Field Performance of a Rapid Diagnostic Test for Influenza in an Ambulatory Setting

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Provided test characteristics are adequate, point-of-care rapid antigen detection tests for influenza could improve the timeliness and appropriateness of clinical decisions. Our objective was to estimate the field sensitivity and specificity of the Quidel QuickVue Influenza A+B test in an ambulatory setting. The sensitivity and specificity of the Quidel QuickVue test was evaluated against reverse-transcriptase PCR (RT-PCR) on nasopharyngeal specimens collected over two consecutive influenza seasons from ambulatory patients consulting for influenza-like illness (ILI) within 7 days of ILI onset. A total of 491 patients with ILI (180 in 2006 to 2007 and 311 in 2007 to 2008) provided specimens that were tested both by PCR and by the Quidel QuickVue test. Among the 267 patients positive by PCR (55%), 52 were also positive by the QuickVue test, for an overall sensitivity of 19.5% (95% confidence interval [95% CI], 14.7% to 24.2%). Among the 221 PCR-negative patients, 2 were positive for influenza B virus by the rapid test (<1%), for an overall specificity of 99.1% (95% CI, 97.9 to 100%). The field sensitivity of the test varied little with the age or gender of the patient, immunization status, delay since the onset of symptoms, or influenza season. The sensitivity of the test was slightly but nonsignificantly higher for influenza B virus (23%) than for influenza A virus (18%). Despite its high specificity, the low sensitivity of the Quidel QuickVue Influenza A+B test is too poor to direct clinical decisions for ambulatory patients with ILI. Negative results cannot rule out the diagnosis of influenza, and in that context, this test is of questionable utility for routine application in the clinical setting.

Due to its nonspecific presentation, the diagnosis of influenza infection based on clinical features alone remains difficult (13). Confirmed diagnosis of influenza has relied heavily on the results of viral culture, antigen detection, and serology testing. Viral culture was historically established as the gold standard for influenza identification (8, 12, 26). More-recent nucleic acid detection methods, such as reverse transcription-PCR (RT-PCR), are more sensitive than culture but remain unavailable to many physicians (13). Unfortunately, none of these methods provide results in a timely manner to guide clinical decisions for ambulatory patients (16). Point-of-care antigen detection tests are meant to address this problem (9, 15, 23). These rapid tests can influence patient management decisions by reducing the use of unnecessary tests or antibiotics and facilitating timely administration of antiviral drugs (1, 3, 6, 14, 17). The usefulness of rapid tests for influenza is highly dependent on their sensitivity and specificity (16). Estimates of test performance differ with a number of variables, such as patient age, delay since symptom onset, type of specimen, and the influenza type/subtype (5, 23). Given these factors, sensitivities ranging from 60 to 90% and specificities from 65 to 100% have previously been reported for rapid tests, mostly based on comparison with viral culture (16). Estimates of the sensitivity of rapid tests may drop if they are measured against RT-PCR, which detects lower levels of influenza viruses and is itself more sensitive than culture (13).

Few studies have evaluated the performance of rapid antigen detection tests among ambulatory adults (18, 20, 22, 29). The Quidel QuickVue Influenza A+B test has reported sensitivities and specificities ranging from 44 to 95% and 76 to 98%, respectively (10, 15, 19). However, sensitivities as low as 22 to 33% have also been reported (18, 22). In this study, we estimated the sensitivity and specificity of the Quidel QuickVue Influenza A+B test based on PCR detection of influenza virus in ambulatory patients seeking care for influenza-like illness (ILI) during two consecutive influenza seasons.

MATERIALS AND METHODS

Selection of participants. A sentinel network was established to assess the incidence of influenza consultations in ambulatory settings, as part of provincial surveillance activities for influenza in Quebec, Canada. Four private family medicine clinics participated during the 2006–2007 influenza season, and eight participated in the 2007–2008 season. For both seasons, any patient seeking care for an ILI was eligible. ILI was defined as the acute onset of fever and cough. Physicians were advised to favor testing among ILI patients presenting within the first 7 days of symptom onset. After providing consent, patients were asked to complete a self-administered questionnaire on symptoms, underlying medical conditions, and other demographic information.

Specimen collection. Nasopharyngeal aspirates (NPA) were collected by trained nurses. A syringe fitted with an 8FR feeding tube was filled with 2 to 3 ml of sterile saline. With the patient’s head hyperextended, saline solution was instilled into one nostril and aspirated back into the syringe. To prevent cross-contamination, the specimen was promptly divided in two; 0.3 ml was added to the extraction tube of the QuickVue test. The remainder was put in a 1.5-ml sterile container, immediately frozen at −20°C, and sent to the Provincial Public Health Laboratory (Laboratoire de Santé Publique du Québec) for influenza A and B virus nucleic acid testing.

