

Low Sensitivity of Rapid Diagnostic Test for Influenza

Timothy M. Uyeki,¹ Ramakrishna Prasad,² Charles Vukotich,² Samuel Stebbins,² Charles R. Rinaldo,² Yu-hui Ferng,³ Stephen S. Morse,³ Elaine L. Larson,³ Allison E. Aiello,⁴ Brian Davis,⁴ and Arnold S. Monto⁴

¹Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia; ²University of Pittsburgh, Pittsburgh; ³Columbia University, New York, New York; and ⁴University of Michigan, Ann Arbor

The QuickVue Influenza A+B Test (Quidel) was used to test nasal swab specimens obtained from persons with influenza-like illness in 3 different populations. Compared with reverse-transcriptase polymerase chain reaction, the test sensitivity was low for all populations (median, 27%; range, 19%–32%), whereas the specificity was high (median, 97%; range, 96%–99.6%).

Rapid influenza diagnostic tests that detect influenza viral antigens are used to screen patients with suspected influenza and offer the advantage of providing a timely result, compared with other tests for influenza, that can influence clinical decision making [1]. Use of rapid influenza tests can help to reduce unnecessary diagnostic testing, to facilitate antiviral treatment, to decrease inappropriate antibiotic use, and to reduce the duration of treatment in the emergency department or hospitalization [2–8]. Rapid influenza tests have also been used for public health purposes to investigate suspected influenza outbreaks. Identification of influenza virus infection by rapid tests can facilitate prompt implementation of control measures before confirmatory test results are available from shell vial culture, tissue cell viral culture, or RT-PCR.

As diagnostic tests, the accuracy of rapid influenza tests is less than that of “gold standard” reference tests, such as viral culture or RT-PCR [1]. In particular, a wide range of sensitiv-

ities of the rapid influenza tests have been reported, whereas specificities have been reported to be high, compared with reference tests [1, 8–12]. In the context of enrolling participants in studies to better understand transmission of influenza virus, a rapid influenza diagnostic test was used to screen persons with influenza-like illness at 3 separate study sites in the United States during the 2007–2008 influenza seasons. Here, we report our findings that compared the accuracy of the rapid test with that of RT-PCR or viral culture and an investigation of these results.

Methods. During 2007–2008, studies at 3 different sites were conducted to assess influenza transmission and non-pharmaceutical interventions among different populations. In each study, the QuickVue Influenza A+B Test (Quidel) was used to test respiratory specimens from ill participants. The study target populations and the case definitions used at each site to recruit study participants were as follows: site 1 (Ann Arbor, MI), university students who presented to an outpatient clinic with influenza-like illness “ILI1,” defined as cough plus at least 1 of the following: fever or feverishness, chills, or body aches; site 2 (New York, NY), children and adults at Headstart programs, preschools, day care centers, pediatric clinics, and Women, Infants, and Children program centers who were identified by study personnel as having influenza-like illness “ILI2,” defined as temperature of $\geq 37.8^{\circ}\text{C}$ and cough and/or sore throat in the absence of a known cause other than influenza virus infection; and site 3 (Pittsburgh, PA), elementary school students with ILI2 identified by study personnel at school visits. At all sites, a foam swab supplied with the test kit was used to collect a nasal swab sample from participants who met the case definitions, and the specimen was tested immediately or within a few hours by study personnel (sites 1 and 3) or was placed in standard sterile viral transport media on ice and then refrigerated and tested within 4–8 h (site 2). Testing was done using the QuickVue Influenza A+B Test in accordance with the manufacturer’s instructions.

Additional respiratory specimens for confirmatory assays were collected at the same time that nasal swabs were obtained from participants. At site 1, a throat swab specimen was collected from participants who met criteria for ILI1 by using a sterile polyester swab and was placed in sterile, veal-infused viral transport media on cold packs and then transported to a laboratory and refrigerated for up to 72 h before testing for influenza A and B viruses by real-time RT-PCR and by tissue cell viral culture with standard laboratory methods (2007–2008). At site 2, a deep nasal swab specimen was collected by

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Reprints or correspondence: Dr. Timothy M. Uyeki, Epidemiology and Prevention Branch, Influenza Div., MS A-20, Centers for Disease Control and Prevention, 1600 Clifton Rd. NE, Atlanta, GA 30333 (tuyeki@cdc.gov).

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a sterile polyester swab and either was placed on a cold pack, refrigerated up to 1 day, and transported to a commercial laboratory for tissue cell viral culture (November 2007–February 2008) or was shipped on cold packs to a public health laboratory, where the specimen was aliquotted and frozen at -70°C before being thawed and tested for influenza A and B by multiplex PCR (March–May 2008). At site 3, an additional nasal swab specimen was collected from participants who met ILI2 criteria by using a sterile dacron swab and was transported to the laboratory for influenza A and B virus testing by multiplex PCR assay within a few hours after specimen collection (2007–2008).

