

Comparison of Flocked and Rayon Swabs for Collection of Respiratory Epithelial Cells from Uninfected Volunteers and Symptomatic Patients

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Significantly more epithelial cells were collected by flocked swabs than by rayon swabs in parallel nasopharyngeal and nasal swabs taken from 16 volunteers. Nasopharyngeal sampling of 61 symptomatic patients also yielded more cells by flocked than rayon swabs, providing better clinical specimens for diagnosis.

Acute upper respiratory tract infections are the most common cause of illness in children (3) and in the institutionalized elderly. Timely laboratory diagnosis may allow specific antiviral therapy, avoidance or discontinuation of antibacterial agents, appropriate institutional infection control measures, and improved surveillance. Rapid testing with direct fluorescent antibody (DFA) shortens the time to laboratory diagnosis for upper respiratory viruses, but sampling must maximize the collection of respiratory epithelial cells, since the sensitivity varies with the number of infected cells examined (7).

We reasoned that the quantitation of total and infected respiratory epithelial cells would measure the adequacy of an upper respiratory tract sample but could identify no reports of using quantitative epithelial cell yield as an outcome measure to ascertain the efficacy of sampling or to compare different swab designs. Our primary study objective was to compare total respiratory epithelial cell yield of two swab designs among volunteers and symptomatic patients. Our secondary objectives were to compare nasal versus nasopharyngeal swabs (NS and NPS, respectively) among volunteers and to compare the number and proportion of infected cells between swab types among symptomatic patients.

Sixteen healthy volunteers were recruited from laboratory staff, and four operators were recruited from investigators and trained research personnel. We directly compared a new, flocked swab (Copan Diagnostics, Corona, CA) to our standard rayon swab (Copan Diagnostics) (Fig. 1). The flocked swab contains short nylon fiber strands attached to molded plastic, with a hydrophilic layer of nylon pile that results in the efficient collection and release of particulate matter.

Each volunteer was swabbed four times, using both swab designs in opposite nares in randomized order. The NPS was inserted at a distance equivalent to that from the nares to earlobe, and the NS was inserted 4 to 5 cm. Volunteers assessed discomfort from lowest to highest on a 100-mm visual analog scale (VAS). The VAS consists of a horizontal line, 100 mm long, with “no discomfort” at one end and “the worst imaginable discomfort” at the other. The volunteer marked the

point in the line corresponding to their perception of discomfort. For analysis, the number of millimeters from the “no discomfort” line was measured.

Nurses sampled 61 patients seen at St. Joseph’s Hospital, Hamilton, Ontario, Canada, for respiratory symptoms in January 2005, as part of routine care, using either swab type (flocked or rayon) according to availability. Samples were chosen by stratified systematic sampling from all consecutive NPS submitted to the laboratory during the study month by the two swab types and three etiologic groups: influenza virus positive, respiratory syncytial virus (RSV) positive, or DFA negative (10 samples in each of the six strata for a planned total of 60; 11 samples were obtained in one stratum). Twenty-one were adults over the age of 15 years, and forty were children. Personal identifiers were removed. Patient NPS specimens were collected using either swab according to the availability of each type on each nursing unit. Both types were in use concurrently during the study period. All swabs were placed in Universal Transport Media (Copan Diagnostics) and coded to maintain blinding.

Swabs were processed identically, according to current DFA protocols. After vortexing for 20 s to release the cells, the swabs were discarded. The medium was centrifuged, the pellet was resuspended in 1 ml of buffered saline, and then 25 μ l of suspension was added to the wells on a glass slide. Slides were air dried, fixed, and stained with fluorescein isothiocyanate-labeled monoclonal antibody against seven respiratory viruses and negative control (Diagnostics Hybrids, Inc., Athens, OH). Two independent readers, blinded to swab type, used a fluorescence microscope at $\times 400$ magnification and recorded the number of fluorescent and nonfluorescent cells per high-powered field (hpf).

The mean cell yield and visual analog data were log transformed as required to improve normality and compared by using a two-tailed paired (for volunteer) or unpaired (for patients) *t* test. Cell yield results from symptomatic patients were also adjusted for virus and age group in a multivariable linear regression model (SPSS for Windows 11.5). A *P* value of <0.05 was taken as statistically significant.

Among volunteers (Table 1), the flocked NPS collected significantly more respiratory epithelial cells than the rayon NPS (geometric mean of 58.6 versus 23.9 cells/hpf; *P* = 0.02) or the

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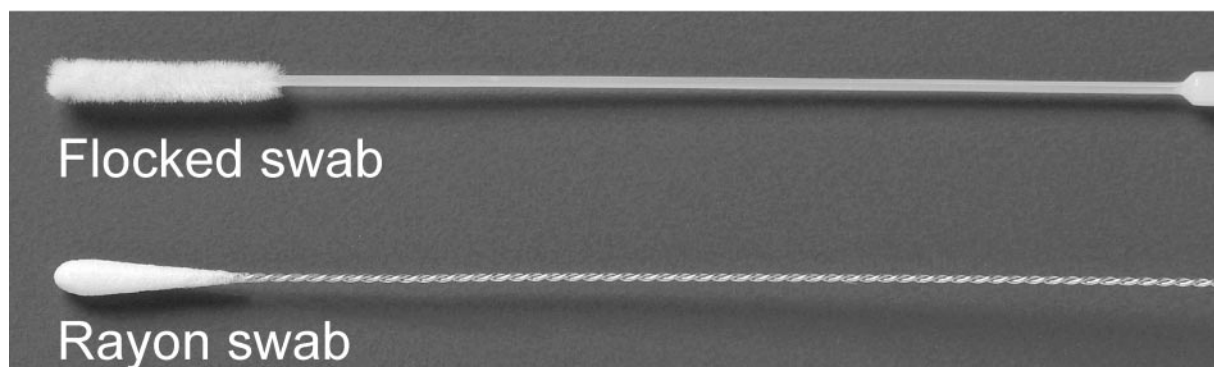


FIG. 1. Flocked and rayon swab designs.

flocked NS (31.3 cells/hpf; $P = 0.03$ for the comparison with NPS). Volunteers found the flocked NPS to be somewhat more uncomfortable than the rayon (VAS = 61.5 versus 43.8 mm; $P = 0.06$), whereas both flocked and rayon NS were rated equally uncomfortable (VAS = 43.4 and 47.8 mm, respectively; $P = 0.45$).

