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Evaluation of the Binax NOW, BD Directigen, and BD Directigen EZ Assays for Detection of Respiratory Syncytial Virus

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The Binax NOW assay (Binax, Inc., Portland, Maine) and the BD Directigen EZ assay (Becton Dickinson and Company, Sparks, Md.), two new rapid immunoassays for detection of respiratory syncytial virus (RSV), as well as the BD Directigen RSV assay (DRSV) (Becton Dickinson and Company) and direct immunofluorescence staining (DFA) were compared with culture for detection of RSV in fresh specimens from both children and adults during the 2002-2003 respiratory virus season. The majority (95%) of specimens were nasal or nasopharyngeal washes or aspirates. A total of 47 (26%) were culture positive for RSV. The overall sensitivities of DFA ($n = 149$), NOW ($n = 118$), EZ ($n = 88$), and DRSV ($n = 180$) compared with culture ($n = 180$) were 93, 89, 59, and 77%, respectively. The specificities of DFA, NOW, EZ, and DRSV were 97, 100, 98, and 96%, respectively. However, when results were separated into those from children and those from adults, DFA was the only rapid test adequate for detection of RSV (sensitivity of 100% compared to 0, 0, and 25% for NOW, EZ, and DRSV, respectively) in adults. For children the sensitivities of DFA, NOW, EZ, and DRSV were 93, 94, 72, and 81%. The NOW assay was the most sensitive and specific and the easiest to perform of the kit tests for detecting RSV in children. None of these three rapid kit tests was sensitive for detecting RSV in specimens from adults. DFA remains the rapid method of choice for detecting RSV in the adult population.

Every year respiratory syncytial virus (RSV) is a major cause of severe respiratory tract infections in infants and young children. RSV is now also recognized as a pathogen in adults, with frail elderly and severely immunocompromised people at greatest risk for serious infections (4). Rapid detection of RSV infections in both children and adults is important to guide therapeutic decisions and to prevent nosocomial transmission. Although direct immunofluorescence staining (DFA) of nasal or nasopharyngeal washes or aspirates is frequently reported to be the most sensitive rapid method of RSV detection (5, 8, 9), this method requires highly trained technologists and high-quality reagents and equipment for optimal sensitivity (7). Many laboratories, unable to meet these requirements, have chosen less technically demanding kit immunoassays for rapid testing.

The BD Directigen RSV enzyme immunoassay (DRSV) (Becton Dickinson and Company, Sparks, Md.) has been a commonly used, rapid RSV assay. Reported sensitivities range from 61 to 86%, with specificities of 74 to 95% (1, 5, 8, 9). In a study in our laboratory during the 2001-2002 respiratory virus season, DRSV had a sensitivity of 71% and a specificity of 90% compared with DFA and culture (M. J. Ohm-Smith, P. S. Nassos, and B. L. Haller, Abstr. 103rd Gen. Meet. Amer. Soc. Microbiol., abstr. C357, 2003).

Because both false-negative and false-positive results can have adverse consequences, we were concerned with the performance of DRSV. Therefore, we chose to evaluate two new immunochromatographic assays for detection of RSV: BD Directigen EZ (Becton Dickinson and Company) and NOW (Binax, Inc., Portland, Maine). These FDA-approved tests require fewer technical skills to perform than DFA and offer rapid results. This study reports on the performance of these two new tests as well as DRSV and DFA compared with that of cell culture for detection of RSV during the 2002-2003 respiratory virus season.

MATERIALS AND METHODS

Specimen collection and processing. Nasal and/or nasopharyngeal wash, aspirate, or swab specimens were collected from adults and children evaluated at San Francisco General Hospital Medical Center and its associated clinics. The specimens were submitted to the laboratory at room temperature for routine rapid testing for RSV by DRSV. Protocols for use of patient specimens and evaluation of the RSV assays were approved by the Committee on Human Research of the University of California, San Francisco.

Nasal or nasopharyngeal aspirates and washes, collected in sterile saline, were treated in one of two ways depending upon the volume. When the volume was less than 1 ml, 1 ml of sterile 0.85% saline was added and vortexed 20 to 30 s with the specimen. When the volume was greater than 1 ml, the specimen was vortexed 20 to 30 s without any additional saline. In either case, 0.5 ml of the vortexed specimen was mixed by vortexing with 1.5 ml of transport-decontamination medium (Eagle's minimal essential medium with 2% fetal bovine serum, gentamicin, vancomycin, and amphotericin B) for culture. The remaining specimen was diluted as necessary (following the manufacturer's instructions) with sterile 0.85% saline for testing by DRSV.

