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Rapid Detection of Respiratory Syncytial Virus in Nasopharyngeal Specimens Obtained with the Rhinoprobe Scraper

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We compared the Rhinoprobe scraping technique for collection of superficial nasal mucosa epithelial cells and rapid detection of respiratory syncytial virus by immunofluorescence with paired, swab-collected specimens for virus culture from 1,257 infants and children with acute respiratory infections. Compared with viral culture as the reference test, the sensitivity, specificity, and accuracy of the immunofluorescence test were 83.6, 93.6, and 91.3%, respectively. We found the Rhinoprobe method safe, easy to use, and helpful in obtaining large quantities of epithelial cells for detection of respiratory syncytial virus and other respiratory viruses.

Swabbing the nose (2) or throat (7), washing and suctioning the nasopharynx (16), and aspirating nasopharyngeal secretions (9) have been used by other investigators to collect infected respiratory epithelial cells for rapid virus detection. These techniques frequently yield a small proportion of respiratory epithelial cells, require time-consuming procedures for removing excess mucus, or fail to produce an adequate specimen (6). Ahluwalia et al. (1) demonstrated that nasopharyngeal aspiration may produce a better specimen than does nasal swabbing for viral culture and rapid immunofluorescence (IF) techniques, without the discomfort experienced by patients undergoing nasopharyngeal washings.

In 1987, we described a new application of the Rhinoprobe scraper (3M Diagnostic Systems Inc., Santa Clara, Calif.) for rapid detection of respiratory syncytial virus (RSV) in a small number of hospitalized pediatric patients (10). The results of this new method of specimen procurement were very encouraging. On the basis of a much larger patient population, we have compared the Rhinoprobe scraping technique for rapid detection of RSV by IF with simultaneous virus culture. We have found the Rhinoprobe method to be reliable and suitable for rapid diagnosis of RSV and possibly other respiratory agents.

MATERIALS AND METHODS

Hospitalized patients, with a variety of upper and lower respiratory symptoms, in several institutions of metropolitan San Diego participated in the study. Rhinoprobe specimens were transported to the University of California, San Diego, Epidemiology and Infectious Disease Laboratory (Rapid Diagnostic Laboratory). These specimens were prepared primarily for RSV IF, and each was accompanied by a swab simultaneously collected for virus culture.

Patients. Patients consisted of 1,257 infants, children, and a small number of adults having acute respiratory disease requiring hospitalization between July 1986 and March 1988. The numbers of patients from each participating institution and percentages of total patients were as follows: Children's Hospital and Health Care, 737 (60.2%); University of California, San Diego, 343 (28.0%); Mercy Hospital Medical Center, 128 (10.5%); other institutions, 16 (1.3%). The tests were ordered by their physicians for diagnostic purposes only; rapid diagnostic tests were performed by the University of California, San Diego, Rapid Diagnostic Laboratory, and cultures were performed by the University of California, San Diego, Viral Diagnostic Laboratory. All patients underwent a Rhinoprobe nasal scraping for rapid detection of RSV and other respiratory virus antigens. A second specimen was collected at the same time from the nasopharynx with a Calgiswab (Spectrum Diagnostics, Inc., Glenwood, Ill.) for virus culture, and a taxi-courier system brought the refrigerated specimens to the laboratory. These specimens accompanied a request for both rapid diagnosis and culturing during the study; only paired specimens were included in the study.

Specimen collection. The specific methods for collecting nasal and pharyngeal specimens have been described elsewhere (10); important aspects of the collection technique along with minor changes are described here for emphasis. Before specimens were obtained, excess secretions were cleared from the nares with an aspirating bulb and were discarded. A gentle Rhinoprobe scraping of the posterior nasal cavity followed. This was accomplished in all infants and young children (≤4 years) without visual inspection of the nasal cavity. We still recommend that visual inspection of the nasal cavity be performed in older children and adults. Scrapings, contained in the Rhinoprobe cusp, were immediately put into a plastic tube containing 1.5 ml of Hanks balanced salt solution with gentamicin sulfate (Whittaker M. A. Bioproducts Inc., Walkersville, Md.) (50 μg/ml) and amphotericin B (Fungizone; E. R. Squibb & Sons, Princeton, N.J.) (1.2 μg/ml) but without phenol red. The sample for viral culture was obtained from either nostril with a Calgiswab Type 1 (Spectrum Diagnostics, Inc.) and was placed in a vial containing veal infusion broth; the aluminum shaft was snipped, and the vial was tightly capped prior to refrigeration. Both specimens were then transported in ice-water to the laboratory for analysis.

