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Consistency of Influenza A Virus Detection Test Results across Respiratory Specimen Collection Methods Using Real-Time Reverse Transcription-PCR

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In our prospective cohort study, we compared the performance of nasopharyngeal, oropharyngeal, and nasal swabs for the detection of influenza virus using real-time reverse transcription-PCR assay. Joint consideration of results from oropharyngeal and nasal swabs was as effective as consideration of results from nasopharyngeal swabs alone, as measured by sensitivity and noninferiority analysis.

In a prospective cohort study of 1,781 health care personnel (HCP) at two medical centers during the 2010-2011 influenza season, which is described elsewhere (1), we collected three respiratory specimen types for acute respiratory illnesses (ARI) (with fever and cough) within 8 days of onset and tested them separately using real-time reverse transcription-PCR (rRT-PCR) assay. Each swab was placed in a separate viral transport tube and shipped frozen to a CDC reference laboratory for testing. Influenza A virus-positive results (threshold cycle [C_T] values of <40.0) identified by any swab type were considered true-positive results.

During the surveillance period, 268 HCP reported one or more ARI and provided all three upper respiratory tract specimens (nasopharyngeal [NP], oropharyngeal [OP], and nasal swabs [NS]). The mean age of participants was 42 years old, and most were female (87%), white (75%), in very good or excellent self-rated health (77%), and received the 2010-2011 seasonal influenza vaccine (82%) (descriptive supplemental Table A, available upon request).

Table 1 presents the distribution of influenza A virus-positive results by swab type. Of the 53 influenza A virus-positive results, 33 (62%) had consistent results across all swab types (Table 2). NP results considered alone and OP results alone each failed to detect 7 (13%) specimens deemed positive by another collection method, while NS alone did not detect 13 (25%). Sensitivity for NP and OP results considered alone were both 87%; NS sensitivity was 75%. The sensitivity of influenza A virus detection increased to 94 to 96% when data from two swabs were considered jointly (Table 2); all confidence intervals overlapped.

Negative predictive value was similar (>95%) across swabs considered alone or in pairs (Table 2). Using previously published standards for interpreting the kappa metric (2), agreement was "substantial" for the OP/NS swab pair (0.78) and "almost perfect" for the NP/NS swab pair (0.81) and NP/OP swab pair (0.87). Results of noninferiority analysis showed that, within a 2% margin of error, the sensitivity of joint consideration of OP-plus-NS swabbing results is not inferior to the NP-only method ($\alpha = 0.05$).

The mean C_T value was lower, indicating more viral RNA, among the 33 consistently influenza A virus-positive specimens compared with inconsistent positive results; this was statistically significant for NP and OP swabs (P values of <0.01; data not shown).

TABLE 1 Distribution of influenza A virus-positive results by swab type

Detection of influenza A virus by PCR by the following swab type[a]:			No. of positive samples
NP	NS	OP	
+	+	+	33
+	+	−	3
+	−	+	8
+	−	−	2
−	+	+	2
−	+	−	2
−	−	+	3

[a] All possible combinations of influenza A virus-positive (+) or influenza A virus-negative (−) results from rRT-PCR results for influenza A virus-positive samples from nasopharyngeal (NP), nasal (NS), and oropharyngeal (OP) swabs tested separately.

Inconsistent influenza virus results across swab types were associated with younger age, more days between the onset of illness and swab collection, and taking antiviral medication prior to swab collection (P values of <0.01) (supplemental Table B, available upon request). The association between age and inconsistency remained significant after controlling for the number of days since onset ($P < 0.05$). Illnesses with consistent versus inconsistent swab results did not differ in duration of illness, subjective ratings of illness severity, vaccination status, or other characteristics we examined (data not shown).

