Diagnostic Efficacy of Two Rapid Tests for Detection of Respiratory Syncytial Virus Antigen

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With the availability of ribavirin therapy for serious respiratory syncytial virus (RSV) infections, rapid diagnostic tests for the detection of RSV antigen are increasingly important. Efficacies of a commercially available enzyme immunoassay (EIA) (Abbott Laboratories, North Chicago, Ill.) and a fluorescent-antibody assay (FA) were evaluated in a study involving 135 specimens from children with respiratory symptoms. A nasal wash specimen was cultured immediately on RSV-sensitive A549 cells; the nasal wash was also used for EIA. FA was performed on a nasopharyngeal swab specimen with bovine anti-RSV and anti-bovine immunoglobulin G antisera (Burroughs Wellcome Co., Research Triangle Park, N.C.). A total of 39 specimens (28%) were tissue culture positive, including 35 EIA-positive and 37 FA-positive samples (sensitivities, 90 and 95%, respectively). All 96 tissue culture-negative specimens were EIA negative (specificity, 100%); 94 of these 96 specimens were FA negative (specificity, 98%). Positive and negative predictive values for the tests were as follows: 90 and 96% for EIA, respectively, and 95 and 98% for FA, respectively. Other viruses, including influenza A virus, adenovirus, enterovirus, and herpes simplex virus, were isolated in nine cases. One adenovirus-positive specimen had a false-positive RSV FA result; all nine specimens were RSV EIA negative. Both tests performed well in our study and provide cost-effective alternatives to tissue culture. The RSV EIA, in particular, uses standard serologic techniques and equipment and does not require expertise in virology. More widespread availability of rapid diagnostic tests for RSV will hopefully result in early and appropriate use of antiviral therapy in patients at risk for serious RSV infections.

With the availability of ribavirin therapy for serious respiratory syncytial virus (RSV) infections (4, 5, 10, 12), rapid diagnostic tests for RSV are increasingly important. Ribavirin treatment at the present time requires hospitalization for near-continuous aerosolization of the drug. Hospitalization is considered early in the course of the disease in certain infants at risk for severe RSV infections, such as infants with congenital heart disease or bronchopulmonary dysplasia. Therefore, diagnostic testing for RSV needs to be readily available for pediatric patients. The present report describes an evaluation of two rapid RSV diagnostic tests performed on pediatric outpatients who had respiratory symptoms and were seen at Cleveland Metropolitan General Hospital from February through April 1985. The tests evaluated were a commercially available enzyme immunoassay (EIA) (Abbott Laboratories, North Chicago, Ill.) and a fluorescent-antibody assay (FA).

MATERIALS AND METHODS

Specimens. Clinical specimens for RSV testing were obtained by a research nurse or one of us (M.L.K., D.M.S., or R.M.L.). Specimens were obtained from children less than 13 months of age who had symptoms of either upper or lower respiratory tract infections and were seen in the pediatric outpatient clinic. Specimens were obtained in the following manners:

(i) Nasal wash specimens. A 5-ml nasal wash specimen for RSV culture and EIA was obtained by the method of Hall and Douglas (3). The nasal wash specimen was immediately added to 2 ml of viral transport medium (Hanks balanced salt solution with 1% bovine serum albumin) and vortexed for 1 min. In the examining room, 0.5 ml of this suspension was inoculated into each of two monolayer tubes of A549 cells (gift from G. D. Hsiung). Although less frequently used for RSV isolation than HEP-2 cells, A549 cells are sensitive to RSV, with the formation of typical RSV-induced syncytia (7). Following inoculation, the tissue culture monolayers were transported to the laboratory. After 1 h of incubation at 36°C for viral absorption, the nasal wash specimen was decanted, and the monolayers were refed with minimal essential medium containing 2% fetal calf serum. Monolayers were incubated at 36°C and examined daily for 10 to 12 days for the appearance for RSV cytopathic effect (CPE). Negative specimens were blind passed and observed for another 10 days.

The remaining nasal wash specimen was frozen at -70°C until tested. The EIA was performed in accordance with the instructions of the manufacturer. In brief, the assay used goat anti-RSV-coated beads. Following a 2-h incubation of 200 μl of specimen material with the coated beads, two additional 1-h antibody incubations were performed. The second antibody was rabbit anti-RSV, and the final antibody was horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G. Beads were washed three times with distilled water between each incubation. Following the final wash, 300 μl of o-phenylenediamine substrate was added;
the reaction was stopped after 30 min by the addition of 1 N H₂SO₄. Reactions were quantitated in a spectrophotometer (Quantum II; Abbott Laboratories) at 492 nm. Three negative controls and one positive control were included in each run. A cutoff value for positive reactions was determined by adding 0.10 to the mean of the three negative controls. Cutoff values in the assays performed during the study ranged from 0.138 to 0.149 with a mean of 0.142.

(ii) Nasopharyngeal swab specimens. Nasopharyngeal swab specimens for FA were obtained with flexible wire swabs (Calgiswab; Spectrum Diagnostic, Glenwood, Ill.). After the swab was positioned in the nasopharynx, it was rubbed carefully over the mucosal surface. Material from the swab tip was applied directly to a two-well FA slide. Slides were transported to the laboratory within 1 h and fixed in cold acetone for 10 min prior to being stained. Antiseras used were bovine anti-RSV and fluorescein-conjugated anti-bovine immunoglobulin G (Burroughs Wellcome Co., Research Triangle Park, N.C.). Slides were examined under a Leitz epifluorescence microscope by two independent observers. The criterion for identification of RSV was typical fluorescence of either large cytoplasmic inclusionlike bodies or fine cytoplasmic particles. The FA was considered positive if any cell exhibited typical RSV fluorescence.