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RT-PCR was performed by laboratory personnel. Hemagglutinin (HA) gene of influenza virus B or the HA gene of the H1 or H3 subtype of influenza virus A or the conventional agarose gel electrophoresis using in-house assays targeting the fluorescence detected to exceed a preset threshold and is inversely proportional to the number of PCR cycles required for the specific gene. The annealing temperature and data collection steps were performed at 57°C. The estimated cycle threshold (Ct) is the number of PCR cycles required for the specific fluorescence detected to exceed a preset threshold and is inversely proportional to the copy number of the target gene. Nucleic acid preparations from samples found positive by real-time detection assays were further analyzed by RT-PCR and conventional agarose gel electrophoresis using in-house assays targeting the hemagglutinin (HA) gene of the H1 or H3 subtype of influenza virus A or the HA gene of influenza virus B. RT-PCR was performed by laboratory personnel blinded to rapid influenza test results.

**Data analysis.** Proportions were compared using the χ² test or Fisher’s exact test. Continuous variables that were normally distributed with equal variances were compared with Fisher’s t test; Satterthwaite’s statistic was used for unequal variances. Continuous variables that were not normally distributed were evaluated with Wilcoxon’s U test. All statistical tests were two-tailed, and P values of 0.05 or less were considered significant. Sensitivity values and their binomial 95% confidence intervals (95% CI) were calculated.

### RESULTS

Specimens were collected between 2 February and 10 April in 2006 to 2007 and between 15 January and 19 April in 2007 to 2008, corresponding with other indicators of peak influenza activity provincially in Quebec. A total of 491 patients (180 in 2006 to 2007 and 311 in 2007 to 2008) with ILI provided specimens that were tested both by PCR and by the Quidel QuickVue test. Although children of any age were also eligible to participate, our sample of patients consisted mostly of adults. The ages of the patients tested ranged from <1 year to 82 years. A total of 94 patients (19%) were children less than 14 years old. The characteristics of patients were generally similar in the two years (Table 1) except for a shorter delay between symptom onset and consultation in the second year (4.3 versus 5.3 days; \(P = 0.0001\)).

Overall, 270 (55%) patients were positive for influenza by PCR (Table 1). Patients who consulted during the second year were more likely to have had a positive PCR than those who consulted during the first year (59% versus 48%; \(P = 0.01\)), but there was no significant difference in the distribution of viruses types identified between the two years. Among patients positive for influenza virus by RT-PCR, 65% were found positive for type A and 34% for type B. Two patients were coinfected...
with influenza viruses A and B (0.7%). Three specimens interpreted as influenza virus A positive by the QuickVue test but found positive for influenza virus B by PCR (1%) were excluded from the analyses of test sensitivity. Among the remaining 267 patients positive by PCR (55%), 52 were positive by the QuickVue test, for an overall sensitivity of 19.5% (95% CI, 14.7–24.2%). Among the 221 PCR-negative patients, 2 were positive for influenza virus B by the rapid test (1%), for an overall specificity of 99.1% (95% CI, 97.9 to 100%). The sensitivity of the QuickVue test was similar between the two influenza seasons (19%) and between genders (17 to 22%) (Table 2). Sensitivity was lower for patients who had been vaccinated against influenza virus for the current season (15%) than for those who had not been vaccinated (20%), although this difference was not statistically significant. Sensitivity was similar for younger adults (23 to 25%) but was reduced by about half for patients aged 40 years and over (10 to 12%). The QuickVue test’s sensitivity was greater for patients without preexisting medical conditions (20% versus 14%) and those not vaccinated against pneumococci (12% versus 20%), although immunization status is strongly associated with the presence of underlying medical conditions ($P < 0.001$). The sensitivity of Quidel’s QuickVue test decreased with a longer delay between the onset of symptoms and the collection of specimen (5 to 25%). It was highest for patients who consulted 3 to 4 days after the onset of symptoms (24%) and dropped to 5% for patients who consulted more than 7 days after onset. Sensitivity was slightly but not significantly higher for influenza B virus than for influenza A virus (23% versus 18%, respectively; $P = 0.17$).

As expected, the sensitivity of the Quidel assay increased with the relative concentration of RNA targets in the sample. There was a significant association between higher concentrations of viral particles and sensitivity ($P < 0.001$). When $C_T$ values were lower than 25 cycles, the sensitivity of the QuickVue test was 50% (38 to 62%), but it dropped to 15% when $C_T$ values were between 25 and 32 and to 1% when $C_T$ values were greater than 32.

**DISCUSSION**

Point-of-care rapid tests for influenza are now available to physicians in both hospital and ambulatory settings. Previous reports have suggested that rapid tests are associated with sufficient sensitivity and specificity to be used, albeit with caution, in the routine detection of influenza virus among ILI patients (7, 11, 27). The results obtained from our study indicate that while the specificity of the test was nearly perfect

