Evaluation of the Quidel QuickVue Test for Detection of Influenza A and B Viruses in the Pediatric Emergency Medicine Setting by Use of Three Specimen Collection Methods

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The Quidel QuickVue influenza test was compared to viral culture and reverse transcriptase PCR by the use of three different respiratory specimen types. Of 122 pediatric subjects enrolled, 59 had influenza virus infections: 44 were infected with influenza A virus and 15 were infected with influenza B virus. The sensitivity of the QuickVue test was 85% with nasopharyngeal swabs, 78% with nasal swabs, and 69% with nasopharyngeal washes. Specificities were equivalent (97% to 98%) for all three collection methods.

During annual outbreaks of influenza A and B virus infection, it is estimated that 10 to 20% of the residential population of the United States may be affected and that the highest attack rates occur in school-age children (1, 5, 6, 16, 18, 20). The rapid and accurate diagnosis of cases of influenza is desirable for management of children presenting to a pediatric hospital's emergency department (ED). A laboratory-confirmed diagnosis of influenza supports the appropriate use of antiviral therapy for patients to be admitted to the hospital as well as for patients to be discharged from the ED and managed as outpatients. A specific diagnosis also decreases the inappropriate use of antibiotics, other diagnostic testing, and the patient's length of stay in the ED (1, 15, 18, 20).

Several methods for laboratory diagnosis of influenza are available; these include viral culture, direct antigen detection, and direct nucleic acid amplification and detection. Viral culture is still considered the "gold standard" method, but traditional culture generally requires at least 3 days or longer to obtain a positive result. Rapid viral culture methods using "shell vial" assays have been applied to speed up the time to detection (8–11). Nucleic acid amplification methods offer very high test sensitivities and same-day results, but there are no FDA-cleared nucleic acid amplification tests for influenza virus detection. Respiratory virus antigen detection by direct or indirect immunofluorescence assay is widely used in laboratories with expertise in fluorescence microscopy, but these assays are often performed as "batch tests" a few times per day and are thus not truly rapid. Antigen detection methods which utilize single-use immunoassay test devices offer the potential for widespread test availability, relative ease of testing, and rapid turnaround (2–4, 8, 10, 12–14, 17, 21).

There are limited published data on the impact of specimen

type from the respiratory tract on the relative efficiencies of rapid immunoassay tests for influenza diagnosis (7, 12). Studies have examined nasopharyngeal (NP) aspirates and washes, posterior NP and throat swabs, and sputa; however, most studies have not included the collection of multiple specimen types from the same subjects. Aspirates and washes are generally considered superior to swab collections for detection of a variety of respiratory viruses, but swab samples are easier and faster to collect and may be preferred by healthcare providers in a busy ambulatory setting. In this study, we evaluated the performance characteristics of the QuickVue influenza test (Quidel Corp., San Diego, CA) in a pediatric ED setting on respiratory specimens collected by NP washing, NP swabbing, and anterior nasal swabbing.

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This study was conducted with approval of the Columbus Children's Hospital Institutional Review Board, and informed consent was obtained for all subjects. Eligible subjects were children presenting to the Children's Hospital ED with symptoms suggestive of influenza virus infection during select times in the annual winter outbreaks of influenza in 2003–2004 and 2004–2005. There were 122 subjects enrolled in the study, including 64 males (52%) and 58 females (48%). The mean age of the subjects was 5 years (range, 2 weeks to 18 years). About 10% of the subjects were <3 months old, 30% were 3 to 24 months old, and 60% were >24 months old. All specimen collections were performed on each subject in a standard fashion by a single research nurse or physician, using all three collection techniques. Anterior nasal swab collection was performed first with an absorbent foam swab on a plastic shaft (Quidel Corp.), posterior NP swab collection was performed second with a Dacron swab on a flexible aluminum shaft (Puritan Medical, Guilford, ME), and NP washing was performed last with sterile saline. Both the anterior nasal swab and NP wash methods are FDA cleared for the QuickVue test; the NP swab collection method is considered an inves-

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tigational collection technique for which there is no FDA clearance with this test.