Virus culture. The procedures for processing collected specimens were the same as those described previously (10). In brief, antibiotics were added to the specimen in veal infusion broth, and the specimen was then incubated at room temperature for 1 h. Samples (0.2 ml) of the specimen were

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inoculated into tubes of human embryonic kidney, HEp-2, and primary monkey (rhesus) kidney cells. Tubes showing cytopathic effects were identified by morphology and staining for RSV and herpes simplex virus and by neutralization for enterovirus and adenovirus.

IF procedure. The Rhinoprobe specimen in Hanks balanced salt solution was broken up into a fine cell suspension by gently aspirating and expelling the specimen 5 to 6 times through the tip of a wide-bore Pasteur pipette. The cells were centrifuged at room temperature for 3 to 5 min at 1,500 to 2,000 rpm with a counter-top clinical centrifuge (International Equipment Co., Needham Heights, Mass.), and most of the supernatant was discarded, leaving 0.2 to 0.3 ml of the Hanks balanced salt solution-cell suspension in the test tube. After gentle mixing, a drop of the cell suspension was placed on each of several wells on microscope slides (Cel-Line Associates, Inc., Newfield, N.J.) with a Pasteur pipette. The slides were air dried, fixed in anhydrous acetone, and air dried again before processing.

An indirect IF procedure was used for RSV, parainfluenza viruses 1 and 3, and influenza virus A. A two-step method was used with the appropriate bovine anti-serum and bovine negative control, followed by fluorescein-conjugated anti-bovine serum (Burroughs Wellcome Co., Research Triangle Park, N.C.). Similarly, a two-step method with chicken anti-serum and chicken negative control, followed by fluorescein-conjugated anti-chicken serum (Burroughs Wellcome Co.), was used to detect parainfluenza virus 1. For influenza virus B and parainfluenza virus 2 detection, a one-step method with fluorescein-conjugated monoclonal antibodies from purified mouse ascites fluid (Whittaker M. A. Bioproducts, Inc.) was utilized in a direct IF procedure. Adenovirus detection required a one-step direct method with fluorescein-conjugated goat anti-adenovirus serum, type 2 adenoid 6 virus strain (Whittaker M. A. Bioproducts, Inc.).

Microscopy. Slides were initially screened at a magnification of ×100 and were confirmed at a magnification of ×400 (dry). A specimen was considered adequate if it contained 100 or more cells per well. At least two wells per specimen were examined, and the specimens were scored for the number of positive cells per well as follows: 1+, 1 to 10 positive cells; 2+, 11 to 50 positive cells; 3+, 51 to 100 positive cells; and 4+, >100 positive cells per well.

Test characteristics and statistics. The results of the IF test were compared with the results of viral culture, with the latter as the "gold standard." The sensitivity, specificity, accuracy, prevalence, positive predictive value, and negative predictive value were calculated by standard formulas. A two-sample median test, a *t* test, and one-way analysis of variance were performed to compare age, sex, RSV-positive, RSV-negative, and reporting-time groups. Additionally, McNemar's nonparametric chi-square test was used to compare the results of the RSV IF assays and viral culture.

RESULTS

Available data on 94% of patients show an age range from 5 days to 27 years, with a median age of 6 months. Seventy-eight percent were 1 year of age or younger. The sex distribution was 59% males and 41% females. The median age for all RSV culture-negative patients was 6.0 months, in contrast to 4.0 months for RSV-positive patients, by either culture or IF method ($P < 0.0001$). The age differences between sexes within RSV-positive or RSV-negative groups were not found to be statistically significant.

