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Nasopharyngeal Swabs and Nasopharyngeal Aspirates Equally Effective for the Diagnosis of Viral Respiratory Disease in Hospitalized Children

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Paired nasopharyngeal swab and nasopharyngeal aspirate specimens from 125 patients were compared for viral diagnosis. The viral isolation rates were comparable for the two types of specimens. There was a high level of agreement between the two specimens in overall positivity rate by immunofluorescence and positivity in culture-confirmed patients.

Acute respiratory infections are the commonest cause of illness in children (9). Confirmation of a viral etiology for respiratory infections is important, particularly as more antiviral therapy becomes available (11, 13). The success of detection of a respiratory virus depends on many variables, including the site of collection and the type of specimen used (1, 4, 6, 8, 10, 12, 14–18, 20). The most commonly recommended respiratory specimens for viral isolation in children are nasal washes and nasopharyngeal aspirates (NPAs) (1, 8, 12, 17, 18, 20). Although nasal washes are routinely performed for the diagnosis of respiratory infections in some centers, they are rarely done in our community (Hamilton, Ontario, Canada) because they are believed to cause discomfort to the children and pose a risk of aspiration. NPA collection requires a suction unit and therefore may be impractical in an office setting, where such units may not be available. Throat and nasal swab specimens are more practical to obtain and involve less expense than either nasal washes or NPAs. However, these swabs do not access the nasopharynx and therefore do not provide an optimal specimen for the diagnosis of respiratory viruses (12, 20). The limitations posed by the different collection techniques may be obviated by using a nasopharyngeal swab (NPS).

In this study, we compared NPA and NPS specimens for the diagnosis of respiratory viruses by the indirect immunofluorescence assay (IFA) and cell culture in children hospitalized with upper and/or lower respiratory tract disease.

Paired specimens were obtained from 125 hospitalized children. Inclusion criteria were (i) age ≤16 years, (ii) presence of respiratory symptoms for ≤3 days, (iii) no preexisting cardiopulmonary disease or immunosuppression, (iv) no medical contraindication to sampling of respiratory specimens, and (v) parental consent to participation in the study. Specimens were collected by either the principal investigator or the trained research nurse, using a standardized approach. The order of specimen collection was randomized for the first 92 patients. In the remaining 33 patients, the order of collection was not noted. NPA specimens were collected by suction of nasopharyngeal secretions from one nostril into mucous traps, as described previously (1, 4, 8). Secretions remaining in the catheter were washed off by suctioning 1 ml of viral transport medium (minimal essential medium containing 25 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid [HEPES; pH 7.4], 5.4 mM sodium bicarbonate, 10 μg of gentamicin per ml, and 0.6% fetal bovine serum) through the catheter. The NPS specimens were collected through the other nostril with a flexible cotton-tipped wire swab which does not contain calcium alginate (Pro Lab Inc., Scarborough, Ontario, Canada). The swab was pushed gently into the nasopharynx until the tip reached a distance equivalent to that from the ear to the nostril of the patient. The swab was then rotated three times, removed, and placed into a tube containing 1 ml of viral transport medium. The swab tip was agitated several times in the transport medium, and then the tube was sealed. All specimens were delivered immediately to the laboratory, which is located within the same hospital.

All specimens were processed in an identical fashion. (i) For cell culture, the specimen was centrifuged, and portions of the supernatant were inoculated into one tube each of rhesus monkey kidney cells, green monkey kidney cells, and human skin fibroblasts. The tubes were incubated in a roller drum at 37°C and examined daily for cytopathic effect. The presence of respiratory syncytial virus (RSV) was confirmed by performing an IFA on the cell culture. A hemadsorption assay with guinea pig erythrocytes was carried out on days 5 and 10 to detect influenza and parainfluenza viruses. (ii) For IFA, a smear was made from the cell pellet of the centrifuged specimen, and an IFA was performed as described previously (7). We used bovine immune sera against RSV, parainfluenza virus type 3, and influenza virus type A and chicken immune sera against adenovirus, parainfluenza virus type 1, and influenza virus type B (Burroughs Wellcome Co., Research Triangle Park, N.C.). Fluorescein isothiocyanate-conjugated antispecies immunoglobulin (at optimal dilutions determined by staining positive and negative monolayers) was used (Burroughs Wellcome). Slides were read under a UV microscope by an experienced technologist. A specimen was considered adequate if ≥1 nasopharyngeal epithelial cell per field was present and positive if fluorescence in >1 epithelial cell was seen: cytoplasmic fluorescence for RSV and parainfluenza virus types 1 and 3, and nuclear fluorescence for influenza virus and adenovirus.

For analysis, a patient was considered to have a viral infection if the virus was isolated in cell culture from either the NPA or NPS specimen and/or if both specimens were positive by IFA for the same virus. Cohen's kappa (19) was

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TABLE 1. Comparison of NPA and NPS specimens for the diagnosis of respiratory viruses by culture or IFA

Diagnostic test	Specimen	% Positive (no. positive/total no.)	% Sensitivity[a]	% Specificity[b]	% PPV[c]	% NPV[d]
Culture	NPA	46.2 (55/119)	93.2 (55/59)	93.7 (60/64)	93.2 (55/59)	93.7 (60/64)
	NPS	42.0 (50/119)	84.7 (50/59)	86.9 (60/69)	84.7 (50/59)	86.9 (60/69)
IFA	NPA	58.7 (74/126)	97.3 (74/76)	96.1 (50/52)	97.3 (74/76)	96.5 (50/52)
	NPS	58.7 (74/126)	97.3 (74/76)	96.1 (50/52)	97.3 (74/76)	96.1 (50/52)

[a] Sensitivity is the percentage of positives in one type of specimen in relation to the total number of positives from both specimens.
[b] Specificity is the percentage of negatives in one type of specimen in relation to the total number of negatives from both specimens.
[c] Positive predictive value (PPV) is the percentage of true-positives in relation to the total number of true-positives and false-positives.
[d] Negative predictive value (NPV) is the percentage of true-negatives in relation to the total number of true-negatives and false-negatives.

calculated as a measure of agreement of the results obtained from the NPA and NPS specimens by IFA.

