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NOTES

Comparison of Respiratory Virus Detection Rates for Infants and Toddlers by Use of Flocked Swabs, Saline Aspirates, and Saline Aspirates Mixed in Universal Transport Medium for Room Temperature Storage and Shipping[▽]

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A nylon flocked swab/universal transport medium collection method developed for bacterial sexually transmitted infections was adapted to detect respiratory viruses in infants and toddlers. This method significantly outperformed the traditional use of nasal aspirates in terms of PCR-based virus detection ($P = 0.016$), and the samples were easier for clinicians to evaluate, store, and transport.

Collection of nasal secretions from infants and toddlers for viral testing is typically performed using the nasal saline aspirate (NA) technique described by Hall and Douglas in 1975 (7). This technique is less invasive and performs similarly to nasopharyngeal aspirates for its most common indication, respiratory syncytial virus (RSV) testing (1). However, NA sampling tends to be highly variable in terms of the volume and material collected and is prone to rapid viral RNA degradation. This method is further complicated by the need to divide specimens into aliquots and freeze them to −70°C when immediate PCR analysis is not available. This additional manipulation, coupled with the need for dry ice shipping, adds extra costs and may potentially increase the number of false-negative results, thereby decreasing the usefulness of PCR-based testing and compromising clinical decision-making.

Nylon flocked swabs (NFS), in combination with universal transport medium for room temperature storage and shipping (UTM-RT; Copan Diagnostics, Inc., Murrieta, CA), are a proven collection and transport method for bacteria responsible for sexually transmitted infections. This method also allows for room temperature storage and shipping (3). RSV remains viable in UTM-RT for up to 96 h (2) and, in this regard, has been reported as superior to other transport media (9). Adapting the NFS/UTM-RT collection method to nasal secretion sampling would provide a better method of nasal secretion sampling.

Our primary hypothesis was that nasal secretions collected using NFS stored in UTM-RT would lead to a higher PCR

TABLE 1. Detection rates for individual viruses by each method[a]

Sample type	No. of samples (%) positive for:							
	RSV A and B	RSV A only	RSV B only	Influenza viruses A and B	Influenza virus A only	Influenza virus B only	hMPV	At least one virus
Unpreserved saline wash	31 (17.1)	30 (16.6)	2 (1.1)	12 (6.6)	7 (3.9)	5 (2.8)	28 (15.5)	64 (35.4)
UTM-RT/saline wash	43 (24)	42 (23.2)	6 (3.3)	10 (5.5)	7 (3.9)	3 (1.6)	29 (16.0)	77 (42.5)
Flocked swab in UTM-RT	49 (27.1)	47 (26.0)	8 (4.4)	12 (6.6)	7 (3.9)	5 (2.8)	25 (13.8)	82 (45.3)

[a] Because more than one virus may be detected per patient, the last column is not the sum of the others $n = 181$.

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TABLE 2. Sensitivity and specificity for each virus using each method[a]

	Unpreserved saline aspirate		UTM-RT preserved saline aspirate		Flocked swab in UTM-RT	
	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
RSV (A or B)	59 (42–74)	95 (90–98)	98 (87–100)	98 (94–100)	90 (77–97)	91 (86–96)
RSV A only	60 (43–75)	96 (91–98)	98 (87–100)	98 (94–100)	90 (76–97)	92 (86–96)
RSV B only	20 (1–72)	99 (97–100)	100 (48–100)	99 (97–100)	100 (48–100)	98 (95–100)
Influenza virus (A or B)	25 (5–57)	98 (94–99)	70 (35–93)	100 (98–100)	58 (28–85)	100 (98–100)
Influenza virus A only	43 (10–82)	98 (94–99)	100 (59–100)	100 (98–100)	100 (59–100)	100 (98–100)
Influenza virus B only	TFTA	TFTA	TFTA	TFTA	42 (15–72)	100 (98–100)
hMPV	93 (76–99)	98 (94–100)	100 (87–100)	99 (95–100)	93 (76–99)	100 (98–100)
At least one virus	71 (59–81)	88 (81–94)	99 (93–100)	95 (88–98)	92 (83–97)	85 (77–91)

[a] Test characteristics assuming a true positive is a case positive by at least two of the three methods. TFTA, too few positives to assess.

detection rate of RSV A and B, influenza viruses A and B, and human metapneumovirus (hMPV) for children younger than 18 months than would traditional nasal aspirates. To test this, we obtained both NFS and NA samples from alternate nostrils (order and side randomized) and compared the detection rates. Our secondary hypothesis was that nasal aspirates stored in UTM-RT would have a higher PCR viral detection rate than unpreserved saline nasal aspirates. To test this, we evaluated detection rates from NA samples and NA samples diluted 1:1 in UTM-RT.