Subjects were accommodated for swab collections by being placed supine on examining beds (infants) or held by a parent or patient care assistant in a sitting position (toddlers); older children and adolescents were allowed to sit unassisted. For anterior nasal swab collection, the foam swab was removed from the plastic sheath of the swab transport device, inserted into one anterior naris, and rotated several times to maximize the amount of secretions and cellular material obtained. The swab was then returned to the plastic sheath and transported to the lab immediately for testing. For NP swab collection, the Dacron swab was inserted through an anterior naris to the posterior nasopharynx until resistance was met, left in place for a few seconds, and removed slowly while being rotated. The swab was then placed in a tube containing 0.5 ml normal saline, the swab tip was cut off in the saline, and the tube was immediately transported to the lab for testing. Nasopharyngeal wash collection was performed on toddlers by placing them on one side on an examining bed; older children and adolescents were allowed to sit. Three to five milliliters (depending on patient age) of sterile saline was instilled through one naris into the posterior nasopharynx by using a 5-ml syringe with a catheter adapter, and the subject's head was positioned to allow the wash fluid (typically 1 to 3 ml) to drain out the other naris into a small emesis basin before being transferred to a sterile container for transport to the laboratory.

The QuickVue influenza test used in this evaluation is an FDA-cleared, single-use, lateral-flow immunoassay which detects both influenza A and B viruses but does not differentiate between the two. All testing, which requires about 15 min, with no more than 5 min of hands-on time, was performed in the Children's Hospital Diagnostic Virology Laboratory by one of several technologists within 15 min of receipt in the laboratory. Nasal foam swab samples and NP washes were processed per the instructions in the package insert; NP swab samples received in 0.5 ml saline were mixed thoroughly by vortexing, the swab tip was removed, and the sample was then processed per the NP wash sample procedure. The QuickVue influenza test involves an initial extraction of viral antigens from fluid or swab specimens followed by allowing the extracted material to migrate on a test strip to react with specific mouse monoclonal antibodies for influenza A or B virus. A positive patient sample produces a pink to red line on the test strip, while a negative sample does not produce a pink to red line. The presence of a blue line serves as a positive on-board control for the procedure. In addition, both external positive and negative control samples for influenza virus were run once per day of testing.

Viral culture was performed on all NP wash specimens, using the R-Mix (Diagnostic Hybrids, Athens, OH) rapid culture method. Briefly, duplicate shell vials of mixed monolayer cultures containing mink lung (Mv1Lu) and human adenocarcinoma (A549) cells were inoculated with 0.2 ml of NP wash material and incubated at 35°C for 36 to 48 h. One vial was then processed and stained with a direct fluorescent antibody reagent for detection and differentiation of influenza A and B viruses (SimulFluor Flu A/B; Chemicon, Temecula, CA). The companion R-Mix vial was similarly stained about 24 h later.

Because viral culture may be negative in cases of influenza for a variety of reasons, reverse transcriptase PCR (RT-PCR)

TABLE 1. Comparison of nasal swab, nasopharyngeal swab, and nasopharyngeal wash specimens with an expanded gold standard in the Quidel QuickVue influenza test^a

Specimen type	No. of positive samples/total no. of samples (%)				
	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Efficiency
Nasal swab	46/59 (78)	61/63 (97)	46/48 (96)	61/74 (82)	107/122 (88)
NP swab	50/59 (85)	62/63 (98)	50/51 (98)	62/71 (87)	112/122 (92)
NP wash	41/59 (69)	62/63 (98)	41/42 (98)	62/80 (78)	103/122 (84)

^a The expanded gold standard for a case of influenza was either a culture or RT-PCR-positive result with the nasal wash specimen.

was also performed on all NP wash specimens to determine if additional cases of influenza could be detected by this technique. Briefly, the RT-PCR procedure involved extraction of 0.2 ml of NP wash specimen by use of a miniMag extractor (bioMérieux, Durham, NC) and an elution volume of 0.05 ml. Amplification was performed on a LightCycler platform (Roche Molecular Diagnostics, Indianapolis, IN) using a LightCycler RNA amplification kit (Roche Molecular Diagnostics) with primers and hybridization probes targeting a 300-nucleotide fragment of the influenza A virus matrix protein gene or a 184-nucleotide fragment of the influenza B virus nucleoprotein gene (19).

