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Comparison between Pernasal Flocked Swabs and Nasopharyngeal Aspirates for Detection of Common Respiratory Viruses in Samples from Children▽

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In this prospective study we compared the use of pernasal flocked swab samples with the use of nasopharyngeal aspirate (NPA) samples for the detection of respiratory viruses from 455 children less than 5 years of age. Overall, the sensitivity and the specificity of the pernasal flocked swab samples were 98.5% and 100%, respectively. The excellent sensitivity of the flocked swab samples in combination with the rapid means by which they may be collected makes them an alternative to NPA samples, whose collection is more invasive.

Specimen collection and transport to the laboratory are two of the pillars for the rapid and accurate diagnosis of respiratory viral infections. Indeed, the identification of respiratory viruses in patient samples is highly dependent on the source of the clinical specimen. Ciliated epithelial cells and cell-free virus have been collected from nasal, throat, and nasopharyngeal swabs; nasopharyngeal aspirates (NPAs); and nasopharyngeal washes (NPWs) for the detection of respiratory viruses (2). It has been well established that NPAs and NPWs are superior to other types of samples for the detection of respiratory viruses since a large number of epithelial cells are aspirated during the collection process (1, 6, 9, 15, 16). However, the NPA and NPW collection process is unpleasant and time-consuming, causes patient discomfort, and requires a suction device, which is not practical in a physician's office setting.

Recently, Copan Diagnostics Inc. introduced flocked swabs, which are designed for the collection of respiratory samples. The flocked swabs utilize an exclusive spray-on nylon flocked fiber technology. The perpendicular nylon fibers act like a soft brush to allow the improved collection and release of patient samples (5).

In the prospective study described here, the performance characteristics of the pernasal flocked swabs was compared to those of NPAs from 455 children hospitalized at Caritas Baby Hospital (CBH) with respiratory tract illness between November 2006 and January 2007. The male/female ratio was 1.6:1, and the patients' ages ranged from few days to 5 years (mean age, 7.4 months). Two samples were collected from the respiratory tract of each patient by well-trained nurses after approval was obtained from the CBH medical ethics team. Pernasal flocked swabs were collected first from the right nostril, after the distance between the patient's nose and ear loop was measured with a disposable cartoon ruler supplied by Copan Diagnostics. Briefly, the patient's head was tilted to a 70° angle before the flocked swab was inserted half the distance to the nasopharynx. Depending on the patient, the flocked swabs were inserted distances that ranged from 2.5 to 6 cm (average, 4.5 cm). The flocked swab was rotated five times before it was pulled out and inserted in 3 ml Copan Diagnostics Universal Transport Medium (UTM). NPAs were collected from the left nostril as described previously (1, 4). Briefly, a soft polyethelyene no. 8 French catheter connected to a disposable aspiration trap was inserted half the distance to the nasopharynx. While intermittent suction was applied to collect mucus and cells, the catheter was gently removed from the nasopharynx. Saline (2 to 5 ml) was suctioned through the catheter into the trap, thereby washing any of the patient sample remaining in the catheter. Both specimens from each patient were sent to the laboratory at room temperature within 30 min of specimen collection.

Specimens collected by both methods were processed in a similar way. Pernasal flocked swabs were removed from the UTM after they were vortexed for 30 s to release trapped cells. The cells were then centrifuged at 300 × *g* for 10 min and washed once with phosphate-buffered saline (PBS). The cell pellets were resuspended in 0.5 ml PBS, and ~20 μl cell suspension was spotted in the wells of an acetone-cleaned glass slide. The slides were air dried before they were fixed in cold acetone for 10 min. The NPAs were vortexed for 30 s and centrifuged at 300 × *g* for 10 min. The cell pellets were washed three times with PBS, and the cells were resuspended in 0.5 ml saline before ~20 μl cell suspension was spotted in the wells of an acetone-cleaned glass slide. The slides were air dried before they were fixed in cold acetone for 10 min. The cell concentrations were adjusted in NPA samples with large amount of secretions. A Light Diagnostics respiratory direct fluorescent-antibody assay (DFA) viral screening and identification kit (Chemicon International; now part of Millipore) was used to stain for common respiratory viruses (influenza A and B viruses; parainfluenza viruses 1, 2, and 3; respiratory syncytial

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TABLE 1. Evaluation of sensitivity, specificity, PPV, and NPV pernasal flocked swab for use for detection of different respiratory viruses by DFA