Among symptomatic patients (Table 2), the flocked swab collected a mean of 67.2 respiratory epithelial cells/hpf compared to a mean of 29.3 cells/hpf for the rayon swab, for a mean difference of 42.0 cells (95% confidence interval [CI] = 30.2 to 54.0; $P < 0.001$). Among children, the flocked swab collected a mean of 69.3 cells/hpf versus 21.8 cells/hpf by rayon, for a mean difference of 47.5 cells (95% CI = 30.5 to 64.5; $P < 0.001$). Similarly, among adults, the flocked swab collected a mean of 61.0 cells/hpf compared to 29.4 cells/hpf by rayon, for mean difference of 31.6 cells/hpf (95% CI = 18.3 to 45.0; $P < 0.001$).

The total epithelial cells collected were greater for influenza virus than for RSV, although the number of infected cells was greater for RSV (Table 2). In all cases, cell yields were better for flocked than for rayon swabs. Since we stratified our sampling by viral etiology and swab type, but not by age, there was some imbalance between age category and viral diagnosis, with more influenza virus among sampled adults and more RSV among sampled children. To adjust for this imbalance, we performed a multivariable linear regression. The total epithelial cell yield did not change on adjustment for pediatric status and viral etiology, with an unadjusted mean difference of 42.0 cells/hpf and an adjusted mean difference of 42.2 cells (95% CI = 30.7 to 53.6; $P < 0.001$). The number of infected cells was also greater with the flocked design in both unadjusted (mean difference of 10.6 [95% CI = 3.4 to 17.9]; $P = 0.005$) and

adjusted analyses (mean difference of 10.2 [95% CI = 4.6 to 15.9]; $P = 0.001$). Our study found that the flocked design swab collected significantly more total respiratory epithelial cells among both volunteers and symptomatic patients. This improvement comes without a significant increase in subject discomfort.

An ideal swab design collects many cells and allows for their release into media. Our method of counting cells on DFA slides offers a simple comparison of swab efficiency. Because DFA sensitivity depends on the detection of fluorescent cells, a larger sample available for staining increases the probability that fluorescent cells will be detected. The importance of cell yield may be even more important for rapid antigen or nucleic acid kits. Our results here apply only to intracellular virus detection, whereas the detection of extracellular virus may also be pertinent for PCR detection. Our recent comparison of flocked versus kit swabs for the detection of *Chlamydia trachomatis* suggests that the flocked swab adsorbs and releases both cellular and cell-free material more effectively than comparator swabs (2).

Several reports (5, 6, 8, 9) have suggested that sensitivity increases when the nasopharynx is sampled instead of the nasal cavity. Our data would agree that deeper sampling yields more cells. Deeper insertion of swabs increased discomfort in previous reports (9), but we did not observe this.

We did not examine nasopharyngeal aspirates (NPA), since our laboratory does not receive a significant number of NPA samples. We have previously demonstrated equivalent positivity rates between NPA and NPS (4), although several other studies have shown differences (1, 6, 9). Swab sampling may be preferable to aspirate due to lower cost, less mucus contamination, and the lack of availability of a suction apparatus in many clinical settings.

We observed a cell yield among flocked NS that was similar to that from rayon NPS, indicating that flocked NS may offer an adequate yield. Although the flocked NPS has become our specimen type of choice for diagnosis in individual symptomatic patients, the less-invasive flocked NS technique may be preferred in other settings, such as outbreak investigation, or among neonatal or long-term care settings where the deeper sample may be more difficult to collect.

In summary, the new flocked swab design yielded significantly more total respiratory epithelial cells and more infected respiratory epithelial cells. This two- to threefold increase in

TABLE 1. Mean respiratory epithelial cell yield among volunteers sampled by collecting NPS and NS using flocked or rayon swabs^a

Swab type (n)	Geometric mean no. of respiratory epithelial cells/hpf (95% CI)		P
	Flocked swab (P = 0.03)	Rayon swab (P = 0.38)	
NPS (15)	58.6 (45.7–75.1)	23.9 (13.2–43.5)	0.02
NS (16)	31.3 (20.1–48.6)	15.2 (9.1–25.5)	0.03

^a P values were determined by using a paired t test on log-transformed values. n, number of volunteers swabbed.

TABLE 2. Mean of total and infected respiratory epithelial cells from nasopharyngeal samples collected by flocced and rayon swabs

Type of viral infection (n) ^a	Total no. of cells/hpf (95% CI)		No. of infected cells/hpf (95% CI)	
	Flocced swab	Rayon swab	Flocced swab	Rayon swab
Influenza A virus (20)	67.2 (55.6–78.8)	29.3 (19.9–38.7)	15.8 (9.7–21.9)	7.2 (3.6–10.8)
RSV (21)	51.7 (36.9–66.6)	19.6 (12.9–26.3)	32.6 (18.7–46.7)	11.0 (6.1–15.9)
DFA negative (20)	82.4 (53.1–112.0)	24.8 (15.2–34.4)	0	0

^a n, number of samples.

cell yield with the flocced design is likely to have a greater effect on diagnostic sensitivity than the differences in sensitivity between test designs.

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