

Use of Throat Swab or Saliva Specimens for Detection of Respiratory Viruses in Children

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Background. Nasopharyngeal (NP) specimens are commonly used for the detection of respiratory viruses, but throat and saliva specimens are easier to obtain. The objective of this study was to compare the viral yield of direct fluorescent antigen detection of NP specimens and nucleic acid amplification tests (NAT) of direct fluorescent antigen-negative NP specimens with the viral yield of NAT of throat swab and saliva specimens.

Methods. NP, throat swab, and saliva specimens were obtained from children and adolescents aged ≤ 17 years. Direct fluorescent antigen testing of the NP specimen for respiratory syncytial virus, influenza A and B viruses, and parainfluenza virus was performed. If no virus was detected, NAT was performed for these 4 viruses, adenovirus, and human metapneumovirus. If a virus was detected by either method, NAT for the same virus was performed for the corresponding throat swab and saliva specimens.

Results. A virus was detected in 105 of the 137 NP specimens. The same virus was detectable by NAT in 87 (83%) of 105 throat swab specimens and 77 (74%) of 104 saliva specimens. The likelihood of viral detection among throat swab and saliva swab specimens was higher when the NP specimen tested positive by direct fluorescent antigen testing, compared with NAT alone, and when the specimens were obtained within 3 days after symptom onset, compared with later in the illness.

Conclusions. Throat swab and saliva specimens are inferior to NP specimens for the detection of respiratory viruses but might be acceptable for screening in a setting where it is impractical to obtain an NP specimen.

Determining the etiologic agent of viral lower respiratory tract infections in children is increasingly recognized to be of value, because it allows for the implementation of ideal infection-control precautions and for the appropriate use of antiviral drugs for treatment and prophylaxis of influenza virus infections. Proving a viral diagnosis potentially prevents hospitalization and the inappropriate use of antibiotics. From a public health point of view, accurate determination of the etiologic agent associated with an outbreak of respiratory disease can be of value in managing the outbreak.

Traditional methods of detecting viruses in respiratory specimens include culture and antigen detection. These methods are reasonably sensitive for the detection of respiratory syncytial virus (RSV), influenza vi-

rus, and parainfluenza virus but much less so for adenovirus. Compared with culture and antigen detection assays, nucleic acid amplification tests (NATs), including PCR and nucleic acid sequence-based amplification, have been shown to improve the detection of influenza virus in upper respiratory tract specimens [1, 2] and of RSV, influenza, parainfluenza virus, and adenovirus in lower respiratory tract specimens. NATs also detect viruses that are difficult to culture and for which antigen detection is not commercially available, such as human metapneumovirus, human coronaviruses, and rhinovirus.

The most practical specimens for antigen detection of respiratory viruses are NP specimens, because the nasopharynx provides a high concentration of infected cells. Because NATs can detect a lower concentration of viruses than can conventional methods and does not require the presence of cells, it is possible that a throat swab or saliva specimen would be an appropriate specimen for analysis, despite the lower concentration of viruses and the absence of appropriate cellular materials in these types of samples. For example, high concentrations of severe acute respiratory syndrome co-

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ronavirus were detected by NAT of throat swab and saliva specimens from patients with severe acute respiratory system [3], suggesting that these specimens have great potential for detection of respiratory viruses by NAT. The purpose of this study was to determine whether the detection of respiratory viruses in throat swab or saliva specimens using NAT is equivalent to detection using an algorithm combining direct fluorescent antigen (DFA) detection and NAT of NP specimens from children.

METHODS

Patients and procedures. This study was approved by the Health Research Ethics Board of the University of Alberta. Children and adolescents aged ≤ 17 years who were admitted to the Stollery Children's Hospital (Edmonton, Alberta, Canada) emergency department or inpatient ward and who had an NP specimen obtained for detection of presumed lower respiratory tract infection were eligible. After written informed consent was obtained, throat swab and saliva samples were obtained within 24 h after the time that the NP aspirate specimen was obtained. The saliva specimen was obtained by rubbing a sponge on a stick (Oracol Saliva Collection System; Malvern Medical Developments Limited) on the inside of the child's mouth until the sponge was saturated (typically for ~ 25 s). Data recorded on the patients included the date of onset of symptoms and hospital admission status following the emergency department visit.

Laboratory testing. DFA detection for RSV, influenza A and B viruses, and parainfluenza virus was performed for the NP specimens, as described elsewhere [2]. If a virus was detected by DFA testing, NAT was not performed for the NP specimen. However, if no virus was detected, NAT was then performed for detection of RSV, influenza A and B viruses, parainfluenza virus, adenovirus, and human metapneumovirus, as described elsewhere [2]. Detection of rhinovirus and human coronaviruses was not attempted because of the cost and the perceived lesser clinical significance of these viruses. If a respiratory virus was detected by DFA testing or NAT, NAT of a throat swab and saliva specimens from the patient was performed for the same virus. Antigen detection tests were not performed for the throat and saliva samples, because the sensitivity of these tests was predicted to be low for these specimens. The crossing threshold of RSV NAT-positive NP specimens was obtained by review of the amplification curve using the ABI 7500 analyzer (Applied Biosystems) and the associated software.