<table>
<thead>
<tr>
<th>TABLE 2. Sensitivity of the QuickVue influenza test by select covariates</th>
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<tbody>
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<td>Covariate</td>
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<tr>
<td>Overall test sensitivity</td>
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<td>Patient age (yr)</td>
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<td>&lt;19</td>
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<td>40–49</td>
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<td>≥60</td>
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<td>Patient gender</td>
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<tr>
<td>Male</td>
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<tr>
<td>Female</td>
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<tr>
<td>Vaccination against influenza virus</td>
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<td>Yes</td>
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<td>No</td>
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<tr>
<td>Vaccination against <em>Pneumococcus</em></td>
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<td>Yes</td>
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<tr>
<td>No</td>
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<td>Any condition requiring medical treatment</td>
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<td>Days since onset</td>
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<td>Influenza infection (as determined by PCR)</td>
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<td>Positive for influenza A virus</td>
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<td>$C_T$</td>
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Analyses were performed on patients with concordant single viral infections ($n = 265$).
(99%), there was suboptimal field performance of the sensitivity of the rapid test, even among patients for whom a high index of suspicion for influenza exists. Our results show that less than 1 in 5 influenza patients with clinical ILI were correctly identified by the rapid test performed in the physician’s office. This is lower than the rate in previous studies. Since most studies assessing the sensitivity of the rapid test compared it to viral culture, lower sensitivity relative to the more-sensitive PCR assay is reasonable, although it is even lower than we had anticipated (13).

Our results are consistent with other reports suggesting lower sensitivity among adults (16, 18, 22). Stein et al. conducted a study among 258 adults presenting to the emergency room with a new illness associated with cough, sinus pain, nasal congestion, rhinorrhea, sore throat, or fever. In this setting, the Quidel QuickVue test had an overall sensitivity of 33%, which was comparable to the performance of clinical judgment (22).

In a 2005-2006 study of 555 United Kingdom pilgrims attending the Hajj, the overall sensitivity of the test was 22%. Both studies were carried out with patients who presented within 7 days after onset of a respiratory illness associated with cough, sore throat, rhinorrhea, or fever (18). Our study has the advantage of testing based on a consistent clinical case definition within a discrete population of ambulatory patients visiting general practitioners’ offices.

Other factors may contribute to the low sensitivity found in our study. While the majority of our patients were above the age of 14 years (81%), most previous studies of the field performance of rapid tests used specimens from pediatric patients; since children shed considerably more virus than adults, the sensitivity for the latter group may be lower. We used NPA from all patients, irrespective of age, which is the type of specimen expected to yield the largest number of virally infected epithelial cells (28). Our procedure required the instillation of 2 ml of sterile solution into one nostril, which may have diluted the final sample and contributed to lower sensitivity. However, our procedures were carried out in accordance with the manufacturer’s instructions included in the package insert, which suggested the instillation of 2.5 ml of saline. To date, few studies have attempted to specifically compare the effect of clinical specimen type on rapid test performance. The manufacturer’s insert presents higher test sensitivity results for nasal swabs (94% and 70% for influenza A and B viruses, respectively) than for nasal washes and aspirates (77% and 82% for influenza A and B viruses, respectively), but our results remain much lower (5).

Results from other studies suggest that the delay since the onset of symptoms is an important factor driving test sensitivity, and our results also support this hypothesis. In a study conducted with adults, the overall sensitivity of the QuickVue test was 74%, ranging from 86% on day 1 of illness to 50% on the second day and 0% on the fourth day (2). In our study, most patients diagnosed with influenza virus infections presented 3 to 4 days after symptom onset. For this group of patients, sensitivity was only 24%. In another study of three distinct populations of children and adults, the QuickVue test had an overall sensitivity of 27% relative to PCR (24).

Our results are consistent with those previously published suggesting lower sensitivity when patients were tested 48 h or more following the onset of symptoms (2, 4, 22, 24). Previous reports suggesting that rapid tests have sufficient sensitivity to be used in ambulatory settings were typically based on tests conducted with pediatric patients presenting within 48 h of illness onset. Because antiviral treatment is most effective when administered within 48 h of symptom onset, that threshold is of great clinical relevance. In the real-world context, few patients actually seek outpatient medical care within such a short time, further limiting the clinical usefulness of the Quidel QuickVue Influenza A+B test in ambulatory settings.

Point-of-care rapid tests are designed and marketed for use in the ambulatory setting, in order to guide physicians in making the best possible clinical decisions. Our sentinel physicians considered the Quidel QuickVue Influenza A+B test insufficient for the needs of ambulatory adult patients due to its poor sensitivity. Negative results for patients with classic ILI created uneasiness, because physicians felt that they had to justify their diagnoses to their patients. Because the Quidel QuickVue Influenza A+B test is easy to perform and has good specificity, it could nevertheless be useful in clinical or epidemiological situations where test sensitivity is not a critical issue. For example, it may be acceptable for community surveillance, because despite its low sensitivity, it may appropriately detect epidemiological trends. It may also be useful in facility outbreaks where multiple specimens are collected so as to identify the causative organism rapidly. The collection of multiple samples per outbreak could compensate for the low sensitivity. In any case, negative results must be interpreted with caution.

In conclusion, the low sensitivity of the Quidel QuickVue Influenza A+B test for ambulatory adult patients with ILI suggests that its use should probably be limited to situations where sensitivity is not an issue.

REFERENCES


