JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2006, p. 1105–1107 Vol. 44, No. 3
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.3.1105–1107.2006

Respiratory Syncytial Virus Detection by Remel Xpect, Binax Now RSV, Direct Immunofluorescent Staining, and Tissue Culture

Anita P. Borek, Susan H. Clemens, Valerie K. Gaskins, Deborah Z. Aird, and Alexandra Valsamakis*

Johns Hopkins Medical Institutions, 600 North Wolfe Street, Meyer Building, Room B1-193, Baltimore, Maryland 21287-7093

Received 7 November 2005/Returned for modification 21 December 2005/Accepted 27 December 2005

The performance characteristics of Xpect RSV (XP) and Binax Now RSV (BN) were compared to those of direct fluorescent-antibody staining and/or tissue culture for detection of respiratory syncytial virus (RSV) in nasopharyngeal aspirate and wash samples from children ($n = 110$) and adults ($n = 66$). The sensitivity, specificity, positive predictive value, and negative predictive value of XP were 75%, 98%, 95%, and 90%, respectively; and those of BN were 74%, 100%, 100%, and 90%, respectively. The performances of the assays were similar within a given age group and specimen type (nasopharyngeal aspirate or wash specimen). XP and BN are useful for screening for RSV in respiratory specimens when large volumes are tested or low levels of staffing occur.

Respiratory syncytial virus (RSV) is a negative-strand RNA virus and is a member of the pneumovirus subfamily of the family *Paramyxoviridae*. It is known as a major cause of respiratory tract illness in children, and it is increasingly recognized as a significant respiratory pathogen in adults (3). Nosocomial outbreaks are well recognized (2, 4). Effective approaches to decreasing the rates of nosocomial transmission rely on rapid laboratory diagnosis (5, 6). Rapid direct RSV detection methods vary in their complexities. Lateral flow, membrane-based immunochromatographic assays have certain advantages compared to direct fluorescent-antibody staining (DFA), including an ultrarapid turnaround time and low levels of complexity. The goal of this study was to determine the performance characteristics of two immunochromatographic methods (Xpect RSV [XP] and Binax Now RSV [BN]) to those of a combined standard of DFA and/or culture (shell vial and conventional tube assays) with nasopharyngeal swab specimens collected in an urban tertiary-care setting.

Nasopharyngeal samples ($n = 176$ total samples; nasopharyngeal aspirates [NPAs], $n = 130$; nasopharyngeal washes [NPW], $n = 46$) were collected from children ($n = 110$; age range, 6 months to 18 years) and adults ($n = 66$) for routine medical care. Specimens were received in M4RT viral transport medium (approximate volume, 3 ml; Remel Inc., Lenexa, KS). Specimens were not further diluted prior to testing. Each specimen was tested by XP, BN, DFA (IMAGEN, Dakocytomation, Carpinteria, CA), and culture, according to the manufacturers' instructions. Uncentrifuged specimens were used for testing by XP and BN. NPAs have been approved for use with XP but not BN. Both tests have been approved for use with NPWs. After migration control failure in the XP device, samples were extracted and retested according to the instructions in the package insert. For extractions, 2 drops of proprietary extraction buffer were added to 250 μl of specimen. Following mixing of the contents, XP was repeated by applying 3 to 4 drops of extracted specimen to an XP device. For DFA, specimens were considered adequate if three or more cells were present per ×200 field. Cell culture was performed with R-Mix shell vials (Diagnostic Hybrids, Inc. [DHI], Athens, OH) and tubes containing rhesus monkey cells (DHI). Shell vials were incubated for 2 days and were then stained with fluorescein isothiocyanate (FITC)-conjugated monoclonal antibody (DHI), according to the instructions in the package insert. The tubes were examined daily for 10 days and then biweekly for an additional 11 days. RSV growth was confirmed by staining washed cell pellets with FITC-conjugated monoclonal antibody (DHI).

Data were compiled in Excel software (Microsoft Corp., Redmond, WA). The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the immunochromatographic methods were calculated in comparison to a combined standard of DFA and culture results. A sample with a true-positive result was defined as any sample that was positive by DFA and/or culture (DFA positive and culture positive, $n = 38$; DFA positive and culture negative, $n = 5$; DFA negative and culture positive, $n = 5$; DFA uninterpretable and culture positive, $n = 1$). A sample with a true-negative result was defined as any sample that was negative by DFA and culture ($n = 115$). XP data were calculated on the basis of the extraction results when the initial results were uninterpretable. Specimens that were uninterpretable by DFA and negative by culture were excluded from the final data set ($n = 12$) since a definitive result for RSV could not be obtained. Additionally, specimens that were uninterpretable by XP or BN (Table 1) were not used for calculation of the sensitivity, specificity, PPV, and NPV. No specimens were found to be positive by both immunochromatographic tests but negative or uninterpretable by the combined standard. This study was approved by The Johns Hopkins Medicine Institutional Review Board.