Results of a blinded comparison between detection of RSV by IF and detection of RSV by culture are presented in Table 1. The IF assay for RSV detection was performed in 1,224 of 1,257 specimens. In 33 specimens, the quantity of sample was inadequate and no IF test was performed. The IF method correctly identified 878 specimens that were RSV negative and 239 specimens that were RSV positive by the culture method (Table 1). The following IF assay characteristics, compared with those of the viral culture, were also calculated: sensitivity, 83.6%; specificity, 93.6%; positive predictive value, 79.9%; negative predictive value, 94.9%; accuracy, 91.3; and prevalence, 23.4%.

TABLE 1. Blinded comparison between results of RSV IF assay and results of viral culture[a]

IF assay result	No. of viral culture results		
	+	−	Total
+	239	60	299
−	47	878	925
Total	286	938	1,224

[a] McNemar's nonparametric chi-square value on discrepant results was $\chi = 1.35$ ($P = 0.25$). The following IF assay characteristics, compared with those of viral culture, were calculated: sensitivity, 83.6%; specificity, 93.6%; positive predictive value, 79.9%; negative predictive value, 94.9%; accuracy, 91.3; and prevalence, 23.4%.

Of the 1,224 specimens compared by both methods, 107 (47 + 60), or 9% of the total, gave positive results by one of the tests but not by the other. In 5% of the cases, or 60 of 1,224, the culture method result was negative but the IF assay result was positive. In 4% of the cases, or 47 of 1,224 specimens, the IF assay was negative and the culture result was positive. McNemar's nonparametric chi-square test revealed that these observed frequencies of 47 and 60 were not significantly different from the expected frequencies of 53.5, 107/2 = 53.5 ($\chi = 1.35$ and $P = 0.25$).

Of the 1,224 specimens submitted to the laboratory for RSV detection, 139 also had a request for detection of one or more other viruses, such as parainfluenza viruses 1, 2, and 3, influenza viruses A and B, and adenovirus. The test characteristics for viruses other than the RSV were not calculated because of insufficient number. Of all patients tested, 6% had a positive IF test for antigens other than RSV antigen. In contrast, 8% of patients were positive by culture for viruses other than RSV. The average age of this group of patients was 15.5 months, with more than twice as many males as females (97 males, 43 females). Nine patients had dual infections (RSV infection combined with another respiratory virus infection).

The average period from specimen collection to reporting positive viral cultures was 8.9 ± 5.3 (standard deviation) days and for reporting of IF results was 0.7 ± 0.5 (standard deviation) days or approximately 17 h ($P < 0.001$). Ninety-one percent of all rapid diagnostic tests were reported within 24 h of receipt of the specimen in our laboratory, including weekends and holidays.

A total of 254 RSV-positive specimens were graded on a scale from 1+ to 4+, as described in Materials and Methods. The number of specimens, average number of days to cytopathic effect in culture, and standard deviation (in parentheses) for each grade were as follows: 1+ (108, 9.2 ± 5.8); 2+ (73, 7.6 ± 4.3); 3+ (28, 8.6 ± 5.4); and 4+ (45, 7.1 ± 3.0). Statistical comparison between the average number of days to cytopathic effect for groups 1+ and 4+ was significant at $P \leq 0.05$, while other comparisons were not significant.

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DISCUSSION

In this study, we evaluated the efficacy of the Rhinoprobe in providing adequate numbers of infected nasal epithelial cells for the rapid detection of RSV. The results confirm our earlier findings (10) and provide additional evidence that this method of specimen collection may alleviate many of the inherent problems associated with swabbing, washing, or aspirating secretions from the nasopharynx for the rapid detection of viral antigens. These techniques frequently provide specimens with inadequate numbers of cells, as well as excess mucus that can give nonspecific fluorescence.

