agents. (4, 5) Therefore, the EIA are useful to the frontline clinician who is faced with the decision of whether to administer antiviral agents or to admit the patient for inpatient care.

2. Material and Methods

The purpose of this study was to compare the performance of the Binax NOW Flu A and Flu B immunochromatographic assay to that of Real-Time PCR with TaqMan method, material and reagents sent from theWHO-CC, CDC, Atlanta. The Binax NOW Flu A and Flu B assay consists of separate test strips for detection of a nucleoprotein of influenza A and B viruses in a lateral-flow format and is approved for nasal-wash, nasal-aspirate, and nasopharyngeal-swab specimens. Nasal-wash and nasal-aspirate specimens can be tested directly on the test strips, whereas nasopharyngeal-swab specimens must be treated with an extraction reagent prior to testing. Results are available within 15 min of sample delivery to the test strips. The test kits can be stored at room temperature. The study was performed during the Pandemic of Influenza 2009 when influenza A virus A/H1N1/pdm2009 was the predominant circulating strain. Consequently, the influenza B virus component could not be evaluated.

All specimens were nasal-wash/nasal-aspirate or nasopharyngeal swab specimens submitted to the virology laboratory near our Insitute of Public Health. The majority of the specimens were collected in the emergency section or from inpatient settings near the Infectious Diseases Clinic or the Pediatric clinic located near the Tirana University Hospital "Mother Teresa". The samples in viral transport medium were most often transported immediately to the laboratory through a triple packaging inside a freeze box and were accompanied with a identification form.

Specimens were tested after receiption in the laboratory by use of the Binax NOW Flu A and Flu B test (Binax, Inc., Portland, ME), following the manufacturer's directions. Once EIA testing was completed, the samples were refrigerated at 2 to 8°C until processed for Real-Time PCR. For the extraction of the viral ARN we have used the commercial kit of QIAGEN with the means of spin columns and followed kit instruction's. Meanwhile for the

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detection of the influenza virus RNA we used the one-step TaqMan-based Real-Time Reverse Transcription PCR (rRT-PCR) method developed and provided along with primer/probe sets targeting the haemaglutinin genes, from WHO CC for Influenza, CDC, Atlanta, GA, USA on a platform of ABI 7500 Real-Time PCR machine. Any remaining specimen was stored at 80°C for future use.

3. Results and Discussion

The Binax NOW Flu A component of the EIA had an overall, specificity, sensitivity, positive predictive value, and negative predictive value of 95.8%, 44.6%, 73.5% and 86.9%, respectively (Table 1). However, when analyzed based on the subject age group, the assay sensitivity decreases significantly in the age group 0-4 yrs and 15-64yrs.

Table 1. Perfomance of Binax NOW Flu A EIA versus rRT-PCR.

Age Group (yrs)	*No of Specimens*					*Specificity (%)*	*Sensitivity (%)*	*PPV*[2] *(%)*	*NPV*[2] *(%)*
	Total	TP[1]	FP[1]	TN[1]	FN[1]				
Overall	542	50	18	412	62	95.8	44.6	73.5	86.9
0-4	46	6	0	34	6	100	40	100	85
5-14	420	12	2	35	5	94.5	70.6	85.7	87.5
15-64	54	27	15	330	46	95.6	36.9	64.2	87.2
65>	26	5	1	13	5	92.8	50	83.3	72.2

[1]TP, true positive; FP, false-positive; TN, true negative; FN, false negative
[2] PPV, positive predictive value; NPV, negative predictive value

The sensitivity for the 0-to 4-year-old group was 40% but increases for 2 other age groups (Table 1). The specificity of the assay for all age groups remained above 92%. The positive predictive value ranged from 64.2% for the 15-64-year old group to 100% for the 0-4-year-old group. The negative predictive values ranged from 72.2% for the >65-year-old group to 87.5% for the 5-14-year old group. The remaining 18 samples which resulted false-positive with EIA, were cultured on three repetitive passages, but unfortunately we did not recovered virus.

The Binax NOW Flu A and Flu B test was used primarily to determine whether antiviral therapy should be initiated, to assess patients prior to admission for grouping purposes and to push the importance of the presence of these imunochromatografic assays in hospital or also in different points of care. We performed the rRT-PCR in comparison with Binax NOW EIA because previous influenza surveillance studies in general indicate that the rRT-PCR indentifies more virus with less hands-on time. The results of this study indicate that the Binax NOW Flu A test is a rapid, user-friendly test for the presence of the influenza A virus. The test is easy to perform with minimal hands-on time and is suitable for rapid testing within or outside of the virology laboratory. However, even when testing was limited to nasal-wash/swab specimens or nasopharyngeal aspirates, which are considered the most ideal specimen types (3, 8, 11), the Binax NOW Flu A EIA provided mixed results with regard to test sensitivity. The assay performed well with specimens obtained from children more than 5 years of age but provided unacceptable sensitivity when specimens were obtained from infants or adults. The result of the test is very dependable from many reason such as the way the sample is collected stored and transported. Our results are partially similar to the results of Landry et al. (11) in their study comparing the Binax NOW Flu A and Flu B test and the Directigen Flu A/B test (BD Microbiology Systems, Cockeysville, MD) to viral culture and spin-enhanced fluorescent-antibody stain. Landry et al. noted increased assay sensitivity in young children (2 years of age) and decreased sensitivity with specimens obtained from adult patients, which is the same with our results. Similar results have been reported by Weinberg and Walker (15), who evaluated the Binax NOW Flu A and B assay in two age groups, younger than and older than 9 years of age. This decrease in test sensitivity in adult patients has also been noted with other enzyme immunoassay-based tests for influenza (9, 10, 13, 14) and has been suggested to be a result of less viral shedding in adult patients (9). Nevertheless, a positive Binax NOW Flu A test result in an adult patient does indicate a high likelihood that the patient is infected with influenza virus.

The one obvious weakness of this study is the fact that during the Pandemic of Influenza in 2009 in Albania was predominantly this strain of Influenza A virus. From all those samples tested only 2 of them resulted positive for influenza B virus. Consequently, the performance of the influenza B virus component of the assay could not be determined. The assay format, as tested in this study, consisted of strips marked for the capsid nucleoproteins of influenza A and B viruses on a single test strip. This format

provides the laboratory with the option of testing for either influenza A or B virus, depending on the influenza viruses circulating during the season.

4. Conclusions

In summary, the results of the study indicate that while the Binax NOW Flu A EIA is relatively sensitive in specimens collected from children above the age 4, the assay provides unacceptable sensitivity in younger children and adults and must be used with caution in these populations. Depending on the clinical situation, further testing by culture, or PCR should be considered in the event of an initial negative result. We agree that rRT-PCR must be considered as a first line diagnostic method for detecting 2009 Influenza A/H1N1

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