Rapid Diagnosis of Respiratory Syncytial Virus Infection by Using Pernasal Swabs

PETER L. K. MACKIE,1* PHILIPPA J. MADGE,2 SUSAN GETTY,1 AND JAMES Y. PATON2

Department of Microbiology1 and Department of Child Health,2 Royal Hospital for Sick Children, Glasgow G3 8SJ, United Kingdom

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The sensitivity and specificity of direct immunofluorescence microscopy performed on Pernasal swab specimens and compared with those of nasopharyngeal aspirates were 93 and 99%, respectively. Posterior nasopharyngeal swabs applied immediately to microscope slides allow a rapid and simple screening procedure for the diagnosis of acute respiratory syncytial virus infections.

Annual epidemics of lower respiratory tract infections caused by respiratory syncytial virus (RSV) account for the high incidence of pediatric hospital admissions during winter months. It has become increasingly recognized that hospital-acquired infections pose a significant hazard during RSV epidemics, particularly for compromised infants (7, 8, 11). Further, the therapeutic option of using aerosolized ribavirin (9) in selected high-risk patients makes an accurate and rapid laboratory diagnosis highly desirable.

RSV antigen can be readily detected in respiratory secretions by a direct immunofluorescence technique (DIF) using a pool of specific monoclonal antibody (6). For DIF, specimens are usually obtained by nasopharyngeal suction (NPA) and the aspirated material is examined by incident light fluorescence microscopy following a combined washing and cell concentration step. In an attempt to further simplify and accelerate the diagnosis of RSV infection, we have investigated the characteristics of specimens obtained by nasopharyngeal swabbing (PNS) and applied directly to microscope slides for examination by DIF. By omitting a cell concentration step, the time required for specimen processing and analysis can be significantly reduced.

All children less than 2 years of age admitted to three medical wards of the Royal Hospital for Sick Children, Glasgow, United Kingdom, were screened for RSV infection. The study group, from whom paired NPA and PNS specimens were obtained, consisted of 101 males (55%) and 82 females (45%), with mean ages of 8.75 and 8 months, respectively. Within 24 h of admission, a PNS specimen was obtained from infants by using sterile flexible cotton-tipped swabs (Pernasal swab; Medical Wire and Equipment Company, Corsham, Wiltshire, United Kingdom). The swab was carefully inserted into the posterior nasopharynx and gently rotated. The exudate was applied to a clean labelled microscope slide by a circular motion, creating a smear of approximately 1 cm in diameter. The slides were air dried and fixed in acetone at room temperature.

After the PNS specimens had been taken, NPA specimens were collected with a suction catheter (8 by 45 cm [charnier gauge]; Argyle Sherwood S.A., Petit-Rechain, Belgium). The volume of nasal secretions sampled by swabbing was tiny and did not appear to adversely affect the volume of the NPA samples. Because of this, we did not routinely use the same or alternate nostrils for paired specimens. On arrival in the laboratory, the cannula was repeatedly flushed with phosphate-buffered saline (PBS) until all visible aspirate was cleared. The resulting suspension was centrifuged at 2,000 rpm for 10 min (Heraeus Christ Bactifuge); the supernatant was decanted, and the aspirate was resuspended in an approximately equal volume of PBS, usually between 0.1 and 1.0 ml, depending on the pellet size. The resuspended exudate was dropped onto two wells of a Teflon-coated multiwell slide. Droplets were air dried (approximately 20 min) and fixed in acetone as described above. All specimens were processed in the laboratory within 1 h of sampling. The same commercially available fluorescein isothiocyanate-conjugated monoclonal antibody pool was used to detect RSV antigens in NPA and PNS specimens (RSV Imagen; Novo Nordisk, Cambridge, United Kingdom).

Following preliminary evaluation and validation with the manufacturers, the incubation step for the DIF test in a humidified chamber at 37°C was reduced from 15 to 5 min. After the slides were washed in freshly deionized water and air dried, they were examined by incident light fluorescence microscopy at a magnification of ×400 (Nikon Labophot/mercury lamp power supply HBO-100/2). Slides were coded so that the microscopist could not correlate NPA and PNS results at the time of reading. Specimens showing fewer than 20 exfoliated respiratory cells within the slide well (1 cm in diameter) were not included in the study (eight NPA specimens and one PNS specimen). The appearance of no fewer than two intact respiratory cells showing characteristic intracytoplasmic, granular, and specific fluorescence per specimen was reported as a positive result. Preliminary experimental work showed that the antigens detected in aspirates by using the RSV-specific monoclonal antibody reagent were stable at room temperature and were acetone tolerant for up to 24 h.