The Kern Medical Center Institutional Review Board approved the study design. After we obtained parental consent, 188 children younger than 18 months were enrolled in the study. Seven nasal aspirate specimens were omitted due to insufficient volume, reducing the total to 181 patients. Initially, samples were collected using minitip flocked swabs (Copan Diagnostics, Inc.) typically used for urethral sampling and a disposable paper ruler to achieve a standardized method of sample collection. During the study, the urethral flocked swab was replaced with a specifically designed swab that incorporated a guard 2.5 cm deep to ensure proper sampling of the mid-turbinate region. Transition from the urethral swab to the flocked nasal swab during the course of the study did not significantly alter the experimental findings. The specifically designed swab, now commercially available, showed a nonsignificant trend toward increased virus detection; the nasal and urethral swabs detected a virus in 49/76 (64%) and 52/103 (50%) of the samples collected, respectively (two swab types were not recorded; $P = 0.06$).

NAs and UTM samples were stored at −70 and −20°C, respectively. Viral RNA was extracted with a QIAamp viral RNA purification kit (Qiagen, Valencia, CA) and tested by reverse transcriptase conventional PCR (RSV A and RSV B) or reverse transcriptase real-time PCR (hMPV, influenza virus A, and influenza virus B). All assays were optimized for specific genes and validated in-house for sensitivity, specificity, interference, accuracy, and precision. The stability of each pathogen in UTM-RT was evaluated, replicating the transportation of the sample from the time of collection to the time of extraction and amplification. Viruses were detected in 82, 77, and 64 of the NFS, nasal aspirate/UTM-RT mix, and unpreserved NAs, respectively (Table 1).

We defined a true positive as a positive result by at least two of the three collection methods; therefore, 72 samples were considered true positives. The sensitivity and specificity for the flocked swabs were 91.7% (95% confidence interval [CI] = 82.7 to 96.9%) and 85.3% (95% CI = 77.3 to 91.4%); for nasal aspirates preserved in UTM-RT, they were 98.6% (95% CI = 92.5 to 100%) and 94.5% (95% CI = 88.4 to 98%); and for unpreserved saline, they were 70.8% (95% CI = 58.9 to 81%), and 88.1% (95% CI = 80.5 to 93.5%), respectively. The detection rates for each virus utilizing each of the collection methods are presented in Table 1, while the sensitivity and specificity for each virus using each collection method are reported in Table 2. When virus detection was directly compared, the flocked swab performed significantly better than unpreserved saline ($P = 0.016$), as did the nasal aspirate/UTM-RT mix ($P = 0.032$); there was no statistically significant difference between the flocked swab and nasal aspirate/UTM-RT mix ($P = 0.312$).

Previous comparisons of Dacron and rayon swabs to NAs for influenza antigen detection have favored NAs (5). Alginate swabs inhibit PCR-based detection methods (4). Swabs are nonetheless a more intuitive and more standardized collection method. Using NFS combined with UTM-RT distinguishes our experiment from prior work since NFS offers greater sample release than do spun fiber swabs (6).

We also found that nasal aspirates stored in UTM-RT led to greater detection of viruses than did unpreserved nasal aspirates. UTM-RT prolongs pathogen viability; UTM-RT has been shown to maintain RSV viability for up to 96 h (2, 8). Although more viruses were detected using the flocked swab than NA in UTM-RT, this difference was not statistically significant. Our study demonstrates that NFS and UTM-RT preservation of nasal aspirates leads to improved viral detection rates and increased pathogen stability compared to traditional NAs.

Nasal secretions collection using NFS or NAs stored in UTM-RT leads to greater detection of viral respiratory pathogens by PCR, particularly RSV, for children up to 18 months of age than does collection using unpreserved NAs.

NFS, UTM-RT, and collection tubes were provided by Copan Innovations, who had no control over the results.

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