We calculated the sensitivity, specificity, and positive and negative predictive values, as well as 95% CIs calculated using binomial exact methods, for the QuickVue Influenza A+B Test compared with confirmatory influenza testing by RT-PCR (all sites) and viral culture (sites 1 and 2) for all specimens tested at each site throughout the study period. A standard instrument was used to collect data on the study populations from each site and to compare methods of collecting, processing, and testing respiratory specimens. Descriptive statistics were used to analyze the characteristics of the study populations. Testing of study participants was approved by the institutional review boards of the institutions involved.

Results. The majority of the study participants were children and young adults; the median age was 19 years (range, 18–22 years) at site 1, 4 years (range, 3 months–71 years) at site 2, and 8 years (range, 5–12 years) at site 3. At the time of study enrollment, the median time from illness onset to specimen collection was 3 days (range, 0–7 days) at site 1, 2 days (range, 1 to >3 days; the time from onset to enrollment was >3 days for 6 of the 138 participants) at site 2, and 3 days (range, <1 to 10 days) at site 3. The test results and test parameters for each site are presented in tables 1 and 2. Overall, the calculated test parameters for the 3 sites in testing for either influenza A or influenza B virus by QuickVue Influenza A+B Test, compared with RT-PCR, were as follows: median sensitivity, 26.7% (range, 18.9%–32.3%); median specificity, 97.2% (range, 96.2%–99.6%); median positive predictive value, 87.5% (range, 80.0%–90.9%); and median negative predictive value,

69.4% (range, 62.5%–79.1%). Although the number of specimens positive for influenza B virus was very small at sites 1 and 3, the median sensitivity was substantially higher for influenza A virus than for influenza B virus at site 2 but was still suboptimal.

An investigation of possible explanations for the low sensitivities was initiated. All specimens obtained were nasal swab specimens (the preferred specimen for the QuickVue Influenza A+B Test) collected using the foam swab supplied in the test kit. All study sites used a small number of trained study personnel to collect clinical specimens and to perform the rapid influenza test, and all specimens were tested either immediately or, for a small number of participants, within 4–8 h after specimen collection, in accordance with the manufacturer’s instructions. The test kit lot numbers for all QuickVue Influenza A+B Tests used were different for each site, none of the kits were expired, and all kits were stored under proper conditions as recommended by the manufacturer.

Discussion. We found very low sensitivity of the QuickVue Influenza A+B Test, compared with RT-PCR, for detection of either influenza A or influenza B virus among ill children and young adults in 3 geographically distinct study populations. Although most other reported sensitivities of rapid influenza diagnostic tests were moderate in comparison with confirmatory assays such as RT-PCR or viral culture, we observed sensitivities that were substantially lower than those reported previously for this test or for other commercially available rapid influenza antigen tests [1, 8, 10–12]. Only 1 other published study has reported such low sensitivity for this test in comparison with RT-PCR [11]. The specificity of the QuickVue Influenza A+B Test was very high at all sites ($>96\%$), compared with RT-PCR, which is consistent with results of previous studies [1, 8–12].

We were unable to identify common factors across all sites that might account for the consistently suboptimal test sensitivities. There were no deviations from the manufacturer’s recommended specimen type, sample storage, or test procedures, and most specimens were tested immediately at 2 sites; at the third site, only a small number of specimens were tested within 4–8 h after collection. It is unlikely that the nasal swab specimen

Table 1. Results of diagnostic testing for influenza A and B viruses at 3 US sites, 2007–2008.

QuickVue ^a results	Site 1 PCR results (n = 303)		Site 1 culture results (n = 303)		Site 2 PCR results (n = 67)		Site 2 culture results (n = 71)		Site 3 PCR results (n = 287)	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Positive	14	2	12	4	10	1	11	0	28	7
Negative	60	227	37	252	21	35	21	39	77	175

NOTE. Data are no. of specimens. Results are for influenza A or B virus. The number of specimens positive for influenza B virus by RT-PCR were as follows: site 1, n = 3; site 2, n = 5; and site 3, n = 50.

^a QuickVue Influenza A+B Test (Quidel).

Table 2. Test parameters of the QuickVue Influenza A+B Test at 3 US sites, 2007–2008.