NPA result[a]	Flocked swab result					
	No. of samples with the following result:		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Positive	Negative				
RSV			98.4	100	100	98.0
Positive	251	4				
Negative	0	200				
Influenza A virus			100	100	100	100
Positive	48	0				
Negative	0	407				
Parainfluenza virus			100	100	100	100
Positive	13	0				
Negative	0	442				
Adenovirus			88.9	100	100	99.8
Positive	8	1				
Negative	0	446				
Total			98.5	100	100	96.3
Positive	320	5				
Negative	0	130				

[a] One NPA sample (0.2%) that was positive for RSV but for which the result was reported to be inadequate for the flocked swabs was considered to have a false-negative result in the calculation for the flocked swabs.

virus [RSV]; and adenovirus), as recommended by the manufacturer. Fluorescein isothiocyanate-labeled antibodies against influenza A and B viruses, parainfluenza viruses (types 1, 2, and 3), RSV, and adenovirus were added to the appropriate wells; and the slides were incubated at 37°C for 30 min in a humid chamber. The slide was then washed with 0.5% Tween 20–PBS for 45 s and then with distilled water washing for 15 s. After the slides were air dried and mounting oil was added, the epithelial cells spotted on each slide were evaluated under a Hund H 600 fluorescent microscope at ×10 magnification. Specimens were reported as inadequate if the number of epithelial cells was less than 20 cells per ×10 field and no positive cells were spotted (14). In order to evaluate the effectiveness of both specimen types, the epithelial cells were enumerated semiquantitately at ×40 magnification according to the following criteria: +1, 1 to 10 cells; +2, 10 to 20 cells; and +3, >20 cells. All slides were screened for positive fluorescing cells at ×10 magnification, and the results were confirmed at ×40 magnification. Positive cells were also enumerated semiquantitately at ×40 magnification according to the following criteria: +1, 0 to 1 cell; +2, 1 to 10 cells; +3, 10 to 20 cells; and +4, >20 cells. All slides were read by two well-experienced medical technologists. DFA staining has previously been shown to have a high sensitivity (>95%) for the detection of influenza A and B viruses, parainfluenza viruses (types 1, 2, and 3), and RSV and a sensitivity of 70% for the detection of adenovirus compared to the results of culture (14). All discrepant results were resolved by the highly sensitive real-time molecular assays, as described previously (11, 13).

Of the 455 samples evaluated, 320 samples collected by both methods were positive, 4 samples were positive by use of the NPAs and negative by use of the pernasal flocked swabs, and 1 sample was positive by use of the NPA but was reported to be inadequate by use of the flocked swabs. The result for this sample was considered false negative by use of the flocked swab. Thus, the overall sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the flocked swabs were 98.5%, 100%, 100%, and 96.3%, respectively (Table 1). The difference between virus detection by use of the pernasal flocked swabs and NPAs was not statistically significant (McNemar's test, $P = 0.0625$).

Stratification of the specimens analyzed by virus type detected also revealed that the results obtained with the pernasal flocked swabs compared well to those obtained with the NPAs. Of the 255 RSV-positive samples detected by the use of NPAs, 251 flocked swab samples were positive and 3 flocked swab samples were negative, while 1 flocked swab sample was inadequate and the result was considered false negative (Table 1). Thus, the sensitivity, specificity, PPV, and NPV of the flocked swabs were 98.4%, 100%, 100%, and 98.0%, respectively (Table 1). The difference between RSV detection by the use of flocked swabs and NPAs was not statistically significant (McNemar's test, $P = 0.125$). Our results are similar to those recently reported by Chan et al., who showed a minimal difference between RSV detection by the use of NPAs and flocked swabs after DFA staining (87.2% and 84.6%, respectively) (3).

Analysis of the three samples with discrepant results for RSV revealed that two samples were weakly positive by use of the NPAs (+1 positivity), while the third sample was strongly positive by use of the NPAs (+4 positivity). The inadequate flocked swab sample was weakly positive for RSV by use of the NPA (+1 positivity). No attempt was made to recollect this sample. Resolution of the discrepant results was performed by quantitative real-time RT-PCR (qRT-PCR). Briefly, viral RNA was extracted by use of a QIAamp viral RNA extraction

kit and was subjected to qRT-PCR, as described previously (11, 13). All three samples with discrepant results were positive for RSV RNA by use of the NPAs and the flocked swabs, indicating that the flocked swabs did not collect enough infected cells to be detected by DFA staining but collected enough cell-free viruses to be detected by the more sensitive qRT-PCR. Henrickson and Hall have previously reported that qRT-PCR is more sensitive than DFA staining for the detection of RSV (10). The sample reported to be inadequate by use of the flocked swab was RSV positive by qRT-PCR by use of the NPA and negative by use of the flocked swab. We predict that the flocked swab failed to collect enough infected cells or cell-free virus because the health care provider did not follow our standard specimen collection protocols.