In sum, we found that two-thirds (62%) of the influenza A virus-positive results were identified consistently across swab

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TABLE 2 Proportion of influenza A virus-positive samples detected, sensitivity, negative predictive value, and kappa statistic for single swabs or two swabs considered together

Swab type or result	No. of influenza A virus-positive swabs/no. of swabs (%)	No. of influenza A virus-positive samples not detected by:		Sensitivity[c] (95% confidence interval)		Negative predictive value[d] (95% confidence interval)		Kappa statistic (standard error)
		Single swab ($n = 53$)[a]	Two swabs[b] ($n = 53$)	Single swab	Two swabs	Single swab	Two swabs	
Any swab	53/290 (18)							
Consistent across all swabs	33/53 (62)							
Not consistent across all swabs	20/53 (38)							
Results for one swab type[e]								
NP only	46/290 (16)	7 (13)		0.87 (0.75–0.95)		0.97 (0.94–0.99)		
NS only	40/290 (14)	13 (25)		0.75 (0.62–0.86)		0.95 (0.91–0.97)		
OP only	46/290 (16)	7 (13)		0.87 (0.75–0.95)		0.97 (0.94–0.99)		
Results for two swab types[f]								
NP or NS	50/290 (17)		3 (6)		0.94 (0.84–0.99)		0.99 (0.96–1.00)	0.81 (0.71–0.91)
NP or OP	51/290 (18)		2 (4)		0.96 (0.87–1.00)		0.99 (0.97–1.00)	0.87 (0.79–0.95)
OP or NS	51/290 (18)		2 (4)		0.96 (0.87–1.00)		0.99 (0.97–1.00)	0.78 (0.68–0.88)

[a] n is the number of influenza A virus-positive swabs by any swab result.
[b] Considering the results for two swabs together.
[c] Sensitivity is the number of swabs identified as influenza A virus positive by single swab or swab combination/number of influenza A virus-positive swabs by any swab result.
[d] Negative predictive value is the number of consistently negative swab results/number of influenza A virus-negative results identified by single swab or swab combination.
[e] Results for one swab type only considered. NP, nasopharyngeal; NS, nasal; OP, oropharyngeal.
[f] Results from both swabs tested separately considered.

types. Given the high sensitivity of the rRT-PCR assay (3) and the use of specific symptom criteria (i.e., fever and cough) associated with influenza illness (4), we are confident that an influenza virus-positive result identified for any swab reflects a true influenza virus infection. Therefore, we were surprised that one in three (38%) influenza A virus-positive results was not detected by one or more swab type if considered alone. Nonetheless, our finding fits with previous studies showing that a modest number of influenza cases are missed if only one specimen type is examined (5, 6, 7).

We also observed that joint consideration of results from more than one swab resulted in higher sensitivity; though these differences were not statistically significant, the direction of the effect was consistent with prior studies showing increased sensitivity with combinations of specimens (6, 8). In addition, the overall trend from the kappa metric suggests that jointly considered swabs detected influenza A virus-positive results that were missed by another swab type. In fact, the lower kappa of the OP-plus-NS combination reveals that this combination detects a more diverse set of influenza A virus-positive results, capturing some missed by NP-plus-OP or NP-plus-NS combinations, which is similar to results from a previous study of adults and children (6). Since 13 (65%) of the inconsistent influenza A virus-positive results had C_T values >30, the lower kappa of the OP-plus-NS combination may indicate the value of this combination in detecting influenza virus-positive results with less viral RNA.

Clinicians and researchers concerned about the discomfort associated with NP swab collection will be reassured by our finding that joint consideration of NS and OP swabs resulted in equivalent sensitivity with statistically noninferior results and fewer undetected cases than consideration of NP swabs alone. Our results indicate that joint consideration of NS-plus-OP swab results is not inferior to the NP-only method.

Prior studies have observed that the sensitivity of rRT-PCR results declines for specimens collected from adults 5 days (9) or 7 days after the onset of the illness (10), which is consistent with observations that virus shedding in adults typically peaks during the first 24 to 72 h of illness and lasts for about 5 to 7 days (11). Our findings complement previous research by noting that the consistency of influenza virus-positive results using rRT-PCR across swab types also declines with greater number of days since the onset of the illness. We also found that consistency increased with older age in our cohort of adults aged 18 to 64 years, and this trend was independent of the number of days since the onset of the illness. Although prior studies have noted higher rRT-PCR sensitivity for specimens collected from children compared to adults (9), we are not aware of research showing differences in sensitivity or consistency of results with age among adults.