The results obtained with XP and BN compared to those obtained by DFA and/or culture are shown in Table 1. Based on these data, the sensitivity, specificity, PPV, and NPV of XP were 75%, 98%, 95%, and 90%, respectively; and those of BN

* Corresponding author. Mailing address: Johns Hopkins Medical Institutions, Meyer Building, Room B1-193, 600 N. Wolfe St., Baltimore, MD 21287-7093. Phone: (410) 955-5077. Fax: (410) 614-8087. E-mail: avalsam1@jhmi.edu.

TABLE 1. Comparative results between immunochromatographic methods and combined standard (DFA and/or culture) for RSV

Test (specimen) and result	No. of specimens with the following RSV combined standard result:		
	Positive	Negative	Uninterpretable[a]
XP (all specimens)			
Positive	36 (32/4)[b]	2 (1/1)	
Negative	12 (11/1)	110 (60/50)	10 (3/7)
Uninterpretable	1 (1/0)	3 (1/2)	2 (1/1)
BN (all specimens)			
Positive	36 (32/4)	0	
Negative	13 (12/1)	113 (62/51)	12 (4/8)
Uninterpretable		2 (0/2)	
XP (NPWs)			
Positive	3 (1/2)	0	
Negative	1 (0/1)	33 (4/29)	7 (1/6)
Uninterpretable		1 (0/1)	1 (0/1)
BN (NPWs)			
Positive	3 (1/2)	0	
Negative	1 (0/1)	33 (4/29)	8 (1/7)
Uninterpretable		1 (0/1)	
XP (NPAs)			
Positive	33 (31/2)	2 (1/1)	
Negative	11 (11/0)	77 (56/21)	3 (2/1)
Uninterpretable	1 (1/0)	2 (1/1)	1 (1/0)
BN (NPAs)			
Positive	33 (31/2)	0	
Negative	12 (12/0)	80 (58/22)	4 (3/1)
Uninterpretable		1 (0/1)	

[a] Uninterpretable DFA and negative tissue culture results.
[b] Numbers in parentheses indicate number of specimens for pediatric population/number of specimens for adult population.

TABLE 2. Clinical performance characteristics of rapid detection tests compared to those of the combined standard

Test and patient group or specimen	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
XP				
Total[a] (n = 160)	75	98	95	90
Children (n = 104)	74	98	97	85
Adults (n = 56)	80	98	80	98
NPW (n = 37)	75	100	100	97
NPA (n = 123)	75	97	94	88
BN				
Total[a] (n = 162)	74	100	100	90
Children (n = 106)	73	100	100	84
Adults (n = 56)	80	100	100	98
NPW (n = 37)	75	100	100	97
NPA (n = 125)	73	100	100	87

[a] All specimens with interpretable results.

were 74%, 100%, 100%, and 90%, respectively (Table 2). The prevalence of RSV in the total population was 27%. The performance characteristics within a single age group were almost entirely equivalent between the two immunochromatographic tests (Table 2). In a comparison between specimen types (i.e., NPWs versus NPAs for XP and NPWs versus NPAs for BN), the sensitivity, specificity, and PPV were equivalent for each individual immunochromatographic assay (Table 2). In addition, the sensitivity, specificity, and PPV were equivalent for each individual sample type when the results of XP and BN were compared (i.e., NPAs by XP and BN and NPWs by XP and BN) (Table 2). The NPVs were lower for both assays when NPAs were used compared to the NPVs obtained when NPWs were used (Tables 1 and 2).

Analysis of specimens with uninterpretable results demonstrated internal control failure rates of 28% (49 of 176 samples) and 1% (2 of 176 samples) (Table 1) for XP and BN, respectively. After extraction and repeat testing, 3% (six samples) remained uninterpretable by XP (Table 1). For DFA, 7% of specimens contained an insufficient number of cells and were therefore uninterpretable (n = 13 specimens in total; DFA uninterpretable and culture negative, n = 12; DFA uninterpretable and culture positive, n = 1).

