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Detection of Respiratory Syncytial Virus Antigen in Nasal Washings by Abbott TestPack Enzyme Immunoassay

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We compared the new Abbott TestPack (TP) respiratory syncytial virus (RSV) enzyme immunoassay (EIA) with cell culture and two commercial RSV EIAs (from Abbott Diagnostics and Kallestad Laboratories) by using split samples of fresh nasal washings from children with suspected RSV disease. Two tubes of HEp-2 cells were inoculated and observed for cytopathic effect for 14 days, and isolates were confirmed by immunofluorescence. The TP EIA was performed by following the manufacturer's instructions. Specimens positive by TP EIA but negative by culture were examined in a competitive inhibition (blocking) assay using the TP EIA and rabbit anti-RSV serum. Of 218 specimens, 93 were positive by culture, 105 were positive by TP EIA, 80 were positive by the Abbott Diagnostics EIA, and 87 were positive by the Kallestad Laboratories EIA. The sensitivity, specificity, positive predictive value, and negative predictive value of the TP EIA were 92, 86, 81, and 93%, respectively. Of 20 apparently false-positive TP EIAs, 10 of 14 that were positive when retested were neutralized in the blocking assay, indicating that they were truly positive. The recalculated sensitivity, specificity, positive predictive value, and negative predictive value of the TP EIA were 92, 91, 90, and 93%, respectively. We conclude that the TP EIA is easy to perform, rapid (<0.5 h), and accurate.

Respiratory syncytial virus (RSV) is the most important cause of acute lower respiratory infection in infants (3, 12). Rapid diagnosis of RSV infection aids clinicians in decisions regarding antiviral therapy with ribavirin and infection control measures (11).

The purpose of this study was to compare a new, second-generation rapid RSV antigen test, the Abbott TestPack (TP) enzyme immunoassay (EIA), with conventional tissue culture and with two other commercially available RSV EIAs (from Abbott Diagnostics [A-EIA] and Kallestad Laboratories [K-EIA]).

MATERIALS AND METHODS

Specimens. Nasopharyngeal washings were obtained from infants and young children hospitalized with pneumonia or bronchiolitis during February and March 1988. Nasopharyngeal washings were collected by irrigation with a small volume (1.0 to 2.0 ml) of sterile saline and aspiration by using a mucus trap (2, 7). Specimens were held at 4°C and transported on wet ice. In the laboratory, specimens were brought to a volume of 3.0 ml with sterile phosphate-buffered saline, pH 7.2, and then split for testing by the four assays.

Tissue culture. A 0.5-ml sample of nasopharyngeal wash fluid was added to 2.0 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. Two tubes of HEp-2 cells were each inoculated with 0.5 ml of veal infusion broth, incubated at 37°C, and examined for cytopathic effect for 14 days. Positive cultures were confirmed by immunofluorescence (Bartels Immunodiagnostic Supplies, Inc., Sacramento, Calif.).

RSV EIAs. The A-EIA and K-EIA were performed by following the manufacturers' instructions.

TP EIA. The solid phase was a reaction disk composed of a focuser and a filter matrix. The capture antibody was bovine anti-RSV serum. The detector antibody was a combination of biotinylated bovine anti-RSV serum and alkaline phosphatase conjugated to rabbit anti-biotin antibody. The substrate was Nitro Blue Tetrazolium chloride and bromcresol indolephosphate.

After an extraction procedure, the specimen was incubated with the capture antibody and biotin reagents in a filtration cup. The solution was clarified by a filtration step and poured through the focuser onto the reaction disk. The focuser was then removed, and the anti-biotin and chromagen steps were performed on the reaction disk. The results were read visually; positive specimens produced a gray-to-purple plus sign, and negative specimens produced a gray-to-purple minus sign. The horizontal bar of the focused area on the reaction disk is coated with inactivated RSV to act as an internal reagent control and is the minus sign when the specimen is negative and the horizontal bar of the plus sign when the specimen is positive.

EIA competitive inhibition (CI) (blocking) test. To determine whether specimens positive by TP EIA but negative by culture or by A-EIA or K-EIA represented false-positive TP EIAs, all discrepant specimens were retested by the standard TP RSV assay. If the result was a negative signal, the initial result was retained as a false-positive. If the result was a repeatable positive signal, a CI (blocking) assay was performed.