Statistical analysis. Specimens were divided into three groups based on the duration of time to the development of characteristic RSV CPE. CPE was noted for group 1 samples between 1 and 3 days, for group 2 samples between 4 and 6 days, and for group 3 samples after 6 days. The EIA mean Optical density (OD) was determined for each group. Differences in mean ODs between groups were analyzed for statistical significance by a one-way analysis of variance after the equality of variance between groups was assured by the Levene test (1). Differences between pairs of means were examined by the LSD procedure (9) at an overall alpha level of 0.05.

RESULTS

A total of 135 specimens from 112 children with respiratory symptoms were tested. Thirty-nine specimens (28%) were culture positive for RSV. The FA and EIA results are summarized in Table 1. Both tests were highly specific (EIA, 100%; FA, 98%). The sensitivity was also high (EIA, 90%; FA, 95%) when compared with that of culture.

Concordance among all three tests occurred in 127 samples (127 of 135 or 94%), including 33 of the 39 culture-positive specimens. The majority of these discordant RSV-positive specimens had antigen readily detectable by EIA; the OD of 22 specimens was >1.0, and only 4 positive specimens had an OD of 0.05. An inverse relationship was present between the length of time to the appearance of CPE and the EIA OD (Table 2). Specimens with a delay in the appearance of CPE of >6 days had a significantly lower mean OD (0.74) than did specimens with positive cultures between 1 and 3 days (1.72) and 4 and 6 days (1.61) (P < 0.05).

Test results for the eight discordant samples are summarized in Table 3. Two culture-positive, FA-negative samples were positive in the EIA, with ODs of 0.819 and 0.356, and appeared to be FA false-negatives. The viral titers in both samples may have been low, as indicated by the prolonged period before the appearance of CPE (12 and 13 days). Four discordant samples were culture and FA positive but EIA negative. Although ODs were just below the cutoff in two of the four samples, all four samples were considered EIA false-negatives. The remaining two samples appeared to be FA false-positives; both were culture and EIA negative.

Other viruses were detected by culture in nine specimens, including two enteroviruses, two influenza A viruses, four adenoviruses, and one herpes simplex virus type 1. One of these specimens was RSV discordant, that is, culture and EIA negative but FA positive (Table 3). The RSV EIA was consistently negative in all nine samples.

DISCUSSION

The laboratory diagnosis of RSV infection requires carefully obtained specimens, albeit for culture, EIA, or FA. In the present study, concordance among all three tests was high (127 of 135 or 94%). Both the EIA and the FA proved highly specific (100 and 98%, respectively). The sensitivity of the commercially available EIA, 90%, compares well with reported sensitivities of other RSV EIAIs developed in research laboratories (2, 6, 8, 11). Ideally, laboratories considering the use of rapid RSV tests should validate the test.
This is particularly important in diagnostic tests for RSV. The titer of virus in nasal wash specimens, as demonstrated by Hall and Douglas (3), is consistently higher than the titer in nasopharyngeal swab specimens. In the present study, nasopharyngeal swabs were carefully obtained by a research study nurse or physician. Specimens were highly cellular and excellent for FA analysis. Nasopharyngeal swabs, however, do not appear to be suitable for EIA analysis. In a small study, McIntosh et al. (8) compared nasopharyngeal swabs and nasal aspirates for the detection of RSV antigen by EIA; only one swab sample was positive, as compared with six aspirate samples. More recently, Masters et al. (H. Masters, B. Lauer, K. Weber, J. Groothuis, and C. Wren, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 82, 1986) compared the efficacy of nasopharyngeal swabs versus that of nasal aspirates for the detection of RSV by both culture and EIA. The detection of RSV by both techniques was significantly lower when nasopharyngeal swabs were used. A nasal wash or aspirate appears to be the specimen of choice for the detection of RSV by EIA. We used a 5-ml nasal wash, which was added to 2 ml of viral transport medium. The sensitivity of the EIA might be further enhanced by use of a smaller-volume nasal wash to avoid dilution of the viral antigen. In the study by Masters et al. (26th ICACAC) that utilized a 1-ml nasal wash, the detection of RSV by EIA was in fact more sensitive than by culture.

In the past, a clinical diagnosis of "probable RSV infection" was considered sufficient by most physicians. However, the availability of specific antiviral therapy with aerosolized ribavirin mandates the development of readily available, rapid RSV diagnostic tests. Laboratory confirmation is particularly helpful in atypical cases, such as infants with apnea or early in the course of infection in infants with congenital heart disease or underlying pulmonary disease. Both of the rapid tests evaluated in the present report performed well as diagnostic tests in our laboratory and provide cost-effective alternatives to tissue culture. Both tests use readily available reagents. The commercial EIA, in particular, uses standard serologic techniques and equipment and does not require expertise in virology. More widespread availability of rapid RSV diagnostic tests will hopefully result in early and appropriate use of antiviral therapy in patients at risk for serious RSV infections.

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