The rapid and accurate detection of influenza viruses is of utmost importance so that appropriate antiviral therapy may be started and infected patients may be isolated to prevent nosocomial infections (8, 12). All 48 samples found to be influenza A virus positive by the use of NPAs were also found to be positive by use of the flocked swabs. Thus, the sensitivity, specificity, PPV, and NPV of the pernasal flocked swabs were each 100% (Table 1). The excellent sensitivity of the flocked swabs for the collection of influenza A virus-positive cells warrants the future evaluation of the use of flocked swabs for the collection of respiratory samples from patients suspected of being infected with the highly pathogenic H5N1 virus. Our results are different from those in a recent report by Chan et al., who showed that NPAs were more sensitive than the flocked swabs (90.2% and 82.9%, respectively) for the detection of influenza A virus by DFA staining (3). The excellent performance of the flocked swabs for the detection of influenza A virus in our study could be in part due to our nurses' strict adherence to the collection protocols.

The rates of detection of parainfluenza virus types 1, 2, and 3 were similar by use of both the NPAs and the pernasal flocked swabs. Of the 454 samples tested, 13 were positive for parainfluenza viruses by the use of NPAs and flocked swabs. All 10 parainfluenza virus type 3-positive samples and 3 parainfluenza virus type 2-positive NPAs were also positive by use of the flocked swabs, giving the flocked swabs a sensitivity, specificity, PPV, and NPV of 100% each (Table 1).

Of the 455 samples tested for adenovirus, 9 were positive by use of the NPAs, while 8 samples were positive by use of the flocked swabs. Thus, the sensitivity, specificity, PPV, and NPV of the flocked swabs were 88.9%, 100%, 100%, and 99.8%, respectively. Resolution of the sample with a discrepant result was performed by real-time PCR analysis, as described previously (11). Adenovirus DNA was detected only in the NPA, indicating that the flocked swabs did not collect enough cells or cell-free virus for detection by either DFA staining or PCR.

In this study, the flocked swabs compared well with the NPAs for the detection of common respiratory viruses by DFA staining. Additional studies are warranted to determine if similar results would be obtained when samples are tested for respiratory viruses by culture or nucleic acid amplification. Chan et al. recently reported that the sensitivities of the flocked swabs for the detection of influenza A virus and RSV by RT-PCR were 100% and 92.3%, respectively (3).

Semiquantitation of the epithelial cells collected by both methods was performed in order to investigate whether the good correlation between the results obtained with the pernasal flocked swabs and the NPAs was in part due to the ability of the flocked swabs to collect an appropriate number of epithelial cells. While 71% of the NPA samples collected large number of cells (>20 cells/×40 magnification field [+3 positivity]), only 19% of the flocked swabs collected that number of cells. On the other hand, 52% of the flocked swabs but only 15% of the NPA samples collected 10 to 20 cells/×40 magnification field (+2 positivity). Of the samples that had 1 to 10 cells/×40 magnification field (+1 positivity), the NPAs and the flocked swabs collected 14% and 28%, respectively. One sample (0.2%) collected by the flocked swabs was reported to be inadequate. The majority of the NPAs collected more cells than the flocked swabs; however, the pernasal flocked swabs were capable of collecting and releasing enough cells to be detected by DFA staining. Chan et al. also reported that NPAs collected more epithelial cells than the flocked swabs (3).

Semiquantitatation of fluorescing cells infected with the two main viruses in circulation, RSV and influenza A virus, revealed that NPAs collected a higher percentage of strongly RSV-positive cells (+4 positivity) than the flocked swabs (28.4% and 9.2%, respectively). These results are consistent with those from earlier reports that large amounts of RSV are collected by the NPAs (7). On the other hand, the majority (50%) of the NPAs collected influenza A virus-positive cells with +2 positivity, while the majority (43.8%) of the flocked swabs collected cells with +1 positivity.

Overall, the use of flocked swabs for the collection of respiratory specimen was highly recommended by the 40 nurses involved in the study. Thirty-nine nurses (97.5%) recommended switching to the flocked swabs because sample collection by use of the flocked swabs was rapid, less traumatic for the pediatric patients, and did not require a lot of training. Eighteen nurses (45%) noted that the children cried while the flocked swabs were collected, while 36 nurses (90%) noted that children usually cried during the NPA collection process.

The excellent sensitivity and specificity of the pernasal flocked swabs complemented by the superb feedback from the nursing staff make them an alternative to NPAs, for which the methods of collection is more invasive. Use of pernasal flocked swabs can be easily implemented in physicians' clinics or emergency rooms, since the collection process is rapid, not a lot of training of personnel is needed, and no special instrumentation is required.

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