For specimens collected by viral Culturette (Becton Dickinson and Company, Cockeysville, Md.), the swab was put into 1 ml of sterile 0.85% saline and vortexed for 20 to 30 s. Then 0.5 ml of this specimen was added to 1.5 ml of a transport-decontamination medium for culture. The remaining specimen was used for testing by DRSV.

Rapid testing. Upon receipt in the laboratory, specimens were prepared as described above and then routinely tested for RSV by DRSV following the manufacturer's instructions. Subsets of these specimens, chosen on the basis of availability of the new test kits and staffing, were tested with NOW and EZ, following the manufacturers' instructions. Since specimen preparation for EZ was similar to specimen preparation for DRSV and since NOW assay instructions did not specify a specimen volume or specimen dilution method, the specimens prepared for DRSV were used for testing with NOW and EZ. Because NOW had not been approved for use with swab specimens, patient specimens collected with swabs were not tested with the NOW assay.

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Specimens were stored at 2 to 8°C (according to manufacturers' instructions) until all testing was completed. All specimens evaluated by NOW were tested concurrently with DRSV or within 24 h, the manufacturer's recommended maximum storage time. Of specimens evaluated by EZ, 80% were tested concurrently with DRSV or within 24 h. The remainder were tested more than 24 h after collection but within 72 h, the manufacturer's recommended maximum specimen storage time.

Briefly, for the NOW and EZ assays, specimen is added to the top of a test strip. As the specimen migrates down the strip, RSV antigen, when present, binds to an anti-RSV-conjugated antibody. The resulting complex is then captured by a line of RSV antibody and appears as a pink or reddish purple line near the bottom of the test strip when RSV antigen is present. A pink or reddish-purple control line, also near the bottom of the strip, must also be present for any result to be valid.

After all of the rapid kit testing was completed, a smear for RSV DFA was prepared from each specimen by spotting a drop of washed cell pellet to a well on a slide (6). A total of 78% of smears were prepared within 24 h of specimen collection; the remainder were prepared from 2 to 4 days after collection. After the specimen had dried on the slide, it was fixed in cold acetone, stained for 30 min with Merifluor RSV identification reagent (Meridian Bioscience Inc., Cincinnati, Ohio), rinsed, air dried, and examined for fluorescent cells at ×250 magnification on a Zeiss fluorescence microscope. A smear was considered positive when there were at least two cells with typical RSV fluorescence. Smears with fewer than 20 to 30 total cells (and no fluorescent cells) were reported as inadequate.

Culture. For respiratory viral culture, one tube of RMK cells (ViroMed Laboratories, Minnetonka, Minn.) and one tube each of MRC-5 and HEp-2 cells (both from Diagnostic Hybrids, Inc., Athens, Ohio) were inoculated with approximately 0.3 ml of specimen in transport-decontamination medium within 1 h of receipt of the specimen in the laboratory. Cells were incubated in a stationary rack at 35 to 37°C and observed daily for 12 to 14 days or until cytopathic effect was evident. When no cytopathic effect was evident, hemadsorption was performed with guinea pig red blood cells (ViroMed Laboratories) at 12 to 14 days on the RMK cells. The identity of viruses from cell lines that showed cytopathic effect and/or were hemadsorption positive was confirmed using direct (for RSV [Meridian Bioscience, Inc.] and cytomegalovirus [Bartels/Trinity Biotech, Carlsbad, Calif.]) or indirect (for influenza virus, parainfluenza virus, and adenovirus [Bartels/Trinity Biotech]) fluorescent antibody staining.

RESULTS

A total of 180 specimens were submitted for rapid RSV testing by DRSV between 16 December 2002, and 30 April 2003. Of the specimens, 127 (71%) were from children (ages 6 days to 32 months) and 53 (29%) were from adults (ages 16 to 91 years). All specimens were nasal or nasopharyngeal washes or aspirates except for nine nasal specimens collected by swab from adults.

All 180 specimens were inoculated into cell cultures and tested by DRSV and DFA. A total of 47 (26%) specimens were culture positive for RSV (34% of specimens collected from children; 8% of specimens collected from adults).

Results for detection of RSV by DFA and the rapid kit tests compared with that by viral culture are shown in Table 1.