Sample size. Because throat swab and saliva specimens are easier and safer to obtain than NP specimens and because the correct determination of the etiologic agent of a viral respiratory tract infection is rarely critical, we postulated that the use of throat swab and saliva specimens for diagnosis of viral respi-

ratory tract infection would be acceptable to clinicians if the viral yield was at least 80% of the yield obtained from testing NP specimens. It has been shown that a midpoint conditional sample size is the best choice to obtain desired power for a 1-sided equivalence test of the sensitivities of 2 diagnostic tests in a matched-pair design [4]. Using the suggested formulas [4] and with the hypothesis that the difference in the sensitivities between testing of NP specimens and testing of a different specimen type would be $<20\%$, the midpoint first-order conditional sample size to achieve a statistical significance of 0.05 and 90% power was 76 samples. A virus was detected in 377 (54%) of 704 NP specimens submitted as clinical specimens from 1 November 2005 through 5 March 2006 in the same laboratory using the same algorithm; thus, it was estimated that 141 children would need to be enrolled to obtain 76 positive NP specimens.

Data analysis. The sensitivity of NAT for the detection of respiratory viruses in throat swab or saliva specimens was calculated using DFA testing or NAT of NP specimens as the reference standard. Using the χ^2 test or Fisher's exact test, as appropriate, sensitivities of the tests were also compared between (1) children for whom respiratory virus was detected by DFA testing versus children for whom virus was detected by NAT only, (2) children who had specimens tested within 3 days after the onset of symptoms versus those who had specimens tested later, and (3) children who required hospital admission versus children who were discharged from the hospital. The crossing threshold for RSV NAT-positive NP specimens was compared with that for RSV NAT-positive throat and saliva samples with use of the Mann-Whitney U test. The crossing threshold values were not compared for other viruses, because the number of cases was too small. A P value of .05 was considered to be statistically significant. The data were analyzed using SPSS software (SPSS).

RESULTS

From 18 November 2006 through 20 February 2007, a total of 137 children were enrolled in the study, with 1 patient being enrolled on 2 occasions 16 days apart. There were 62 male patients (59%), and the median age was 4.9 months (range, 11 days to 8.1 years). Ninety patients were hospitalized, and 47 were discharged from the emergency department. A virus was detected in 105 NP specimens (82 by DFA testing and 23 by NAT); 83 of these isolates were RSV (table 1). Throat and saliva specimens were obtained from these 105 patients, with the exception of 1 saliva specimen, for which there was a problem with the extraction. The virus found in the NP sample was detected in 87 (83%) of the 105 throat swab specimens and 77 (74%) of the 104 saliva specimens (table 1). The viral yield from saliva specimens was higher among patients who had a

Table 1. Sensitivity of nucleic acid amplification testing (NAT) of throat swab and saliva specimens for detection of respiratory viruses, using detection of respiratory viruses in nasopharyngeal (NP) specimens as the gold standard.

Variable	No. of patients with finding/ no. of patients studied (%)	
	Same virus detected by NAT of throat swab specimen	Same virus detected by NAT of saliva specimen
Virus detected in NP specimen (test)		
All	87/105 (83)	77/104 (74)
RSV (DFA)	59/68 (87)	61/68 (90)
RSV (NAT)	10/15 (67)	10/14 (71) ^a
Influenza (DFA)	5/7 (71)	1/7 (14)
Influenza (NAT)	2/2 (100)	0/2 (0)
PIV (DFA)	7/7 (100)	4/7 (57)
PIV (NAT)	3/4 (75)	0/4 (0)
ADV (NAT)	1/2 (50)	1/2 (50)
Any (DFA)	71/82 (87)	66/82 (81)
Any (NAT)	16/23 (70) ^b	11/22 (50) ^{a,c}
Onset of illness within 3 days before testing ^d	52/57 (91)	47/57 (83)
Onset of illness >3 days before testing	34/47 (72) ^e	30/46 (65) ^{a,f}
Child admitted to hospital	59/72 (82)	53/71 (75) ^a
Child discharged from the hospital	28/33 (85)	24/33 (73)

NOTE. Human metapneumovirus was not detected in any of the specimens. ADV, adenovirus; DFA, direct fluorescent antigen; NAT, nucleic acid amplification testing; NP, nasopharyngeal; PIV, parainfluenza virus; RSV, respiratory syncytial virus.