An atraumatic method for obtaining nasal mucosal specimens suitable for histological staining, IF, and electron microscopy was described by Douglas et al. (4) in 1968. This method utilized a Freimuth ear curette, and with it, a gentle scraping of the superficial nasal mucosa was obtained which was shown to provide numerous ciliated columnar epithelial cells. The metallic nature of this curette prevented its widespread use. The Rhinoprobe, in contrast, which is made of inexpensive, disposable, flexible plastic, has provided us with the opportunity to reevaluate this method of collection.

We found that 2.6% (33 of 1,257) of all collected specimens were inadequate. These inadequate specimens originated from one institution, where several members of a specimen procurement team were responsible for collection. Kaul et al. (11) reported 2.5% inadequate specimens (10 of 387) with nasopharyngeal aspirates from only one institution. Other investigators have reported a higher percentage of inadequate specimens, such as 6% by Lauer (13), 11% by Freymuth et al. (5), and 17% by Hendry et al. (9). Minnich and Ray (14) reported only 10 of 567 (2%) specimens inadequate for IF assays; however, 97% of these specimens were pooled swab specimens collected from two different sites. When two separate Rhinoprobe collections are pooled, the chance of getting inadequate specimens is also markedly reduced.

Nasopharyngeal aspirate specimens have been shown to contain more cells than specimens obtained by the swab technique (1). Similarly, swabbing the endocervix and ectocervix produced more inadequate smears and scanty cellular yield when compared with those obtained by a scraping method (15). Recent studies have shown that specimens obtained with the Rhinoprobe contain more respiratory epithelial cells, as described by Zeltzer and Maes at the Allergy and Asthma Prevention and Treatment Center (unpublished data), and more eosinophils and basophilic cells than do those obtained with paired swab-collected specimens (12).

The IF assay characteristics calculated for this study are very similar to those reported earlier (10). When we compared the sensitivity and specificity values of 84 and 94%, respectively, for this study, with those of other investigators (3, 8, 13–15) assaying for RSV detection, we find our results clearly support earlier findings that the Rhinoprobe method is effective in providing cells for detecting RSV.

Since multiple assays can be performed with the larger number of epithelial cells obtained with the Rhinoprobe, we were able to perform IF assays for other respiratory viruses when requested, i.e., with 11.1% of the patient population. Because of an insufficient number of positive IF and culture specimens, the assay characteristics were not calculated; we will continue to accumulate data for future reporting. In our study, 6% of patients who were RSV IF negative tested positive for another virus. Culture-positive results for viruses other than the RSV were obtained in 8% of patients evaluated, including 33 patients that had inadequate specimens for IF diagnosis.

The quality of collected specimens can affect the results of viral culture. Hall and Douglas (8) reported that identification of RSV cytopathic effect was more rapid with aspirates of washed nasal secretions than with specimens obtained by the swab method. Similarly, Ahluwalia et al. (1) found that nasopharyngeal aspirates, when compared with nasopharyngeal swab specimens, provided not only a faster rate of isolation but also a greater percentage of RSV isolates. In this study, we showed that the degree of positivity by IF of Rhinoprobe-collected specimens correlates positively with the rapidity of swab-collected specimens, demonstrating RSV cytopathic effect.

Cheeseman et al. (3) cited the major disadvantage of the IF method as the time consumed in preparing the slides for reading. With increasing availability of commercial reagents for the rapid IF detection of RSV and a variety of other respiratory viruses, it has become more important to collect a larger quantity of respiratory epithelial cells for simultaneous detection of viral antigens. A major advantage of the Rhinoprobe method is the expeditious processing of specimens and the ease of slide preparation while providing numerous intact and clean epithelial cells for multiple IF assays. This is accomplished by avoiding the need for special equipment (no suction apparatus is needed), eliminating much of the mucus and debris known to interfere with accurate evaluation of specimens, and shortening procedural time by providing relatively clean specimens, since there is then no need to repeatedly wash the specimen.

