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Detection of Respiratory Syncytial Virus Antigen in Nasopharyngeal Secretions by Abbott Diagnostics Enzyme Immunoassay

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We compared a rapid respiratory syncytial virus (RSV) antigen enzyme immunoassay (EIA) (Abbott Diagnostics, North Chicago, Ill.) with virus culture and with the indirect fluorescent-antibody test (FAT) by using nasopharyngeal washings from children with suspected RSV pneumonia or bronchiolitis. Fresh washings were used in all three tests. Specimens were inoculated into HEp-2 cells and human embryonic lung fibroblasts and observed for cytopathic effect. Cells in the centrifuged sediments of the nasal washes were examined for typical cytoplasmic fluorescence of RSV by FAT. The EIA cutoff was an optical density (OD) at 492 nm that was greater than the mean OD of the negative controls plus 0.1. An OD within +20% of the cutoff was considered borderline, and these specimens were retested. Of 289 specimens, 118 (41%) were positive by culture, 150 (52%) were positive by FAT, and 154 (53%) were positive by EIA. Eight borderline EIAs were all negative when the specimens were retested after storage at −70°C. Of 17 specimens positive by EIA but negative by culture and FAT, 9 were blocked in a competitive EIA, indicating that they were true-positives and that the culture and FAT were falsely negative. The sensitivity, specificity, and predictive value (positive) of the EIA versus culture, FAT, or blocking assay were 90, 94, and 95%, respectively. We conclude that the Abbott RSV antigen EIA is highly sensitive and specific.

Respiratory syncytial virus (RSV) is the major cause of acute lower respiratory disease in infants worldwide (4, 13). In the United States, approximately 1 in 100 to 1 in 500 infants are hospitalized each year with pneumonia or bronchiolitis due to RSV infection (1, 4). The accurate, rapid diagnosis of RSV infection is important to clinicians who must decide whether to begin antiviral therapy with ribavirin aerosol and when to institute infection control measures. The purpose of this study was to assess the diagnostic accuracy of a commercial RSV antigen enzyme immunoassay (EIA) (Abbott Diagnostics, North Chicago, Ill.) compared with tissue culture and with an indirect fluorescent-antibody test (FAT) by using nasopharyngeal washings.

(These results were presented in part at the 25th Interscience Conference on Antimicrobial Agents and Chemotherapy, Minneapolis, Minn., September 1985.)

MATERIALS AND METHODS

Specimens. Fresh nasopharyngeal washings were obtained from children up to 3 years of age hospitalized with suspected RSV infection in the greater Denver, Colo., area during March and April 1985. Hospital staff collected the specimens by irrigating the posterior nasopharynx with 1 to 2 ml of sterile saline and then quickly aspirating the fluid with suction and a mucus trap. The specimens were held at 4°C and transported to the laboratory on wet ice within 24 h of collection. All specimens were brought to a volume of 2 ml with sterile phosphate-buffered saline before being tested.

Tissue culture. A 0.4-ml portion of the nasopharyngeal washing was mixed with 0.5 ml of veal infusion broth transport medium containing gelatin, penicillin, gentamicin, and amphotericin B. Specimens were inoculated into veal infusion broth within 4 h of arrival in the laboratory and cultured 30 min to 2 h later. Two tubes of HEp-2 cells and one tube of human embryonic lung fibroblasts were each inoculated with 0.3 ml of veal infusion broth and incubated at 37°C. Tubes were examined for cytopathic effect for 21 days. At 19 to 21 days, all cultures still negative for cytopathic effect were blindly passed to a single HEp-2 tube, which was examined for an additional 14 days. All cultures were tested by the FAT to confirm RSV cytopathic effect and to verify negative cultures.