Results for paired NPS and NPA specimens taken from 183 infants in the study were as follows. Nine slides could not be used because cellular material was lost during processing. NPA specimens tended to show a greater number of fluorescing cells per high-power field than PNS specimens. However, PNS specimens were free of the excessive mucus often seen in NPA specimens, and the conformation of exfoliated columnar epithelial cells was more obvious. Of 95 patients, there were six whose PNS specimens showed no evidence of RSV infection when their corresponding NPA specimens revealed positive results. This gave an overall sensitivity of 93% for PNS specimens. Only 1 of 79 PNS specimens was positive when the corresponding paired NPA

* Corresponding author.
specimen remained negative; this resulted in an overall specificity of 99%.

Fifty-two patients with positive NPA and PNS specimens were monitored by both sampling procedures every day until they were discharged from the hospital. This permitted a longitudinal examination of variation in antigen detection between the two sampling techniques. In Fig. 1, boxed numbers along the x axis indicate the number of patients remaining in the hospital and available for testing. (\% antigen detection = [number of positive specimens/total specimens] \times 100.) Antigen detection decreased with time, but the falloff was more pronounced for PNS specimens. An analysis of the difference between the time until the NPA result was negative and the time until the PNS result was negative was made. For 22 patients, the NPA and PNS results became negative on the same day; 11 patients were discharged from the hospital before either result became negative; and 19 patients had NPA specimens yielding positive results for more days than the parallel PNS samples (sign test, \( P = 0.001 \)).

Recently, two studies have compared specimens obtained by PNS and NPA (1, 5). In both these previous studies, PNS specimens were resuspended in virus transport medium and then processed similarly to NPA specimens. In the present study, it was recognized that one of the main advantages of applying PNS smears directly onto microscope slides was that the time-consuming laboratory procedures required to process specimens such as NPA specimens were obviated. An air-dried PNS smear takes 10 min to prepare for examination by DIF, compared with 40 min for an NPA smear. The speed in preparing PNS specimens is slightly greater than for Directigen RSV, a new rapid enzyme immunoassay membrane test (Becton Dickinson and Co., Cockeysville, Md.). However, the latter test has the disadvantages of being five times as expensive as DIF reagents at current prices and of giving uninterpretable or false-positive results with excessively bloody specimens (2).

Indirect immunofluorescence using RSV-specific monoclonal sera and an antispecies conjugate was not considered a satisfactory alternative to DIF to be included in this study. Indirect immunofluorescence takes up to 1 h for the two incubation steps, in addition to slide preparation time. In our experience, problems have also arisen from nonspecific fluorescence, which makes rapid microscopy difficult. Cell culture for RSV was also not included in this study because it is slow and expensive and can give variable results (3). Using the same monoclonal antibody pool employed in the present work (formerly Imagen; Boots-Celltech, Slough, United Kingdom) both Cheeseman et al. and Freke et al. observed a sensitivity of 94% for DIF compared with cell culture (4, 6). Both groups detected more RSV infections in patients by DIF than by cell culture isolation. However, there is evidence that an occasional RSV strain may not be detected by using this DIF reagent (4).

Of 84 paired PNS and NPA specimens, 6 PNS specimens failed to detect antigen; this represents a sensitivity for PNS specimens compared with NPA specimens of 93%. Thus, potential false-negative results will occur marginally more frequently with PNS than with NPA specimens. Because of this, further investigation of infants with negative screening results and with continuing symptoms and signs of lower respiratory tract infection may be best achieved by using NPA specimens for DIF testing against a wider range of respiratory viruses (including RSV), together with culture studies.

In the clinical management of RSV infection, a policy of prospective screening, cohort nursing, and appropriate barrier procedures reduces the probability of nosocomial infection (10). One of the main objectives in using a rapid and economical test is to allow comprehensive RSV screening with the appropriate allocation of children within a ward. These results suggest that there is a small chance of an uninfected infant being placed in the RSV cohort if a PNS method of sampling is used. The overall specificity for PNS specimens compared with NPA specimens was 99%.

Results for sequential specimens show that the overall detection rates for NPA and PNS specimens decrease with time after patient admission, but the effect is significantly more pronounced for PNS samples (Fig. 1). The concentration of RSV antigens will naturally diminish with time but will also be reduced by routine therapeutic aspiration of nasal secretions. Nasal sampling is most useful in the screening of RSV infection for acutely presenting children.

In summary, PNS specimens offer a very rapid and convenient procedure for the initial diagnosis of RSV infection, compared with NPA specimens. The likelihood of a false RSV diagnosis with a PNS sample was minimal, especially for patients presenting acutely. Using this simple technique, we were able to detect RSV antigen within 10 min of receipt of a slide in the laboratory. Such rapid diagnosis may be especially valuable in ensuring that all hospitalized children less than 2 years old are allocated to the appropriate cohort during an RSV epidemic. Definite early diagnosis also allows only RSV-positive children to be offered ribavirin therapy.

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