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LITERATURE CITED

1. **Ahluwalia, G., J. Embree, P. McNicol, B. Law, and G. W. Hammond.** 1987. Comparison of nasopharyngeal aspirate and nasopharyngeal swab specimens for respiratory syncytial virus diagnosis by cell culture, indirect immunofluorescence assay, and enzyme-linked immunosorbent assay. J. Clin. Microbiol. **25**:763–767.
2. **Bell, D. M., E. E. Walsh, J. F. Hruska, K. C. Schnabel, and C. B. Hall.** 1983. Rapid detection of respiratory syncytial virus with a monoclonal antibody. J. Clin. Microbiol. **17**:1099–1101.
3. **Cheeseman, S. H., L. T. Pierik, D. Leombruno, K. E. Spinos, and K. McIntosh.** 1986. Evaluation of commercially available direct immunofluorescent staining reagent for the detection of respiratory syncytial virus in respiratory secretions. J. Clin. Microbiol. **24**:155–156.
4. **Douglas, R. G., Jr., B. R. Alford, and R. B. Couch.** 1969. Atraumatic nasal biopsy for studies of respiratory virus infection in volunteers, p. 340–343. Antimicrob. Agents Chemother. 1968.
5. **Freymuth, F., M. Quibriac, J. Petitjean, M. L. Amiel, P. Pothier, A. Denis, and J. F. Duhamel.** 1986. Comparison of two new tests for rapid diagnosis of respiratory syncytial virus infections by enzyme-linked immunosorbent assay and immunofluorescence techniques. J. Clin. Microbiol. **24**:1013–1016.
6. **Gardner, P. S., and J. McQuillin.** 1980. Rapid virus diagnosis: application of immunofluorescence. Butterworth & Co. (Publishers) Ltd., London.
7. **Gray, K. G., D. E. MacFarlane, and R. G. Sommerville.** 1968. Direct immunofluorescent identification of respiratory syncytial virus in throat swabs from children with respiratory illness. Lancet **i**:446–448.
8. **Hall, C. B., and R. G. Douglas.** 1975. Clinically useful method

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for the isolation of respiratory syncytial virus. J. Infect. Dis. **131**:1–5.
9. **Hendry, R. M., L. T. Pierik, and K. McIntosh.** 1986. Comparison of washed nasopharyngeal cells and whole nasal secretions for detection of respiratory syncytial virus antigens by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **23**:383–384.
10. **Jalowayski, A. A., B. L. England, C. J. Temm, T. J. Nunemacher, J. F. Bastian, G. A. MacPherson, W. M. Dankner, R. C. Straube, and J. D. Connor.** 1987. Peroxidase-antiperoxidase assay for rapid detection of respiratory syncytial virus in nasal epithelial specimens from infants and children. J. Clin. Microbiol. **25**:722–725.
11. **Kaul, A., R. Scott, M. Gallagher, M. Scott, J. Clement, and P. L. Ogra.** 1978. Respiratory syncytial virus infection. Am. J. Dis. Child. **132**:1088–1090.
12. **Lara, A., S. Gonzalez, C. Canseco, and R. N. Hamburger.** 1989. Determination of the eosinophil count in nasal cytology: comparison of two techniques. Ann. Allergy **62**:262.
13. **Lauer, B. A.** 1982. Comparison of virus culturing and immunofluorescence for rapid detection of respiratory syncytial virus in nasopharyngeal secretions: sensitivity and specificity. J. Clin. Microbiol. **16**:411–412.
14. **Minnich, L., and C. G. Ray.** 1980. Comparison of direct immunofluorescent staining of clinical specimens for respiratory virus antigens with conventional isolation techniques. J. Clin. Microbiol. **12**:391–394.
15. **Shen, J., R. H. Nalick, J. B. Schlaerth, and C. P. Morrow.** 1983. Efficacy of cotton-tipped applicators for obtaining cells from the uterine cervix for Papanicolaou smears. Acta Cytol. **28**:541–545.
16. **Treuhaft, M. W., J. M. Soukup, and B. J. Sullivan.** 1985. Practical recommendations for the detection of pediatric respiratory syncytial virus infections. J. Clin. Microbiol. **22**:270–273.

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