A volume of rabbit anti-RSV serum equal to 1/10 of the volume of the specimen was added to the specimen after the extraction step. This mixture was incubated at room temperature for 15 min. The assay was then continued in the usual TP manner.

If the signal of the blocked sample was reduced by 50% or more compared with the signal of the unblocked repeat assay described above, the presence of RSV antigen in the specimen was confirmed, and the initial result was considered a true-positive. If the signal of the blocked sample was not reduced 50% or greater, the initial result was retained as a false-positive. (Blocking was done courtesy of Abbott Diagnostics.)

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TABLE 1. RSV TP EIA compared with tissue culture, K-EIA, A-EIA, and CI (blocking) assay

TP EIA result (n = 218)	No. of results					
	Culture		Culture or K-EIA and A-EIA		Culture or K-EIA and A-EIA or blocked TP EIA	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive (105)	85	20	89	16	95	10
Negative (113)	8	105	8	105	8	105
Total	93	125	97	121	103	115

RESULTS

Of 218 specimens, 85 were positive by tissue culture, 85 were positive by K-EIA, 80 were positive by A-EIA, and 105 were positive TP EIA. The sensitivities and specificities compared with tissue culture were 76 and 90% for the K-EIA, 81 and 96% for the A-EIA, and 91 and 84% for the TP EIA. The correlation between TP EIA and the comparative tests is shown in Table 1. Of 105 specimens positive by TP EIA, 95 (92%) either were positive by tissue culture and both K-EIA and A-EIA or were blocked in the CI assay (Tables 2 and 3).

DISCUSSION

Laboratory diagnosis of RSV infection is commonly done by tissue culture, immunofluorescence or, more recently, by commercially produced EIAs. Tissue culture is slow, requiring 3 to 7 days before specimens become positive. Immunofluorescence requires special equipment and considerable expertise on the part of the microscopist (4, 6). Early RSV EIAs were generally insensitive (5, 10), but recently introduced commercial RSV EIA kits appear to be more sensitive (1, 8, 9, 13).

In this study, the TP EIA detected more positive specimens than did tissue culture, A-EIA, or K-EIA (in a much reduced time frame). We found by doing multiple RSV EIAs and a CI assay that half of the apparently false-positive TP EIA results were true-positives; the tissue culture was false-negative. We believe that including the results of the CI assay with culture, K-EIA, and A-EIA results represents most closely the diagnostic accuracy of TP EIA (Table 1).

Of 218 specimens tested, 7 were positive by tissue culture but negative by TP EIA. Five of the seven were also negative by both K-EIA and A-EIA, suggesting that there was too little antigen present to be detected by EIA. One of the seven specimens was positive by both K-EIA and A-EIA in addition to culture, and one was positive by only A-EIA and culture. Because TP EIA is more sensitive than tissue culture in our laboratory, we do not feel that tissue culture is a necessary backup for TP EIA-negative specimens.

We conclude that TP EIA is both sensitive and specific when fresh nasopharyngeal specimens are used. The TP EIA is also very rapid, requiring only 0.5 h to perform.

TABLE 2. Diagnostic accuracy of TP RSV EIA

Parameter	% Accuracy of TP EIA vs:		
	Culture	Culture or K-EIA and A-EIA	Culture or K-EIA and A-EIA or blocked TP EIA[a]
Sensitivity	91	92	92
Specificity	84	87	91
Positive predictive value	81	85	90
Negative predictive value	93	93	93

[a] This column represents most closely the diagnostic accuracy of TP EIA.

TABLE 3. Results of CI (blocking) assays of apparently false-positive TP RSV EIAs (TP EIA positive, culture negative)

Sample no.	Results of:		
	K-EIA	A-EIA	CI (blocking) assay[a]
1	+	+	Blocked
2	+	+	Blocked
3	+	+	Blocked
4	+[b]	+	Blocked
5	+	−	Blocked
6	−	−	Blocked
7	−	−	Blocked
8	−	−	Blocked
9	−	−	Blocked
10	−	−	Blocked
11	−	−	Not blocked
12	−	−	Not blocked
13	−	−	Not blocked
14	−	−	Not blocked
15	+	−	Repeated negative
16	+	−	Repeated negative
17	−	−	Repeated negative
18	−	−	Repeated negative
19	−	−	Repeated negative
20	−	−	Repeated negative

[a] A TP EIA blocked specimen was defined as a negative or nearly negative signal. Of the 14 specimens positive on repeat testing, 10 (71%) were blocked.
[b] Borderline.

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