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Original Research

Comparison of Nasal and Nasopharyngeal Swabs for Influenza Detection in Adults

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Objective: Examine differences in the detection of influenza by specimen and test type using paired nasal and nasopharyngeal swabs.

Design: Prospective study

Setting: Enrollment took place between January and March 2007 in a central Wisconsin population.

Participants: Adult patients were screened and enrolled by trained research coordinators following medical encounters for acute respiratory illnesses of <10 days duration.

Methods: Paired nasal and nasopharyngeal swabs were collected from consenting patients and tested by both real-time reverse transcriptase polymerase chain reaction (rRT-PCR) and viral culture. A composite measure of positivity was used as the gold standard; cases included any positive result by rRT-PCR or viral culture from either specimen type.

Results: Paired samples were collected from 240 adults; 33 (14%) individuals tested positive for influenza by rRT-PCR. Using rRT-PCR, the sensitivity of the nasal swab was 89% (95% CI, 78%-99%) and the sensitivity of the nasopharyngeal swab was 94% (95% CI, 87%-100%), compared to a composite gold standard.

Conclusion: Test sensitivity did not vary significantly by swab type when using a highly sensitive molecular diagnostic test, but power was limited to detect modest differences.

Keywords: Detection; Influenza virus; Specimen type

Influenza is a major cause of acute respiratory illness worldwide and accounts for thousands of deaths in the United States in a typical season.[1,2] The 2009 pandemic and increasing type-specific antiviral resistance have heightened the need for influenza testing that is accurate, timely, and well-tolerated by patients.[3,4]

A variety of specimens have been used for influenza testing including the nasopharyngeal (NP) swab, oropharyngeal swab, nasal wash, and nasal aspirate. The NP wash or aspirate is generally considered the 'gold standard' for virus isolation, but it is cumbersome to perform and unpleasant for patients.[5-7] Swabs are easier and faster to collect and may be preferred by providers and patients. Comparative data on the sensitivity of influenza sampling procedures are limited; many studies focus on pediatric populations or use older diagnostic methods.[5,8-12] Recent literature has focused on NP sampling compared to oropharyngeal or combined nose-throat swabs.[6,7,10,12] To date, no studies have compared paired nasal and NP swabs collected from adults.

Several methods for laboratory diagnosis of influenza are available. Viral culture has historically been considered the 'gold standard' diagnostic test, but traditional culture can

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require up to 7 days to obtain a positive result.[5] Recent findings from studies utilizing real-time reverse transcriptase polymerase chain reaction (rRT-PCR) for the detection of respiratory pathogens suggest that the use of this current molecular technology may outweigh potential differences in sensitivity due to specimen type.[7,10]

We conducted a prospective study to examine differences in the detection of influenza by specimen type. Paired nasal and NP swabs were tested by both viral culture and rRT-PCR. Study procedures were reviewed and approved by the Marshfield Clinic Institutional Review Board, and all participants gave written informed consent for influenza testing.

Methods

Participants

Enrollment in the study took place between January and March 2007. Adult patients were enrolled by study staff following a medical encounter for acute respiratory illness. Eligible illnesses were <10 days duration and included fever, chills, or cough.[13]

Clinical Specimens

Both shallow nasal and NP swabs were collected from consenting patients. The NP swab was collected using an aluminum/plastic unishaft swab, inserted half the distance from the nares to the base of the ear, or to a depth of approximately 2 inches (Remel, Thermo Fisher Scientific, Lenexa, KS).[14]

As it was less invasive, the nasal swab was collected first, using a large tipped, plastic shafted Dacron swab. The swab was inserted approximately 1 centimeter, rubbed along the septum of the nostril for 3 to 5 seconds, and withdrawn. The swab was then placed in M4-RT viral transport media for testing. The more invasive NP swab was then collected using a smaller Dacron swab on a wire shaft. As per manufacturer's protocol, the swab was inserted to the point where the wire shaft meets the plastic sleeve, rotated, and withdrawn by scraping the septum.[14] The wire-shafted swab was then placed in a separate M4-RT viral transport tube for testing. All samples were refrigerated for <24 hours until aliquots could be taken, which were then frozen until testing.

Diagnostic Testing

Real-time reverse transcriptase polymerase chain reaction (rRT-PCR) and viral cultures were performed at the Marshfield Clinic Research Foundation. rRT-PCR was performed on nucleic acid extracts from 200 µl of clinical sample using the LightCycler Real-Time PCR System (Roche Diagnostics, Indianapolis, IN), and Invitrogen SuperScript III Platinum One-Step Quant RT-PCR chemistry (Life Technologies, Grand Island, NY). All rRT-PCR protocols, primers, and probes are property of and were provided by the Influenza Division of the Centers for Disease Control and Prevention (CDC) (protocols available from the CDC on request).[15] The assay was a TaqMan based, real-time detection of the matrix 1 protein (M1) of influenza A and the non-structural protein 1 (NS1) of influenza B; the sequences of both proteins are highly conserved. The human RNase P gene primer and probe set served as an internal positive control for human nucleic acids.

Viral culture was performed using Madin-Darby canine kidney (MDCK) shell vial (Diagnostic Hybrid, Athens, OH). Cells were inoculated with 200 µl of specimen and 1 ml of culture media. Inoculated shell vials were centrifuged for one hour at 2000 rpm to enhance viral contact and more rapid infection of the MDCK cells. The vials were incubated at 35° to 37°C. Cultures were microscopically examined daily for cytopathic effect (CPE). When CPE was observed or after 5 days of incubation with no CPE, the shell vial monolayer was scraped, and a slide was prepared and stained for Influenza A and B immunofluorescent identification (D^3 Influenza A/ Influenza B DFA Kit, Diagnostic Hybrid, Athens, OH).