FAT. A 1.0-ml portion of the original specimen was centrifuged at ~400 × *g* for 10 min. The pellet was suspended in 0.5 ml of phosphate-buffered saline, spotted on clean glass slides, and air dried. After being fixed in cold acetone, the slides were stored at −70°C and stained and read in batches at the end of the RSV season. The indirect FAT was done with rabbit antiserum (made in our laboratory) and fluorescein-conjugated goat anti-rabbit antiserum (Meloy Laboratories, Inc., Springfield, Va.) (10). All slides were read blindly at a ×400 magnification by a single experienced technologist using a Zeiss incident-light microscope with a mercury light source. Negative slides containing fewer than five respiratory epithelial cells were considered inadequate, and these specimens were deleted from the analysis.

EIA. The Abbott RSV EIA uses goat anti-RSV antibody-coated polystyrene beads as the solid phase. Rabbit anti-RSV antibody-conjugated horseradish peroxidase and *o*-phenylenediamine substrate constitute the development system. The tests were read at 492 nm with a Quantum II spectrophotometer (Abbott Diagnostics). A specimen was considered positive if the optical density at 492 nm (OD) was greater than 0.1 plus the average of three negative controls. An OD within +20% of the cutoff was considered borderline, and these specimens were retested. Specimens positive by EIA but negative by culture and FAT were tested in a blocking assay with rabbit anti-RSV antiserum. Specimens showing a decrease in OD of >50% compared with an unblocked control were considered true-positives by EIA.

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RESULTS

Specimens were obtained from 296 children. Five specimens were excluded from analysis because the FATs were considered inadequate, and two were excluded because the cell cultures were contaminated. Of the remaining 289 specimens, 118 (41%) were positive by culture, 150 (52%) were positive by FAT, and 154 (53%) were positive by EIA (Table 1). One specimen was culture positive only after a blind passage. Eight specimens were borderline by EIA; of these, all eight were negative by culture, seven were negative by FAT, and all eight were negative on repeat EIA after being frozen for 4 to 8 weeks at −70°C. Fifteen specimens grew other viruses, including adenovirus (twelve specimens) and rhinovirus, enterovirus, and herpes simplex virus (one specimen each). Two specimens which grew both RSV and adenovirus were RSV positive by EIA.

Of 17 specimens positive by EIA but negative by both culture and FAT, 13 were positive by repeated EIA; 9 of these 13 (70%) were neutralized by >50% in the blocking assay, indicating that they were true-positive specimens (Table 2).

We evaluated the EIA versus culture alone, FAT alone, either culture or FAT, and either culture, FAT, or blocking assay (Table 3). The sensitivity and specificity of the EIA versus culture, FAT, or blocking assay were 90 and 94%, respectively.

DISCUSSION

The laboratory diagnosis of RSV is most commonly done by tissue culture or immunofluorescence microscopy. Tissue culture is slow, requiring 4 to 7 days before specimens become positive, and the virus may die if transport to the laboratory is delayed or if the specimen is frozen. The FAT, although rapid, requires special equipment and considerable expertise on the part of the microscopist (5, 8).

Early studies of RSV EIAs showed them to be generally insensitive (7, 12), but recently introduced commercial RSV EIA kits appear to be more sensitive (2, 3, 11, 14). Kumar et al. (9) and Freymuth et al. (3) reported high sensitivity (90 and 84%, respectively) with the Abbott EIA compared with culture alone, and Swenson and Kaplan (14) reported an EIA sensitivity of 88% versus a combination of culture and FAT. We found that the Abbott RSV EIA detected more positive specimens than either culture or FAT did (EIA sensitivity, 90%) and that a large percentage of seemingly false-positive EIA specimens were true-positives as determined by the blocking assay. We believe that including the results of the blocking assay with culture and FAT in the analysis best represents the true diagnostic accuracy of the EIA.