We studied 126 paired specimens from 125 patients. Specimens were obtained from one infant during two separate hospitalizations. The mean age of the patients was 10.3 months (range, 26 days to 7 years). Thirty-two children had only upper respiratory tract symptoms, and 93 had lower with or without upper respiratory tract symptoms. The overall virus isolation rate was 41% for NPS specimens and 44% for NPA specimens, which is not a statistically significant difference ($P > 0.10$). RSV was recovered from 47 NPS specimens and 52 NPA specimens, parainfluenza virus type 3 was recovered from 3 NPS specimens and 2 NPA specimens, and influenza virus was recovered from 2 NPS specimens to 2 NPA specimens. In seven instances, the culture of one or both specimens was contaminated and therefore was excluded from the analysis, leaving 119 paired specimens for comparison. There were 46 instances in which both NPS and NPA specimens yielded virus in cell culture, 4 instances in which only the NPS specimens were positive, and 9 instances in which only the NPA specimens were positive. The difference in the isolation rates from the NPS (42.0%) and NPA (46.2%) specimens was not statistically significant ($P > 0.10$). The overall positivity rate, sensitivity, specificity, and predictive values of the two types of specimens are shown in Table 1.

The positivity rate by IFA was similar for the NPS and NPA specimens (74 of 126). Both specimens were positive by IFA in 72 patients; the NPS specimen only was positive in 2 patients; and the NPA specimen only was positive in 2 others (kappa, 0.93; $P < 0.0001$). All four specimens discordant by IFA were positive by culture and thus considered to be true positives. From these data, the sensitivity and specificity of the two specimens are the same at 97.3% (74 of 76) and 96.1% (50 of 52), respectively (Table 1).

Previous comparative studies have presented data favoring NPA specimens (1, 8, 18) or nasal washes (17, 20) over NPS specimens. In a limited study of nine infants excreting RSV, McIntosh et al. (18) found the NPA specimen superior to the NPS specimen for viral isolation and detection of antigen by enzyme immunoassay. Cruz et al. (8) reported higher isolation rates from NPA than NPS specimens in 76 nonhospitalized children from whom the dominant organisms isolated were enteroviruses. Ahluwalia et al. (1) obtained a higher yield of RSV isolates from NPA than NPS specimens collected from 32 hospitalized children with lower respiratory tract infection. In contrast to these studies, the isolation rate of viruses from the two types of specimens in our study were not significantly different (42% for NPS and 46.2% for NPA specimens). Our results indicate that the sensitivity of the NPS in culture (84.7%) is much higher than the 33% (3 of 9), 57% (36 of 63), and 65% (15 of 23) reported by McIntosh et al. (18), Cruz et al. (8), and Ahluwalia et al. (1), respectively. When RSV isolates alone were considered, the sensitivity of the NPS was 83.7% (46 of 55) and that of the NPA was 92.7% (51 of 55). Although other studies (3, 4, 10, 14–17) as well as ours have shown that antigen detection methods provide a positive diagnosis in the absence of positive cultures, our isolation rates were probably influenced by the lack of a continuous HEp-2 or HeLa cell line, which would be more sensitive to RSV (2, 15). In our study, the overall positivity rates by IFA were comparable for the two types of specimens. The same was observed with the culture-confirmed patients: both specimens were positive by IFA in two patients, the NPA specimen only was positive in one patient, and the NPS method detected two additional patients who were missed by the NPA method (kappa = 0.905). These are important findings in view of the increased utilization of detection methods for rapid diagnosis of viral respiratory infections in children (1, 3, 4, 7, 10, 15–17, 20). These findings are similar to those of Ahluwalia et al. (1), in which viral antigen was detected at similar frequencies in the paired specimens, despite the recovery of significantly more cells in the NPA specimen. Thus, it appears that efficient swabbing may dislodge sufficient number of cells containing antigens that can be detected by IFA but may not contain live virus. Alternatively, small numbers of live virus may be sequestered in swab strands or be inactivated by the adhesive holding the material to the shaft, although this is less likely, since we used the same swab throughout and yet had numerous positive cultures. Some of the differences between our results and those of others may be attributed to the type of swabs used and/or the order of specimen collection. In all previous studies (1, 8, 18), calcium alginate swabs were used. Although no data are available for respiratory viruses, certain batches of these types of swabs have been reported to be toxic for herpes simplex virus (6) and *Chlamydia trachomatis* (5). In all previous studies (1, 8, 18), the NPS specimen was collected before the NPA specimen. This raises the possibility that the swab dislodges virus-containing cells into the nasopharyngeal secretions, which are readily recovered by the NPA, thus leading to an improved specimen. However, this is unlikely, since the order of specimen collection did not seem to influence our results. The isolation rates from the two specimens were identical (14 of 15) in the 39 children from whom the NPS specimen was collected first, and these rates were not significantly different (NPS, 24 of 29; NPA, 27 of 29) from those in the 53 children from whom the NPA specimen was collected first ($P > 0.10$). Similarly, IFA positivity rate of the NPS and NPA specimens were the same in the two groups. The most likely explanation for the differences between our results and those of others is that the efficiency of swabbing in our study may have been improved by standardizing the sampling tech-

niques and limiting the number of persons involved in specimen collection. In addition, specimen collection early in the disease process, when the virus load is maximal (18), may have contributed to these differences.

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