Statistical Analysis

Sensitivities and 95% confidence intervals (CI) were calculated from two-by-two tables. Sensitivity was calculated as compared to a composite gold standard, which included any positive result by rRT-PCR or viral culture from either specimen type. Sensitivities were compared using chi-square; *P* values <0.05 were considered statistically significant. Data analysis was performed using SAS 9.2.

Results

Paired nasal and NP swabs were collected from 240 patients. The median age of the patients was 60 years (range 47 to 91

Table 1. Comparison of the sensitivity of nasal and nasopharyngeal swab specimens for the detection of influenza by viral culture and rRT-PCR.*

	Nasal swabs			Nasopharyngeal swabs			
	No. positive samples	Sensitivity (%)	95% CI	No. positive samples	Sensitivity (%)	95% CI	*P* value†
Viral culture	14	40.0	23.8-56.2	18	51.4	34.9-68.2	0.34
rRT-PCR	31	88.6	78.0-99.1	33	94.3	86.6-100	0.40

*Sensitivity calculated as compared to a composite gold standard. Gold standard cases included any positive result by rRT-PCR or viral culture from either specimen type (n=35).

†Sensitivity of nasal swab as compared to NP swab, using same diagnostic testing method.

CI, confidence interval; NP, nasopharyngeal; rRT-PCR, real time reverse transcriptase polymerase chain reaction

years), 151 (63%) were female, 147 (61%) were vaccinated with the 2006–2007 influenza vaccine, and 109 (45%) had a chronic medical condition. The mean interval from symptom onset to specimen collection was 4.8 (range 0–10) days.

A total of 35 swabs tested positive for influenza by either rRT-PCR or viral culture by either swab type (CGS). Thirty-one samples were positive for influenza A; four samples were positive for influenza B. There were 14 (6%) nasal swabs and 18 (8%) NP swabs that tested positive for influenza by viral culture. There were 31 (13%) nasal swabs and 33 (14%) NP swabs that tested positive for influenza by rRT-PCR. The nasal swab had 40.0% sensitivity by viral culture and 88.6% sensitivity by rRT-PCR, when calculated compared to the CGS (P<0.0001). The NP swab had 51.4% sensitivity by viral culture and 94.3% sensitivity by rRT-PCR, compared to the CGS (P<0.0001). The sensitivity differences by swab type when using the same diagnostic test were not significant (table 1).

Of the 18 NP swabs positive for influenza by viral culture, 14 paired nasal swabs tested positive, for a sensitivity of 77.8% (95 % CI, 58.6%–97.0%) for the nasal swab, as compared to the NP swab. Of the 33 NP swabs positive by rRT-PCR, 29 paired nasal swabs tested positive, for a sensitivity of 87.9% (95% CI, 76.7%–99.0%) for the nasal swab, as compared to the NP swab. The difference in sensitivity of the nasal swab as compared to the paired NP swab by diagnostic test was not significant (P=0.35).

Discussion

This study compared the sensitivities of two specimen collection methods using two diagnostic methods for the diagnosis of influenza. The nasal swab was less sensitive than the NP swab, irrespective of diagnostic test, but the difference in sensitivities between sampling methods was not significant. Real-time RT-PCR was significantly more sensitive than viral culture, irrespective of specimen collection method.

Our results, together with findings from the literature, suggest that less invasive methods of specimen collection may be acceptable in the era of molecular testing. A larger study of influenza detection using combined nose and throat swabs versus NP aspirates found that the combined swabs had a higher diagnostic yield, but the performance of nasal swabs alone was not evaluated.[7] A recent large pediatric study reported 88% sensitivity for the detection of influenza A using NP aspirates, and 84% sensitivity with the nasal swab when tested by PCR; the difference in sensitivities was not statistically significant (P=0.72).[12] Lambert and colleagues[10] compared combined nose-throat swabs with NP aspirates in a large pediatric population. Reported sensitivities of the nose-throat swab were 92% for the detection of influenza A and 100% for influenza B.[10]

We are not aware of any other published studies evaluating paired nasal and NP swabs from adults using rRT-PCR as the diagnostic method for detection of influenza. A strength of our study is the use of consistent recruitment procedures and standardized sample collection methods. The most important limitation of the study is the limited power to detect modest differences in sensitivity; only 35 participants tested positive for influenza. Sensitivity of sample collection methods may vary by influenza type/subtype, and our case numbers did not allow for stratified analyses. The paired specimens in this analysis were collected from older adults only; thus, we are unable to generalize our results to younger populations. Finally, specimens underwent an additional freeze-thaw cycle between rRT-PCR and culture testing. While this has the potential to affect the sensitivity of the viral culture, any impact would have been minor. Additionally, the focus of our investigation was the agreement between collection methods, not diagnostic test, as literature has previously demonstrated increased sensitivity of PCR compared to viral culture.

Conclusion

Traditional specimen collection methods for the detection of influenza are based on the use of viral culture as the diagnostic test.[5] A 'gold standard' sampling method has not been identified and validated for influenza detection using rRT-PCR, but emerging evidence suggests that less invasive samples may have comparable sensitivity to nasopharyngeal swabs or aspirates when using molecular diagnostic tests.

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