To compare test sensitivities among the three collection methods, statistical analysis was restricted to the cases of influenza detected by either a positive culture or a positive RT-PCR result. Since the study data consist of results of three tests performed on samples from the same subjects, generalized estimating equation models with exchangeable correlation structures were used to adjust for the correlation among the measurements within subjects. Odds ratios and 95% confidence intervals (CI) for the three pairwise comparisons were also computed.

Of the 122 subjects enrolled in the study, 54 were determined to have influenza based on culture (gold standard), and 59 (44 positive for influenza A virus and 15 positive for influenza B virus) were determined to have influenza if either culture or RT-PCR was utilized (expanded gold standard; prevalence = 48%). Of the five culture-negative, RT-PCR-positive cases of influenza, two were recorded as being toxic in tissue culture.

Based on culture alone, the QuickVue test had an overall sensitivity of 85% (46/54) with specimens obtained by posterior NP swab collection. The test sensitivity with anterior nasal swabs was 78% (42/54), while the sensitivity with NP washes was 69% (37/54). The difference in sensitivity between NP swabs and NP washes was significant (*P* value, 0.005; odds ratio, 2.64; CI, 1.34 to 5.2). There was no difference in sensitivity between anterior nasal swab collection and the two NP collection methods. In contrast, specificities were equivalent for specimens obtained by the three methods and ranged from 91% to 93% (data not shown).

If the expanded gold standard was used to define a case of influenza, the QuickVue test had overall sensitivities of 85% (50/59) with posterior NP swab specimens, 78% (46/59) with anterior nasal swabs, and 69% (41/59) with NP wash specimens (Table 1). The difference in sensitivity between NP swabs and NP washes was significant (*P* value, 0.005; odds ratio, 2.43; CI,

1.30 to 4.55). There was no difference in sensitivity between anterior nasal swab collection and the two NP collection methods. In contrast, specificities were equivalent for specimens obtained by the three methods and ranged from 97 to 98%. Positive predictive values of the test were very high and equivalent (96 to 98%) for all collection methods, while negative predictive values ranged from 78 to 87%; overall efficiencies ranged from 84 to 92% (Table 1).

The sensitivities of the QuickVue test for detection of the 44 cases of influenza A virus infection were 89% with NP swabs, 84% with nasal swabs, and 73% with NP washes; sensitivities for detection of the 15 cases of influenza B virus infection were 73%, 60%, and 60%, respectively, for the three specimen types (data not shown).

In summary, both the anterior nasal swab and posterior NP swab collection methods, as performed in this study, can be recommended as alternative collection methods to NP washes for rapid immunoassay for influenza virus detection using the QuickVue influenza test. The swab specimens yielded reasonably good test sensitivities with high specificities. It should be pointed out that establishing the laboratory diagnosis of influenza and subsequent test sensitivities and specificities for the three collection methods was based on using only nasal wash samples for culture and RT-PCR; we believe this to be a reasonable approach. One of the limitations of the anterior nasal swab collection method is that the sample is used up in the QuickVue test procedure, and thus, additional sample is not available should other testing be required. Because the QuickVue test results are available rapidly, repeat collection by NP swabbing, washing, or aspiration could be done to obtain an additional specimen for further testing.

