Comparison of Three Nasal Collection Specimen Methods for the Detection of Pediatric Respiratory Infectious Disease

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AMENDED ABSTRACT

Background: A prospective controlled trial study was performed to determine the percent agreement between three methods of nasal specimen collection: (1) standard anterior nasal wash, (2) nasal wash transported in UTM-RT media, and (3) anterior nasal swab performed with a flocked swab. Detection of pathogens by PCR was used to validate each method.

Methods: Informed consent was obtained. Children up to 18 months of age with a clinical indication of viral infection or clinical diagnosis of bronchiolitis were selected for inclusion. A flocked swab was inserted to a depth of 25 mm and transferred to a tube containing 1 milliliter (mL) of UTM-RT media (swab and UTM-RT media from Copan Diagnostics, Corona, CA). Nasal washes were performed on the opposite nostril. For comparative studies, nasal washings were either diluted 1:1 in UTM-RT media, whereby 0.5 mL of wash was placed in 0.5 mL of UTM-RT, or submitted directly for analysis. The side and order in which the samples were obtained were randomly assigned. All samples were stored at 4°C prior to freezing at -70°C. Aliquots of the UTM-RT media and nasal wash samples were extracted for RNA (Qiagen QIAmp viral RNA Isolation Kit) and analyzed for the presence of RSV A, RSV B, hMPV, INF A, and INF B by reverse transcriptase treatment followed by RT qPCR or conventional PCR and confirmation by sequencing the amplicon.

Results: A total of 181 specimens were analyzed. Of the three methods, those using UTM-RT media resulted in the highest sensitivity of detection. Flocked swab and nasal wash/UTM-RT methods to test RSV A proved to be more sensitive detecting 36/181 (90% sensitivity; ROC 0.911) and 39/181 (97.5% sensitivity; ROC 0.977), respectively. Nasal washings alone proved to be the most ineffective means of sampling having incorrectly reported 16 specimen as negative for RSV A and a sensitivity of 60% (ROC 0.779). Specificity for flocked swab, nasal wash/UTM-RT and saline wash were 92.2%, 97.9%, and 95.7%, respectively. Testing of RSV B and human metapnuemovirus with the flocked swab had sensitivity rates of 100% and 92.6%, respectively compared to the saline wash method resulting in sensitivity of 20% for RSV B and 92.6% for hMPV.

Conclusion: Transport of collected specimens in UTM-RT resulted in a greater stability of pathogen than that obtained without UTM-RT. Analyses of these collection methods were evaluated for RSV A, RSV B, hMPV, and Influenzae A & B. The results indicate that assays using the flocked swab and/or UTM-RT/wash had significantly higher sensitivity and specificity than using the saline wash method.

BACKGROUND

Collection of nasal specimens via anterior nasal wash has been a standardized method for testing respiratory viruses. However, the saline wash is unpreserved and requires storage at -70ºC for batch PCR processing. Nasopharyngeal swabs are invasive and often diagnostic sensitivity is compromised. A new type of swab, the flocked swab, is designed to maximize the collection of epithelial cells and surrounding pathogens thereby improving the rate of detection. This study evaluates the percent agreement between three methods of nasal specimen collection: (1) standard anterior nasal wash, (2) nasal wash transported in UTM-RT media, and (3) anterior nasal swab performed with a flocked swab.

MATERIALS & METHODS

Patient Selection and Collection Method:
Informed consent was obtained from a parent or guardian. The Institutional Review Board for Kern Medical Center, Bakersfield, CA approved this study. Patients with clinical indication of RSV were enrolled for specimen collection. Patients were subjected to three forms of collection methods: (1) a flocked swab was inserted 25 mm inside the nares and then placed in 1 ml of UTM-RT **(Figure 1),** (2) from the opposite nostril, nasal washing with saline solution was performed **(Figure 2),** and (3) 0.5 ml of the nasal washing was placed in 0.5 ml of UTM-RT. Collection by either method was randomized for which nostril was used.

Transport and Nucleic Acid Extraction:
Specimens were frozen and shipped to the laboratory for analysis on dry ice. Upon thawing, aliquots of the UTM-RT were extracted for DNA (Corbett Robotics X-Tractor Gene) and RNA (Qiagen QIAmp Viral RNA Isolation Kit). Prior to DNA extractions, specimens were spiked with an unrelated, non-cross-reactive virus which was assayed for by real-time PCR prior to the actual respiratory pathogen testing. This step verified a successful DNA extraction procedure and a lack of PCR inhibitors. RNA was assayed by reverse transcriptase conventional PCR (RSV A, RSV B) and reverse transcriptase followed by real-time PCR for hMPV, INF A, and INF B.

Assay Optimization:
Each of the respiratory pathogen assays were developed and validated in-house. Specific genes were targeted for each assay design and thorough validation tests were performed to determine the sensitivity, specificity, and accuracy against the pathogen being tested. Standards were created by PCR amplification of known positives obtained from ATCC. The amplified products were subcloned into a vector backbone. Plasmid preparations were created for use as DNA standards. RNA was generated by transcriptional run-off procedures and purified using the RNeasy method (Qiagen,Valencia, CA).

Sensitivity:
Sensitivity was determined by testing serial dilutions of plasmid preparations or RNA transcriptional run-off of each pathogen assay. Serial dilutions tested in triplicate ranged from 10^5 to 10 copies/reaction with sensitivities for each assay determined to be 10^2 copies/reaction or lower.