Among the study's strengths are its prospective design, weekly surveillance of ARI, and focus on HCP who are at increased risk of influenza exposure. Among the study's limitations is the small number of influenza cases identified, which reduced statistical power and resulted in wide confidence intervals for sensitivity calculations. The generalizability of our findings to studies using combined specimens is also limited because we examined specimens stored and tested separately.

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REFERENCES

1. **Thompson MG, Gaglani MJ, Naleway A, Ball S, Henkle EM, Sokolow LZ, Brennan B, Zhou H, Foster L, Black C, Kennedy ED, Bozeman S, Grohskopf LA, Shay DK.** 2012. The expected emotional benefits of influenza vaccination strongly affect pre-season intentions and subsequent vaccination among healthcare personnel. Vaccine **30:**3557–3565.
2. **Landis JR, Koch GG.** 1977. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. Biometrics **33:**363–374.
3. **Centers for Disease Control and Prevention.** 2009. Evaluation of rapid influenza diagnostic tests for detection of novel influenza A (H1N1) virus - United States, 2009. MMWR Morb. Mortal. Wkly. Rep. **58:**826–829.
4. **Ohmit SE, Monto AS.** 2006. Symptomatic predictors of influenza virus positivity in children during the influenza season. Clin. Infect. Dis. **43:** 564–568.
5. **Lieberman D, Lieberman D, Shimoni A, Keren-Naus A, Steinberg R, Shemer-Avni Y.** 2009. Identification of respiratory viruses in adults: nasopharyngeal versus oropharyngeal sampling. J. Clin. Microbiol. **47:** 3439–3443.
6. **Kim C, Ahmed JA, Eidex RB, Nyoka R, Waiboci LW, Erdman D, Tepo A, Mahamud AS, Kabura W, Nguhi M, Muthoka P, Burton W, Breiman RF, Njenga MK, Katz MA.** 2011. Comparison of nasopharyngeal and oropharyngeal swabs for the diagnosis of eight respiratory viruses by real-time reverse transcription-PCR assays. PLoS One **6:**e21610. doi:10.1371/journal.pone.0021610.
7. **Abu-Diab A, Azzeh M, Ghneim R, Ghneim R, Zoughbi M, Turkuman S, Rishmawi N, Issa AE, Siriani I, Dauodi R, Kattan R, Hindiyeh MY.** 2008. Comparison between pernasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. J. Clin. Microbiol. **46:**2414–2417.
8. **Hammitt LL, Kazungu S, Welch S, Bett A, Onyango CO, Gunson RN, Scott JA, Nokes DJ.** 2011. Added value of an oropharyngeal swab in detection of viruses in children hospitalized with lower respiratory tract infection. J. Clin. Microbiol. **49:**2318–2320.
9. **Harper SA, Bradley JS, Englund JA, File TM, Gravenstein S, Hayden FG, McGeer AJ, Neuzil KM, Pavia AT, Tapper ML, Uyeki TM, Zimmerman RK, Expert Panel of the Infectious Diseases Society of America.** 2009. Seasonal influenza in adults and children–diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: clinical practice guidelines of the Infectious Diseases Society of America. Clin. Infect. Dis. **48:**1003–1032.
10. **Belongia E, Kieke B, Donahue J, Greenlee R, Balish A, Foust A, Lindstrom S, Shay D.** 2009. Effectiveness of inactivated influenza vaccines varied substantially with antigenic match from the 2004–2005 season to the 2006–2007 season. J. Infect. Dis. **199:**159–167.
11. **Aoki FY, Boivin G.** 2009. Influenza virus shedding—excretion patterns and effects of antiviral treatment. J. Clin. Virol. **44:**255–261.