Only 2 of 118 specimens positive by culture were falsely negative by EIA. The matched cultures became positive very late, after 16 and 27 days of incubation, indicating that little virus was present. In contrast, there were 30 falsely negative cultures as determined by FAT and EIA. Because RSV EIA is more sensitive than tissue culture in our laboratory, we do not feel that culture is a necessary backup for negative EIA specimens, as recommended by some investigators (N. Bartholoma, J. McMillan, L. Weiner, and B. A. Forbes, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C-297, p. 377), except to detect respiratory viruses other than RSV. In our study, blind passage and screening of negative cultures by FAT did not significantly increase the yield of tissue-culture-positive specimens.

Only fresh nasopharyngeal washings were used in this study. Similar studies by others have used frozen specimens, and consequently the sensitivities reported may have been falsely low (6, 9, 14). We have had extensive experience with three commercial RSV EIAs using both fresh and frozen specimens and have found that with two of the kits, ODs are consistently lower after storage at −70°C, sometimes changing from positive to negative. With the third kit, frozen specimens gave inconsistent results on retesting out of the

TABLE 1. Results of Abbott RSV EIA versus virus culture and FAT[a]

RSV EIA result (*n*)	No. (%) of specimens							
	Culture		FAT		Culture or FAT		Culture or FAT or blocking assay	
	Pos	Neg	Pos	Neg	Pos	Neg	Pos	Neg
Pos (154)	116	38	135	19	137	17	146	8
Borderline (8)	0	8	1	7	1	7	—[b]	—
Neg (127)	2	125	14	113	16	111	17	118
Total	41	59	52	48	53	47	56	44

[a] Pos, Positive; Neg, negative.
[b] —, Eight of eight borderline EIAs were negative in repeat testing.

TABLE 2. False-positive Abbott RSV EIA[a]

Specimen no.	Initial EIA OD (pos)	Repeat EIA OD	EIA blocking assay	Final interpretation of pos EIA
1	0.659	0.334 (Pos)	Pos	True
2	0.474	0.366 (Pos)	Pos	True
3	0.304	0.191 (Pos)	Pos	True
4	0.342	0.184 (Pos)	Neg	False
5	0.234	0.094 (Neg)	NI	False
6	0.166	0.081 (Neg)	NI	False
7	0.322	0.185 (Pos)	Pos	True
8	0.336	0.099 (Neg)	NI	False
9	0.237	0.141 (Pos)	Neg	False
10	0.188	0.268 (Pos)	Pos	True
11	0.198	0.177 (Pos)	Neg	False
12	0.187	0.159 (Pos)	Pos	True
13	0.252	0.203 (Pos)	Pos	True
14	0.193	0.189 (Pos)	Pos	True
15	0.776	0.639 (Pos)	Pos	True
16	0.399	0.438 (Pos)	Neg	False
17	0.164	0.089 (Neg)	NI	False

[a] All specimens were initially positive by EIA but negative by culture and FAT. Abbreviations: Pos, positive; Neg, negative; NI, not interpretable because the repeat EIA was negative (considered false-positive).

TABLE 3. Diagnostic accuracy of the Abbott RSV EIA compared with virus culture, FAT, and blocking assay[a]

Measure of accuracy	% Accuracy of RSV EIA vs:			
	Culture	FAT	Culture or FAT	Culture or FAT or blocking assay
Sensitivity	98	90	89	90
Specificity	78	86	87	94
Predictive value (+)	75	88	89	95
Predictive value (−)	99	89	87	88

[a] For this analysis, borderline RSV EIAs were considered negative since eight of eight were negative on repeat EIA testing.

freezer; the ODs were sometimes higher and sometimes lower than when the specimens were tested fresh. We have also found that most specimens with an OD reading near the cutoff (borderline) will retest negative after storage at −70°C, and therefore we feel that a second specimen should be obtained when the result is borderline, rather than having the same specimen retested.

We found the Abbott RSV antigen EIA to be highly sensitive and specific. It is a relatively rapid assay, requiring only 5.5 h to complete, and can be read on a spectrophotometer. We prefer the RSV EIA to culture or FAT because in our hands it was more rapid and sensitive than culture and more objective than FAT.

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