Specificity:
The primers and probes in all assays used were cross-referenced by BLAST analysis against all DNA sequences deposited in the Entrez nr database. Analytical specificity was determined by testing for amplification in the presence of DNA and RNA extracted from 52 known bacterial, fungal, and viral pathogens purchased from ATCC. All primers and probe showed 100% specificity for the pathogen against which they were designed with no cross-reactivity with irrelevant pathogens.

Interference:
The addition of 400 ng of DNA or RNA extracted from a patient sample that had tested negative for the pathogen in question was used to examine for potential interference. The exogenous DNA or RNA was added to a dilution of plasmid or RNA transcriptional run-off and was found to have no deleterious effects on the detection of individual pathogens or the sensitivity of the individual assays.

Stability:
Stability testing was performed over five days in triplicate to test the stability of several pathogens in the nasal swabs. Each stability test was completed by the spiking of unextracted pathogen purchased from ATCC into a previously tested negative nasal swab sample. Stability testing was evaluated from Day 0 through Day 5 with storage at room temperature and 4ºC. Each sample was extracted for DNA or RNA and then stored at -20ºC until the final time point extraction. At that time, all sample days were tested with the appropriate test in triplicate.

Statistical Analysis:
Sensitivity, specificity and area under the curve were determined using STATA 9.2 (StataCorp, College Station, TX). Samples were deemed positive if they were called positive by at least two of the three collection methods tested.

RESULTS

Infants and toddlers up to the age of 18 months were selected for this study. Figure 3 represents the age distribution of the 181 subjects who comprised the screening panel. The positive detection rate of two of the three different collection methods were found to be fairly similar between nasal washing mixed in UTM-RT (1:1) and flocked swab. RSV A had a positive detection rate of 39/181(21.5%) in saline/UTM-RT mix and 36/181(19.9%) in flocked swab. The saline wash alone had a positive detection rate of 24/181 (13.3%), more than 5% lower than what was found in the flocked swab and saline mixed in UTM-RT. The positive detection rate for RSV B was similar between the three collection methods. However, sensitivity of RSV B in flocked swab was 80% higher than that in saline wash alone and matched the sensitivity rate for the UTM-RT/wash. Human metapnueumovirus also had similar positive detection rate but, unlike RSV B, the sensitivity rates correlated between the flocked swab and the saline wash, and an 8% decrease in sensitivity compared to the UTM-RT/wash. Influenzae A and B viruses had the same positive detection rate with the flocked swab and UTM-RT/Wash method, but saline wash had significantly lower positvity rate. However, the sensitivity rate varied with the UTM-RT/wash as the highest, followed by the flocked swab then the saline wash alone. A summary of results is presented in Table 1. Statistical analysis was performed where any positive by two of the three different methods was determined to be a true positive. The sensitivity and specificity of each method tested is represented in Table 2.

Figure 1. Collection tools used for nasal swab.

Figure 2. Collection tools used for nasal washing.

Figure 3. Histogram of age distribution of patients.

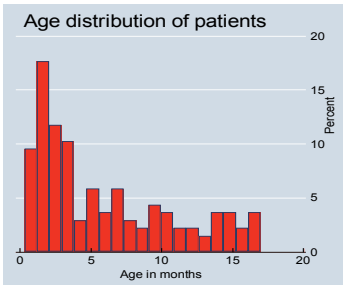

Table 1. Positive Detection rate by 3 different collection methods.

Virus	Flocked Swab (+)	Saline (+)	UTM-RT/Wash (+)
RSV A&B	37 (20.4)	24 (13.3)	40 (22.1)
RSV A	36 (19.9)	24 (13.3)	39 (21.5)
RSV B	5 (2.8)	1 (0.6)	5 (2.8)
hMPV	25 (13.8)	25 (13.8)	27 (14.9)
INF A&B	7 (3.9)	3 (1.7)	7 (3.9)
Any Virus	66 (36.5)	51 (28.2)	71 (39.2)

Table 2. Sensitivity and Specificity for each collection method.

Method	Flocked Swab		Saline		UTM-RT/Wash	
Virus	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
RSV A&B	90.2	91.4	58.5	95.0	97.6	97.9
RSV A	90.0	92.2	60.0	95.7	97.5	97.9
RSV B	100.0	98.3	20.0	99.4	100.0	99.4
hMPV	92.6	100.0	92.6	98.1	100.0	98.7
INF A&B	58.3	100.0	25.0	97.6	70.0	100.0
Any Virus	91.7	85.3	70.8	88.1	98.6	94.5

DISCUSSION

From this study, considerable agreement in positive detection rate, sensitivity and specificity were found between nasal washing mixed in UTM-RT and flocked swab with UTM-RT collection methods, with the exception of Influenzae A and B viruses. Overall, saline wash alone was significantly lower in sensitivity and specificity than nasal washing mixed in UTM-RT and flocked swab. In regards to sensitivity and specificity, concordance between saline/UTM-RT and flocked swab was the highest for RSV-B, followed by RSV A and hMPV.

It appears in this study that specimen collection, in combination with UTM-RT storage, stabilizes the pathogens allowing for better, more exact downstream analyses. Both methods employing the use of UTM-RT in their collection protocols faired much better in downstream respiratory tract infection pathogen detection assays than did the traditional saline wash with freezing method alone.