Of the 149 specimens with adequate cellular content, agreement between DFA and culture was 96% (143 of 149). For three specimens DFA was positive for RSV when culture was negative. The average number of positive cells seen in these specimens was relatively small (≤2 positive cells/field). In three specimens DFA was negative when culture was positive.

Of the 31 specimens for which the cell content was inadequate on DFA, three were culture positive for RSV. Two of these three specimens tested were positive by DRSV, two of two tested by NOW were positive, and zero of one tested by EZ was positive. The other 28 specimens were RSV negative by culture and DRSV and were RSV negative by NOW (14 specimens) and EZ (10 specimens) when those assays were performed.

TABLE 1. Results of rapid tests compared with culture

Test and result	No. of specimens with indicated RSV culture detection result	
	Positive	Negative
DFA (n = 149)		
Positive	41	3
Negative	3	102
DRSV (n = 180)		
Positive	36	5
Negative	11	128
NOW (n = 118)		
Positive	31	0
Negative	4	83
EZ (n = 88)		
Positive	13	1
Negative	9	65

Agreement between DRSV and culture was 91% (164 of 180). For the five specimens for which DRSV was positive and culture was negative for RSV, the DFAs were also negative for RSV for all five; in two cases, viruses other than RSV (herpes simplex virus type 1 and parainfluenza virus type 3) were isolated from culture. Therefore, these five DRSV results were considered to be false positives.

NOW was performed on nasal or nasopharyngeal wash or aspirate specimens from 84 children and 34 adults. Agreement between NOW and culture was 97% (114 of 118). Only two specimens did not initially migrate down the NOW test strip. Both specimens were positive for RSV by DFA and culture; one was positive and one was negative by NOW after the specimen was diluted 1:2 with saline and retested.

EZ was performed on specimens from 66 children and 22 adults. All specimens were nasal or nasopharyngeal washes or aspirates except for three specimens collected by swab from adults. Agreement between EZ and culture was 89% (78 of 88). Both DFA and DRSV were negative for RSV for the one specimen that was EZ positive and culture negative.

Table 2 shows the sensitivity, specificity, and predictive values for DFA, DRSV, NOW, and EZ when culture was used as the reference method. NOW was the most sensitive and specific of the three kit assays. The levels of sensitivity of NOW and DFA on specimens from children were comparable. EZ was the least-sensitive assay; however, fewer specimens were evaluated with this assay. The sensitivity of DRSV was comparable to that previously seen in our laboratory for this assay.

The sensitivity of all of the kit tests for detecting RSV in adults was poor (Table 2). Culture and DFA were positive for RSV in specimens from 4 of the 53 adults. Only one of these four was detected by DRSV, none of the four was detected by EZ, and neither of two specimens tested was detected by NOW.

Viruses other than RSV were isolated from 24 specimens. These included seven influenza A virus isolates, two influenza B virus isolates, five parainfluenza virus type 3 isolates, two enterovirus or rhinovirus isolates, two adenovirus isolates, five

TABLE 2. Evaluation of rapid results compared with culture

Test and population[a]	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
DFA	93	97	93	97
Adults (n = 41)	100	97	80	100
Children (n = 108)	93	97	95	96
DRSV	77	96	88	92
Adults (n = 53)	25	98	50	94
Children (n = 127)	81	95	90	91
NOW	89	100	100	95
Adults (n = 34)	0	100		94
Children (n = 84)	94	100	100	96
EZ	59	98	93	88
Adults (n = 22)	0	100		82
Children (n = 66)	72	98	93	90

[a] First row of data for each test represents combined results for adults and children.

cytomegalovirus isolates, and one herpes simplex virus type 1 isolate. From two specimens, one with cytomegalovirus and one with influenza B virus, RSV was also isolated. DRSV and NOW were positive for these two specimens, and DFA was positive for one specimen and inadequate for one. Neither specimen was tested with EZ. DRSV was positive for 2 specimens of the 22 in which only viruses other than RSV were isolated. NOW and EZ were negative for all specimens tested in which only viruses other than RSV were isolated (16 and 13 specimens tested, respectively), including the two in which DRSV was positive. DFA was negative for 21 of the specimens and inadequate for 1 specimen.

