Nylon Flocked Swabs Versus Rayon Swabs for Respiratory Viral Recovery of Pediatric Nasopharyngeal Specimens: Which Is Better?

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Abstract

Background: When diagnosing respiratory viral illness using nasopharyngeal swabs, specimen quality has always been a concern for the Clinical Virology laboratory and the clinician. The gold standard, the M4 swab, has been used as the universal collecting device. A new flocked swab designed to optimize both the collection and transport of respiratory samples appears to yield more virus. Increased viral recovery suggests we may be able to identify more patients with influenza and respiratory syncytial virus (RSV).

During the 2006-2007 influenza season we prospectively enrolled infants and children up to 18 years of age admitted to the Cleveland Clinic Children's Hospital with respiratory symptoms. After informed consent each patient underwent nasopharyngeal swabbing with the rayon MicroTest M4 swab (placed in M4 transport medium) and the Flocked swab (in Copan universal transport medium). Clinical information was also collected at the time of enrollment such as age, sex, presence of cough, wheezing, nasal congestion, fever and rhinorrhea.

Objective: To evaluate the viral recovery from nasopharyngeal swabs for influenza A, influenza B, RSV, and human metapneumovirus (hMPV) comparing the new nylon flocked swab (COPAN Diagnostic Inc., Corona, CA) to the standard MicroTest M4 swab (Remel, Lenexa, KS) in symptomatic pediatric patients admitted to the hospital.

Materials and Methods: Sixty nasopharyngeal respiratory specimens were collected from 30 symptomatic children admitted to the hospital and extracted by Easy-Mag (NucliSENS®, BioMerieux Inc, Durham, NC) and tested by Real Time-PCR (RT-PCR) and direct fluorescence assay (DFA). ProFlu-1 and Pro hMPV assays (Prodesse, Inc., Waukesha, WI) are one-step RT-PCR assays based on: nucleic acid extraction, reverse transcription to generate complementary DNA (cDNA) from target RNA, and amplification and detection of target cDNA by using specific primers and probes. RT-PCR was performed using Rotor-Gene (Corbett Inc, San Francisco, CA) and DFA was performed using reagents from Diagnostic Hybrids per standard protocol.

Results: Fifty-six nasopharyngeal swabs (56 M4, 56 flocked) were tested by RT-PCR and DFA. M4 and flocked swab results were compared. Preliminary results of RT-PCR and DFA showed 18 out of 56 patients (32.0 %) were positive for respiratory viruses (5 RSV, 3 influenza A, 5 influenza B, and 5 hMPV). RT-PCR appears to be more sensitive than DFA as DFA only detected 13 of 56 positive samples. M4 and flocked swabs showed comparable results.

Conclusion: Although our preliminary numbers are small, at this point we are unable to show increased viral recovery with the flocked swab. Additional study enrollment is currently ongoing. However, M4 and flocked swabs both detected the same number of positive symptomatic patients from the nasopharyngeal samples.

Aim

To evaluate and compare the new nylon flocked swabs (Copan Diagnostic Inc., Corona, CA) with rayon swabs (Remel Inc, Microtest M4, Lenexa, KS) for the detection of influenza A, influenza B, and Respiratory Syncytial Virus (RSV) using Real-time PCR (RT-PCR) and direct fluorescent assay (DFA) in symptomatic pediatric patients admitted to the hospital. A small percentage of the samples were also tested for the presence of human metapneumovirus (hMPV). This study was approved by the Cleveland Clinic Institutional Review Board.

Materials and Methods

Hospitalized pediatric patients and their parents were approached between 8AM – 10AM Monday to Friday for potential enrollment in our study. Informed consent was obtained and nasopharyngeal (NP) sample collection was performed by a study nurse, adult or pediatric infectious disease practitioner. All samples were collected in a similar manner. Rayon M4 and nylon flocked swabs were used together side-by-side to obtain the NP samples. Each swab was then placed in a separate viral transport medium (M4 swabs in M4 transport medium; flocked swabs in Copan Universal Transport Medium [UTM]) and sent to the Clinical Microbiology Lab for processing and testing.

One hundred twelve nasopharyngeal swabs were collected from fifty-six symptomatic children and adolescents (two swabs per patient) admitted to the Cleveland Clinic Children's Hospital and transported to the laboratory.

Four hundred microliters (400 ul) of each viral transport medium was used for nucleic acid extraction with EasyMag (NucliSENS®, BioMerieux Inc, Durham, NC)) and tested by RT-PCR. The remaining viral transport medium from the M4 and UTM was used to prepare DFA slides.

ProFlu-1 and Pro hMPV assays (Prodesse, Inc., Waukesha, WI) are one-step RT-PCR assays based on: nucleic acid extraction, reverse transcription to generate complementary DNA (cDNA) from target RNA, and amplification and detection of target cDNA by using specific primers and probes. RT-PCR was performed using Rotor-Gene (Corbett Inc, San Francisco, CA) and DFA was performed using reagents from Diagnostic Hybrids per standard protocol.

Viral Transport Medium (56 M4 and 56 UTM)

Transported to the Lab via Pneumatic Tube System (10 – 11 AM)

400 ul each for N.A. extraction (EasyMag)

1ml of VTM used to prepare DFA slides

56 M4

56 UTM

56 M4 slides

56 UTM slides

56 ProFlu-1 & 13 Pro–hMPV RT-PCR

Flu A, Flu B & RSV + hMPV results

Flu a, Flu B, RSV and hMPV results

Results

One hundred twelve nasopharyngeal swabs (56 rayon M4 and 56 nylon flocked) were tested for influenza A, influenza B and RSV by RT- PCR and DFA (Diagnostic Hybrids Inc., Athens, OH). A small number of the samples were also analyzed for hMPV (Pro-hMPV RT-PCR and Pro-hMPV DFA).

Type of swabs	Test methods	Flu a	Flu B	RSV	hMPV
Nylon/UTM	RT-PCR	3/56	5/56	5/56	1/13*
	DFA	1/56	3/56	5/56	5/56
Rayon/M4	RT-PCR	3/56	5/56	5/56	2/13†
	DFA	1/56	3/56	5/56	5/56

* Only 13 were tested. 1/13 had no IC.; †Only 13 were tested.

Summary:

* RT-PCR: 13/56 = 23.2 % Positive rate (for influenza A, B & RSV)

* DFA: 9/56 = 16.1 % Positive Rate for DFA (same 3 viruses).

** hMPV by DFA: 5/56 = 8.9% Positive rate.

Conclusions

1. Results from this small study showed that both Flocked & Rayon swabs performed equally well.
2. In this study, the specimens were collected simultaneously by inserting both swabs side-by-side in the same nostril. It is likely that the additional cells/virus collected by the flocked swab bristle with the capillary action was transferred to the adjacent hydrophilic rayon swabs. Thus we observed equivalent results in our study. We will continue and expand this study for the next respiratory season by including separate swab collections.
3. No inhibitory effects observed on the Real-time PCR reactions with either Flocked or Rayon swabs.
4. Positive rate of the four viruses (Flu A, Flu B, RSV and hMPV) from our study is 32.1%. We also detected an 8.9% positive rate of hMPV in our hospitalized pediatric patients.