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Comparison of the Binax NOW Flu A Enzyme Immunochromatographic Assay and R-Mix Shell Vial Culture for the 2003-2004 Influenza Season

Robert C. Fader*

Scott and White Memorial Hospital and the Texas A&M University Health Science Center, Temple, Texas 76508

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The Binax NOW Flu A enzyme immunochromatographic assay was compared to viral culture with R-Mix shell vials for 455 nasal-wash or nasal-aspirate specimens. The overall sensitivity, specificity, positive predictive value, and negative predictive value of the assay were 64.9%, 98.4%, 89.3%, and 93.2%, respectively. However, the assay sensitivity decreased significantly with increasing patient age.

With 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 available to the laboratorian but often suffer from a lack of sensitivity, although specificity is often high (1–3, 6–15). Fluorescent-antibody stains 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, 6, 9, 10). Viral culture with shell vials provides excellent recovery (4, 5) but usually not in the time frame that can impact treatment with antiviral agents. 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.

The purpose of this study was to compare the performance of the Binax NOW Flu A and Flu B immunochromatographic assay to that of viral culture utilizing R-Mix shell vials. 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 2003-2004 influenza season when influenza A virus (H3N2/Fujian) was the predominant circulating strain. Consequently, the influenza B virus component could not be evaluated.

All specimens were nasal-wash or nasal-aspirate specimens submitted to the virology laboratory at Scott and White Memorial Hospital. The majority of the specimens were collected in the emergency department, in the pediatric outpatient clinic, or from inpatient settings and were most often transported immediately to the laboratory through a pneumatic tube system. Specimens were tested within 30 min of receipt 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 nasal-wash or nasal-aspirate specimens were placed into M-4 transport medium (Remel, Lenexa, KS) and were refrigerated at 2 to 8°C until processed for cell culture. Specimens in M-4 transport medium were passed through a 0.45-μm Acrodisc syringe filter (Pall Corp., Ann Arbor, MI) and were inoculated into two R-Mix shell vials (Diagnostic Hybrids, Inc., Columbus, OH) as previously described (6), except that the shell vials were centrifuged at 700 × *g* for 50 min. Any remaining specimen was stored at −80°C for future use. After an overnight incubation at 37°C, the cell monolayer was rinsed with phosphate-buffered saline, fixed in chilled acetone, rinsed again in phosphate-buffered saline, and finally stained with SimulFluor Flu A/Flu B direct fluorescent-antibody (DFA) stain (Light Diagnostics, Temecula, CA), following the manufacturer's instructions. Coverslips were removed to a glass microscope slide and examined with a fluorescence microscope with dual filters (fluorescein isothiocyanate and rhodamine). With this stain, influenza A virus-infected cells demonstrate a green fluorescence, whereas influenza B virus-infected cells stain with yellow-gold fluorescence. For cultures with initial negative results, the second shell vial was processed after an additional overnight incubation as described above but was stained with a SimulFluor respiratory screen kit (Light Diagnostics, Temecula, CA), following the manufacturer's instructions. This stain will cause respiratory syncytial virus-infected cells to fluoresce yellow-gold, while cells infected with influenza A or B virus, adenovirus, or parainfluenza virus will fluoresce green. When virus identification could not be established by the SimulFluor respiratory screen kit, the specimen was retrieved from −80°C storage, thawed, and reinoculated into two additional R-Mix shell vials. The shell vials were incubated for 48 h, and each shell vial was stained with either the SimulFluor Flu A/Flu B DFA stain or the SimulFluor Para 1,2,3/Adeno DFA stain (Light Diagnostics, Temecula, CA), according to the manufacturer's instructions. The parainfluenza virus component of this stain causes infected cells to fluoresce green, whereas the adenovirus component causes infected cells to fluoresce yellow-gold.

The Binax NOW Flu A component of the EIA had an overall sensitivity, specificity, positive predictive value, and negative predictive value of 64.9%, 98.4%, 89.3% and 93.2%, respectively (Table 1). However, when analyzed based on the subject age group, the assay sensitivity decreased significantly.

* Mailing address: Microbiology, Scott and White Memorial Hospital, 2401 South 31st Street, Temple, TX 76508. Phone: (254) 724-2714. Fax: (254) 724-8776. E-mail: rfader@swmail.sw.org.

TABLE 1. Performance of Binax NOW Flu A EIA versus R-Mix cell culture

Age group (yr)	No. of specimens[a]					Sensitivity (%)	Specificity (%)	PPV (%)[b]	NPV (%)[b]
	Total	TP	FP	TN	FN				
Overall	455	50	6	372	27	64.9	98.4	89.3	93.2
0–5	229	33	4	186	6	84.6	97.9	89.2	96.9
5–20	61	9	1	44	7	56.3	97.8	90.0	86.3
21–50	55	3	0	48	4	42.9	100	100	92.3
>50	110	5	1	94	10	33.3	98.9	83.3	90.4

[a] TP, true positive; FP, false positive; TN, true negative; FN, false negative.
[b] PPV, positive predictive value; NPV, negative predictive value.

The sensitivity for the 0- to 5-year-old group was 84.6% but fell to 33.3% for those patients over the age of 50 (Table 1). The specificity of the assay for all age groups remained above 97%. The positive predictive value ranged from 83.3% for the over-50-year-old age group to 100% for the 21- to 50-year-old age group. The negative predictive values ranged from 86.3% for the 6- to 20-year-old age group to 96.9% for the 0- to 5-year-old age group. Of the six false-positive EIA results, one culture was positive for respiratory syncytial virus, one culture grew a parainfluenza virus, and the remaining four were negative for virus isolation.

This is the largest reported study of the Binax NOW Flu A and Flu B immunochromatographic assay. During the 2003-2004 respiratory virus season, Scott and White Memorial Hospital served as a study site for the FluMist nasal vaccine (Aviron, Mountain View, CA). Consequently, a large number of specimens were processed for viral culture (2,159 total specimens with 860 total specimens positive for respiratory viruses). The Binax NOW Flu A and Flu B test was used primarily to assess patients prior to admission for grouping purposes or to determine whether antiviral therapy should be initiated. Viral culture was performed with R-Mix shell vials because previous influenza surveillance studies in our laboratory indicated that the R-Mix FreshCells recovered more respiratory viruses than 14-day rhesus monkey kidney cell cultures with less hands-on time (unpublished data). 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 clinical virology laboratory. However, even when testing was limited to nasal-wash specimens or nasopharyngeal aspirates, which are considered the most optimal 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 less than 5 years of age but provided unacceptable sensitivity when specimens were obtained from older children or adults. This outcome is 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. also noted increased assay sensitivity in young children (<2 years of age) and decreased sensitivity with specimens obtained from adult patients. 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 the 2003-2004 influenza season in Texas was predominantly one of influenza A virus. Of the 773 positive influenza virus cultures recovered during the 2003-2004 respiratory season, only one was 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 individual test strips for influenza A and B viruses. 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. However, the company has now begun to manufacture an assay in which both influenza A and B viruses can be identified on a single test strip.

In summary, the results of the study indicate that while the Binax NOW Flu A EIA is relatively sensitive in specimens collected from children under the age of 5, the assay provides unacceptable sensitivity in older children and adults and must be used with caution in these populations. Depending on the clinical situation, further testing by culture, fluorescent-antibody stain, or PCR should be considered in the event of an initial negative result.

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