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Comparison of Three Enzyme-Linked Immunosorbent Assays and a Direct Fluorescent-Antibody Test for Detection of Respiratory Syncytial Virus Antigen

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We prospectively evaluated three enzyme immunoassays (EIAs) and a direct fluorescent-antibody (DFA) test for respiratory syncytial virus detection. Of 90 specimens, 79% gave the same results in all four tests (30 positive and 41 negative) and 97% were in agreement in three of the four assays. The agreement between the direct fluorescent-antibody test and each enzyme immunoassay was ≥86%.

Respiratory syncytial virus (RSV) is the major cause of acute lower respiratory tract disease in infants and young children (4) and of nosocomial respiratory infections (10). Rapid and accurate diagnosis of infection with this agent has become increasingly important for the prompt consideration of specific antiviral therapy (aerosolized ribavirin) and in controlling the nosocomial spread of the virus. Enzyme immunoassays (EIAs) (12) as well as direct and indirect immunofluorescent-antibody techniques (13) have been used to test for the presence of RSV antigen in nasal secretions. This study prospectively compares three commercially available EIAs and a direct fluorescent-antibody (DFA) test for RSV detection.

Nasopharyngeal wash specimens from children with suspected RSV disease during the winter of 1986 to 1987 were obtained by the bulb suction technique of Hall and Douglas (9) and transported on ice to the microbiology laboratory. Within 24 h of receipt of a specimen, 0.75-ml aliquots were snap-frozen to −70°C for the EIAs. The cells were pelleted from the remainder of the specimen by washing the specimen several times at 350 × *g* for 10 min in a tabletop centrifuge (Beckman Instruments, Inc., Fullerton, Calif.). After being air dried, the slides were fixed for 10 min at 4°C in acetone. Nasopharyngeal secretions were assayed by three EIA systems (Ortho EIA from Ortho Diagnostics, Inc., Raritan, N.J.; Abbott EIA from Abbott Laboratories, North Chicago, Ill.; and Kallestad EIA from Kallestad Diagnostics, Austin, Tex.). In each system the absorbance value for the specimen was spectrophotometrically determined and compared with high and low cutoff values established from the negative controls included in each evaluation. A specimen was considered positive if the absorbance value was above the value of both cutoff points. The reading was deemed equivocal if it was between the two cutoff values and negative if it fell below the lower cutoff value. Specimens with equivocal readings were retested in a subsequent run of the same EIA in accordance with the instructions of the manufacturer. Although the Kallestad EIA product insert suggests that visual interpretation of the reaction is possible; we noted many equivocal results, and only results from spectrophotometric determinations are reported.

The Ortho DFA test (Ortho Diagnostics) is a DFA test that uses a mixture of two different fluoresceinated monoclonal antibodies to RSV to detect the presence of RSV antigen in fixed nasopharyngeal cells. Stained slides were read on an epifluorescence microscope (Leitz/Opto-Metric Div. of E. Leitz Inc., Rockleigh, N.J.) at a magnification of 630× and were considered positive if one or more cells stained with characteristic cytoplasmic fluorescence. Slides with less than 200 cells and no specific fluorescence were reported as inadequate and omitted from the datum analysis. All slides were read independently by two observers.

Of the 95 specimens submitted during the study period, 92 were considered evaluable. Three specimens had insufficient cells visualized upon DFA staining and were not included in the test comparisons, although they were negative in all three EIAs. The intertest agreement among the RSV antigen detection tests is summarized in Table 1. If the two instances in which complete evaluation was unavailable (one that was tested in only three assays and one with a persistent equivocal result in the Kallestad EIA) are eliminated, 79% of the specimens gave the same results in all four tests. At least three of the four assays had the same results for 96.7% of the specimens.

Of the seven instances in which only one assay had a negative result, four were negative in the Ortho DFA test, two were negative in the Kallestad EIA, and one was negative in the Ortho EIA. Upon retesting with frozen samples, six of seven slides were again negative; one slide stained positive upon repeat Ortho DFA testing.

In the nine cases in which only one assay had a positive result, three were positive in the Abbott EIA, three were positive in the Ortho DFA test, two were positive in the Ortho EIA, and one was positive in the Kallestad EIA. Upon retesting, all nine of these specimens gave negative results in the previously discordant assays.

Data comparing each EIA with the Ortho DFA test are presented in Table 2. The overall agreement between the Ortho DFA test and each of the EIAs was ≥86%. There was

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TABLE 1. Intertest comparison of four RSV antigen detection assays

Test result	No. of specimens
All four tests positive	30
All four tests negative	41
Three tests positive and one test negative	7
Three tests negative and one test positive	9
Two tests positive and two tests negative	3
Three tests negative and one test QNS[a]	1
Three tests positive and one test equivocal[b]	1

[a] QNS, There was an insufficient quantity of one specimen to run the Abbott EIA.
[b] Persistently equivocal in the Kallestad EIA.

no statistical difference among these results, as determined by chi-square analysis ($P > 0.5$).

In this comparison of three EIAs and a DFA test for RSV antigen detection, we have shown that all of these assays are reliable for the rapid detection of RSV. Other evaluations of EIAs and/or immunofluorescent-antibody staining (1–3, 5–8, 11–15) for RSV have also shown sensitivities and specificities of 80 to 90% or greater when compared with culturing. This study enabled us to make a direct comparison of four different RSV antigen detection assays with a single specimen. Viral culturing is not presently available at Long Island Jewish Medical Center.

Of the four commercially available assays compared, no one test was clearly superior. The Ortho DFA test gave the most discrepant results; in 7 of the 16 samples in which three of four assays agreed, the one discordant result was in the Ortho DFA test. Still, this assay had an overall agreement with each EIA of ≥86%. This technique offers the advantage of visualizing the adequacy of a specimen (i.e., the presence of nasopharyngeal cells) and is the fastest of the methods available (one 30-min incubation followed by a rinse in phosphate-buffered saline). The performance of the test requires personnel with expertise both in interpreting the slides and with a fluorescence microscope.

The EIAs as a group offer the advantage of an objective determination of the reaction and, thus, require less technical expertise to perform. They are more labor intensive and require a spectrophotometer or enzyme-linked immunosorbent assay reader. The readers available with the Ortho and Abbott systems include programs that determine cutoff values and adequacy of the runs with negative and positive control wells. The Kallestad EIA provides a formula for similarly determining the results in comparison with the optical densities of the controls.

TABLE 2. Comparison of each RSV antigen EIA with the Ortho DFA test

Ortho DFA test result	No. of results					
	Kallestad EIA[a]		Ortho EIA		Abbott EIA[b]	
	+	−	+	−	+	−
+	33	5	33	5	34	4
−	5	48	7	47	9	44

[a] One specimen gave equivocal results on two separate occasions in the Kallestad EIA.
[b] There was an insufficient quantity of one specimen to run the Abbott EIA.

In the Kallestad EIA, the detector antibody is added with the clinical specimens. This assay is therefore faster to perform than the other EIAs (75 min versus 5 to 6 h), but the larger tubes used make rinsing between incubation steps more difficult and running a large number of specimens at one time more unwieldy.

The ability to rapidly identify the presence of RSV antigen, as opposed to waiting 3 to 7 days for culture results, is especially important in the consideration of antiviral therapy in infected children. In addition, in centers that do not have an on-site facility for the culturing of viruses, these methods offer an alternative to transporting the specimens for viral culturing.

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