Parameter	Site 1		Site 2 ^a			Site 3 PCR-tested specimens (n = 287)
	PCR-tested specimens (n = 303)	Culture-tested specimens (n = 303)	PCR-tested specimens (n = 67)	Culture-tested specimens (n = 71)	PCR- and culture-tested specimens (n = 138)	
Sensitivity, % (95% CI)						
Influenza A or B	18.9 (10.8–29.7)	24.4 (13.3–38.9)	32.3 (16.7–51.4)	34.4 (18.6–53.2)	33.3 (22.0–46.3)	26.7 (18.5–36.2)
Influenza A	19.7 (11.2–30.9)	25.5 (14.0–40.4)	55.6 (21.2–86.3)	44.4 (21.5–69.2)	48.2 (28.7–68.1)	30.9 (19.2–44.8)
Influenza B	0 (0–70.8)	0 (0–84.2)	22.7 (7.8–45.4)	21.4 (4.7–50.8)	22.2 (10.1–39.2)	22.0 (11.5–36.0)
Specificity, % (95% CI)						
Influenza A or B	99.1 (96.9–99.9)	98.4 (96.0–99.6)	97.2 (85.5–99.9)	100 (91.0–100)	98.7 (92.8–100)	96.2 (92.2–98.4)
Influenza A	99.1 (96.9–99.9)	98.4 (96.1–99.6)	100 (93.6–100)	100 (93.3–100)	100 (96.7–100)	92.2 (88.0–95.3)
Influenza B	100 (98.8–100)	100 (98.8–100)	97.8 (88.2–99.9)	100 (93.7–100)	99.0 (94.7–100)	89.9 (85.3–93.4)
PPV, % (95% CI)						
Influenza A or B	87.5 (61.7–98.5)	75 (47.6–92.7)	90.9 (58.7–99.8)	100 (71.5–100)	95.5 (77.2–99.9)	80.0 (63.1–91.6)
Influenza A	87.5 (61.7–98.5)	75 (47.6–92.7)	100 (47.8–100)	100 (63.1–100)	100 (75.3–100)	48.6 (31.4–66.0)
Influenza B	NA	NA	83.3 (35.9–99.6)	100 (29.2–100)	88.9 (51.8–99.7)	31.4 (16.9–49.3)
NPV, % (95% CI)						
Influenza A or B	79.1 (73.9–83.7)	87.1 (82.7–90.8)	62.5 (54.4–72.5)	65.0 (51.6–76.9)	64.0 (54.4–72.5)	69.4 (63.4–75.1)
Influenza A	80.1 (75.1–84.6)	87.8 (83.5–91.4)	93.6 (84.3–98.2)	84.1 (72.7–92.1)	88.8 (81.9–93.7)	84.9 (79.9–89.1)
Influenza B	99.0 (97.1–99.8)	99.3 (97.6–99.9)	72.1 (59.2–82.9)	83.8 (72.9–91.6)	78.3 (70.2–85.1)	84.5 (79.5–88.8)

NOTE. NA, could not be calculated; NPV, negative predictive value; PPV, positive predictive value.

^a At site 2, viral culture was used as the reference standard comparison test from 14 November 2007 through 29 February 2008; RT-PCR was used as the reference standard from 1 March 2008 through 19 May 2008.

obtained for rapid testing had substantially less viral antigen than did the specimen collected at the same time for the comparison assay for participants consistently across all 3 sites. The choice of a reference standard test can affect the test sensitivity. For example, sensitivities have generally been higher when rapid influenza tests were compared with viral culture [9, 10] than when they were compared with RT-PCR [10–12]. However, this does not account for the low sensitivities that we observed using either method. At site 1, the QuickVue Influenza A+B Test (using nasal swab specimens) in comparison with RT-PCR (using throat swab specimens) resulted in a slightly lower sensitivity (19.7%) for either influenza A or influenza B virus than was found in the comparison with viral culture (24.4%), whereas at site 2, the sensitivities for the 2 comparisons were nearly identical (34.4% for comparison with culture and 32.3% for comparison with RT-PCR). These sensitivities were much less than those previously reported in other studies of the QuickVue Influenza A+B Test [9, 10].

Our findings have clinical and public health implications because this rapid influenza diagnostic test is used to promptly detect influenza virus infections to prescribe antiviral treatment or to implement outbreak-control interventions. Because of the very low sensitivity of the QuickVue Influenza A+B Test, the negative predictive value was moderate, indicating that there were false-negative results. Even though the specificity was very high, the positive predictive value was moderately high across all sites, which indicates that although a positive result was reasonably predictive of a diagnosis of influenza for the patients tested, there were also false-positive results. For rapid influenza

diagnostic tests with moderate sensitivity and high specificity compared with RT-PCR, positive and negative predictive values vary by the prevalence of circulating influenza viruses among the population being tested [8]. Other factors that might decrease the sensitivity and accuracy of a rapid influenza antigen test are improper specimen collection; not testing the recommended clinical specimen, because results may vary by the kind of respiratory sample tested [12]; use of a swab that is not recommended (e.g., using an unapproved tip or shaft material or not using the foam swab supplied with the test); prolonged time from illness onset to specimen collection, because viral shedding may have decreased to undetectable levels; and improper handling or storage procedures before testing of specimens. Some possibilities to increase the test sensitivity include use of flocked swabs or testing of combined specimens (e.g., pooled nasal and throat swab specimens). Although we evaluated only 1 of a number of commercially available rapid influenza tests, our findings highlight the need to develop more-sensitive rapid influenza diagnostic tests to detect influenza A and B viruses in respiratory specimens from patients of all ages and to inform patient management.

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