This study demonstrated that the performance characteristics of XP and BN were comparable for the total study population and within stratified age groups. There are no previously published reports on the performance of XP. For BN, the high specificity (1, 7), positive predictive value (7), and negative predictive value (7, 8) with samples from children were consistent with previously published data. In this study the sensitivity of BN was lower than that previously reported with samples from children (1, 7, 8). In contrast, with samples from adults, we observed a greater number of positive results than was previously described for BN (4 positive results of 66 tested). In the only other study describing the performance of BN with samples from adults, BN failed to detect RSV in any adult specimens (n = 34) (7).

One potential explanation for the decreased sensitivities of BN and XP that we observed is diminished performance with NPAs, the predominant specimen in the study set. No other comparative data on immunochromatography test performance with NPAs are available. Previously published studies of BN used a combination of nasal or nasopharyngeal aspirates and washes; however, the data were not stratified by specimen type. Of note, we observed that the sensitivity of BN with NPWs (75%) was lower than that reported in the package insert (89%). Therefore, whether our data reflect diminished assay sensitivity with NPAs or variable performance in different clinical settings is unclear.

One advantage of BN compared to XP and DFA was the low rate of uninterpretable results after initial testing. For XP, internal control failure was observed in approximately one-third of specimens. This high rate of uninterpretable results resolved after extraction; however, the additional extraction steps decreased the ease of use and the time to retrieval of the results.

Immunochromatographic tests such as XP and BN are ideal during the respiratory virus season, when rapid, simple tests can help virology laboratories effectively handle large increases in testing volumes. Additionally, these low-complexity tests are extremely useful during off shifts, when staffing is low. However, our data suggest that these ultrarapid methods are best performed as screening tests, given their relative insensitivities compared to the sensitivities DFA and culture. Laboratories should continue to perform additional, sensitive diagnostic tests with specimens that test negative.

We thank the staff of The Johns Hopkins Clinical Virology Laboratory for technical support and Remel for providing reagents. We also thank William Merz and Michael Forman for comments and suggestions on this work.

REFERENCES

1. **Aldous, W. K., K. Gerber, E. W. Taggart, J. Thomas, D. Tidwell, and J. A. Daly.** 2004. A comparison of Binax NOW to viral culture and direct fluorescent assay testing for respiratory syncytial virus. Diagn. Microbiol. Infect. Dis. **49:**265–268.
2. **Berner, R., F. Schwoerer, R. F. Schumacher, M. Meder, and J. Forster.** 2001. Community and nosocomially acquired respiratory syncytial virus infection in a German paediatric hospital from 1988 to 1999. Eur. J. Pediatr. **160:**541–547.
3. **Falsey, A. R., P. A. Hennessey, M. A. Formica, C. Cox, and E. E. Walsh.** 2005. Respiratory syncytial virus infection in elderly and high-risk adults. N. Engl. J. Med. **352:**1749–1759.
4. **Forster, J., G. Ihorst, C. H. Rieger, V. Stephan, H. D. Frank, H. Gurth, R. Berner, A. Rohwedder, H. Werchau, M. Schumacher, T. Tsai, and G. Petersen.** 2004. Prospective population-based study of viral lower respiratory tract infections in children under 3 years of age (the PRI. DE study). Eur. J. Pediatr. **163:**709–716.
5. **Leclair, J. M., J. Freeman, B. F. Sullivan, C. M. Crowley, and D. A. Goldmann.** 1987. Prevention of nosocomial respiratory syncytial virus infections through compliance with glove and gown isolation precautions. N. Engl. J. Med. **317:**329–334.
6. **Madge, P., J. Y. Paton, J. H. McColl, and P. L. Mackie.** 1992. Prospective controlled study of four infection-control procedures to prevent nosocomial infection with respiratory syncytial virus. Lancet **340:**1079–1083.
7. **Ohm-Smith, M. J., P. S. Nassos, and B. L. Haller.** 2004. Evaluation of the Binax NOW, BD Directigen, and BD Directigen EZ assays for detection of respiratory syncytial virus. J. Clin. Microbiol. **42:**2996–2999.
8. **Zheng, X., S. Quianzon, Y. Mu, and B. Z. Katz.** 2004. Comparison of two new rapid antigen detection assays for respiratory syncytial virus with another assay and shell vial culture. J. Clin. Virol. **31:**130–133.