DISCUSSION

In our laboratory, the results of DFA showed excellent correlation with culture, and DFA was the most sensitive and specific rapid method for detecting RSV in a population of both adults and children. However, because our laboratory is not staffed to offer DFA outside of regular weekday work hours, a less technically complex assay is necessary to provide rapid results at other times. DRSV has been the assay used in our laboratory, and although the sensitivity and specificity of this assay in this study were slightly higher than those determined in the previous season (Ohm-Smith et al., 103rd Gen. Meet. Amer. Soc. Microbiol.), there continued to be a number of false-negative and false-positive results.

Of the rapid kit immunoassays that we evaluated, the NOW assay performed the best, with an overall sensitivity and specificity of 89 and 100%, respectively. This sensitivity was similar to the levels of sensitivity (85 and 86%) reported by others for specimens from children and adults (M. W. Arroyo, S. Ikemoto, N. Sapigao, and H. Margesson, Abstr. 19th Annu. Clin. Virol. Symp., abstr. S13, 2003; A. Borek, D. Aird, and A. Valsamakis, Abstr. 19th Annu. Clin. Virol. Symp., abstr. S11, 2003). The NOW sensitivity (94%) for specimens from children fell between the 86% reported by S. L. Barton and R. L. Hodinka (Abstr. 19th Annu. Clin. Virol. Symp., abstr. S7, 2003) and the 100% reported by K. R. Fiebelkorn and K. M. Lawless (Abstr. 19th Annu. Clin. Virol. Symp., abstr. S22, 2003) for pediatric specimens. Although we found no false-positive results with NOW (specificity, 100%), others have reported specificities of 96 and 90% when the assay was compared with culture (Barton and Hodinka, 19th Annu. Clin. Virol. Symp., abstr. S7; Borek et al., 19th Annu. Clin. Virol. Symp., abstr. S11).

In this study EZ was the least-sensitive assay, but this outcome may have been a reflection of the smaller total number of specimens evaluated by EZ than by the other methods. However, the EZ sensitivity of 72% for pediatric specimens was in the range (71 to 84%) of sensitivities reported by others in studies with large numbers of pediatric specimens (Y. B. Yoshi and R. L. Hodinka, Abstr. 19th Annu. Clin. Virol. Symp., abstr. S12, 2003; C. Robinson, N. Jones, J. Hanson, A. Ballweber, K. Carlson, D. Fortnstrom, and N. Maldeis, Abstr. 19th Annu. Clin. Virol. Symp., abstr. S14, 2003).

While the DRSV package insert does not specify the age of the population for which the test is intended, DRSV has previously been found to be insensitive for detecting RSV in adults from nasal brush specimens (3) or combined nasal wash and throat swab specimens (2). NOW and EZ package inserts both indicate that the tests are for use in neonatal and pediatric populations (for subjects less than 5 years of age for NOW and less than 20 years of age for EZ). Although the number of adults tested and the number of adult specimens positive for RSV were not large in our study, the results support limiting the use of the new tests to pediatric populations. With sensitivities of 0 to 25%, these tests performed poorly for RSV diagnosis in adults.

Both NOW and EZ were easy to perform and read. NOW required no specimen preparation prior to application to the strip, and only 2 of 143 specimens did not migrate down the strip at first application. Both of these specimens migrated when retested after dilution. For EZ, the specimens were extracted and filtered (in similarity to those in the DRSV) before being applied to the strip, and there were no problems with specimen migration. The control and test lines on the NOW device were consistently darker than those on EZ. In general, the specimens that were falsely negative by EZ were those that gave weakly positive results for NOW. No false-positive results were found with NOW; and with this assay, all test lines, even when very faint, correlated with positive culture results.

At the time of our study, the NOW assay was limited by several conditions which may restrict its use or require laboratories to do validations. These conditions include lack of approval for specimens other than nasal washes and for specimens in viral transport media. In addition, the assay results must be read at exactly 15 min, which decreases flexibility in performing the test. The EZ assay is approved for specimens and conditions similar to those for DRSV: nasopharyngeal washes, aspirate, and swabs in a variety of transport media. Also, EZ results may be read at any time from 15 to 60 min after specimen addition.

In summary, of the rapid kit tests that we evaluated, the Binax NOW assay was the most sensitive, specific, and the easiest-to-perform test for detecting RSV in children. The EZ assay was also easy to perform, but it was the least sensitive of the assays evaluated. The rapid kit assays were not sensitive for detecting RSV in specimens from adults. Laboratories should consider performing direct immunofluorescence and/or cul-

ture testing of specimens from adults that give negative results with any of these rapid kit tests.

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