For patients presenting to the ED but not being admitted and for patients seen in various ambulatory settings, rapid antigen testing alone offers a cost-effective and clinically useful approach for confirming a diagnosis and basing therapeutic decisions at the time of the visit. However, although rapid antigen tests for influenza virus are generally highly specific, they lack sensitivity compared with viral culture or nucleic acid amplification (11, 12). For acutely ill patients with suspected respiratory virus infection who require hospitalization, it is imperative to follow up a negative influenza virus rapid antigen test with more comprehensive and definitive diagnostic methods.

The relative sensitivity of the NP wash collection method (69%) compared to the swab collections was surprising to us given that in our experience, this collection method generally yields large numbers of respiratory epithelial cells. However, there are relatively few published studies comparing the impacts of specimen type on influenza virus detection. Covalciuc et al. evaluated a 14-day cell culture method and a rapid optical immunoassay for influenza virus with four specimen types, namely, nasal aspirates, NP swabs, throat swabs, and sputa (not all specimen types were collected from each patient) (7). Sputa and nasal aspirates were significantly superior to throat swabs for influenza virus detection by both culture and immunoassay; more cases of influenza were detected by both culture and immunoassay performed on NP swabs than those performed on throat swabs, but this difference was not significant (7). Well over 50% of subjects in this study were >16 years of age. A recently published study by Cazacu et al. which examined NP wash specimens collected in the ED of a large pediatric hos-

pital supports our findings on the sensitivity obtained with NP wash specimens for rapid influenza virus detection by immunoassay (2). In that study, the QuickVue influenza test had a sensitivity and specificity of 70% and 98%, respectively, with NP wash specimens, which are very close to our results. It is possible that the volume of saline used in NP wash collection procedures is sufficient to dilute out target influenza virus antigens for rapid immunoassay-based procedures. This effect might not be seen in direct fluorescent antibody or culture assays for influenza virus because specimens for fluorescent antibody assays are commonly centrifuged to concentrate cellular material and relatively large inocula are used in culture assays. This supposition is supported by the observation that four of five NP wash specimens initially tested and reported in the data as negative by the QuickVue test were positive when the test was repeated after the NP wash material was centrifuged and the pellet resuspended in a smaller volume of saline (data not shown). This observation may also have relevance to rapid antigen testing of swab collections that are diluted by being placed in a volume of viral transport medium larger than is needed to perform the rapid test.

One of the caveats of testing an NP swab specimen with the QuickVue test is that this specimen type is not FDA cleared for use and there is no recommended NP swab collection/transport device for this application. It is therefore incumbent on users to verify the clinical utility of a specific NP swab collection system in their own clinical setting. Because NP swab collections yielded the best overall test sensitivity, it would be useful to compare NP aspirates with NP swab collection procedures with the goal of recommending a standardized NP swab collection device for the QuickVue test.

Previously published data suggest that the sensitivities of rapid antigen tests for influenza B virus infection are lower than those for influenza A virus infection (21). The results of our study support these findings, although there were only 15 patients with influenza B virus infection detected. Additional studies are needed to better characterize the sensitivity of this test for detection of influenza B virus. These studies should include evaluation of newer influenza virus antigen tests, such as the Quidel QuickVue Influenza A+B test, which is designed to detect and differentiate these viruses.

It is important that a well-trained research nurse collected the vast majority of specimens in this study. Thus, the results obtained here might not be reproducible when testing is performed in a busy ambulatory setting with multiple individuals collecting specimens. In addition, the study was conducted during time periods of high influenza disease activity (prevalence = 48%). The predictive values of the test may differ significantly when applied to a pediatric population with low disease prevalence or to nonpediatric patient groups. In all situations where swab collections are used, emphasis should be placed on obtaining as large a sample of respiratory secretions as possible. Regarding the use of NP wash specimens for rapid influenza testing, we recommend that concentration of the specimen by centrifugation and resuspension in a smaller volume be considered whenever feasible. Alternatively, the collection of NP aspirates should yield a high-quality, undiluted specimen for improved antigen detection testing. Whichever collection method is used, laboratory testing personnel should

work closely with clinical services to ensure that test results are used effectively to manage patients.

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