^a One saliva specimen was not available for testing.

^b For viral detection by DFA testing, compared with NAT, of throat swab specimens, $P = .07$, by Fisher's exact test.

^c For viral detection by DFA testing, compared with NAT, of saliva specimens, $P < .05$, by χ^2 test.

^d The day of illness onset was not recorded for 1 patient.

^e For the comparison of yield of testing of throat specimens on the basis of the time of onset of illness, $P < .05$, by χ^2 test.

^f For the comparison of yield of testing of saliva specimens on the basis of time of onset of illness, $P < .05$, by χ^2 test.

positive result of DFA testing of an NP specimen than among those who had a positive result of NAT of an NP specimen only ($P < .05$). There was a similar trend for throat swab specimens ($P = .07$) (table 1). The sensitivity of viral detection in throat swab and saliva specimens was higher for children who presented within 3 days after symptom onset than for those who presented later ($P < .05$) (table 1). Sensitivity did not vary between children who required hospital admission and those who were discharged from the emergency department (table 1). For the 20 patients who had NP specimens that tested positive for RSV by NAT, the amount of RSV was higher in the NP specimens when the throat swab specimens also tested positive by NAT (7 patients), compared with when the throat swab specimens tested negative by NAT (13 patients), as demonstrated by a lower crossing threshold (crossing threshold, 30 vs. 35; $P < .05$, by Mann-Whitney U test). A similar result was observed for the saliva samples (8 patients had saliva specimens that tested positive by NAT, and 11 patients had saliva speci-

mens that tested negative by NAT; crossing threshold, 31 vs. 35; $P < .05$; by Mann-Whitney U test).

DISCUSSION

To our knowledge, this is the first study to determine the sensitivity of detection of respiratory viruses in throat swab or saliva specimens that used detection of respiratory viruses in NP specimens as the reference standard. The yield of testing of throat swab specimens was 83%, and the yield of testing of saliva specimens was 74%. Yield appears to be higher when the concentration of viruses is higher; this finding is supported by the higher rate of detection in the throat or saliva specimens when the virus was detected by DFA testing, rather than by NAT, of the NP specimen; a higher detection rate when specimens were obtained shortly after the onset of respiratory symptoms; and a higher yield when the amount of RSV was higher in the NP specimen than in the throat or saliva specimen. Using

hospital admission as a surrogate marker for the severity of illness, no relationship was demonstrated between the severity of illness and the sensitivity of testing of throat swab or saliva specimens. The number of cases was too small to compare the yields of tests for different viruses.

Inexperienced personnel may avoid obtaining an NP specimen or may obtain a suboptimal NP specimen, because they are not familiar with the procedure, but they would be very accustomed to obtaining a throat swab specimen and could easily be instructed on how to obtain a saliva specimen. Coughing ensues once an NP specimen is obtained, which probably increases the risk of nosocomial spread of respiratory viruses, with the risk being highest for viruses such as influenza, in the context of which small particle aerosolization is thought to occur [5]. Obtaining a throat swab or saliva specimen is less likely to precipitate coughing.

The utility of throat swab or saliva specimens, which were obtained and processed in our study for diagnosis of respiratory viral infections, is dependent on the accuracy required by the submitter. In our initial calculation of sample size, we postulated that a minimum sensitivity of 80% would be adequate in some contexts. For example, if an influenza pandemic occurred, throat swab specimens could be rapidly obtained by personnel with minimal training, and the 83% sensitivity of testing of throat swab specimens that was demonstrated in our study would likely be considered to be adequate. It is unfortunate that the yield of testing of saliva specimens was <80%, because saliva specimens could be obtained at home by parents or, in the case of older children, by the individuals themselves. This would prevent the need for physician visits for patients with milder illness and would decrease the chance of influenza being transmitted in physician offices or emergency departments, while still leading to appropriate use of antiviral medication in the majority of cases. Throat swab or saliva specimens should not currently be used in routine settings, where a yield of closer to 90% is likely to be acceptable to clinicians.

It is important to note that the yield from throat or saliva specimens might be higher for viruses that were not tested for in our study, in which RSV predominated. For instance, there is some evidence that the H5N1 strain of avian influenza that is considered by many experts to be the most likely source of the next influenza pandemic may have a higher concentration in the throat than in the nasopharynx [6]. Future studies should analyze the sensitivity of NAT of throat swab and saliva specimens from adults and from large numbers of children and adults infected with viruses other than RSV. A recent study demonstrated excellent sensitivity of NAT of nasal swab specimens for RSV; thus, this site should also be studied for detection of other respiratory viruses [7]. In addition, future studies should perform cost analysis of obtaining and processing alternate specimen types.

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