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Poor Positive Accuracy of QuickVue Rapid Antigen Tests during the Influenza A (H1N1) 2009 Pandemic[▽]

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We assessed the accuracy of positive QuickVue rapid influenza virus antigen test results. Using reverse transcription (RT)-PCR as the gold standard, 17 (37.8%) of 45 QuickVue-positive specimens were determined to be false positives. We report an unexpectedly high rate of false-positive QuickVue results during a period of high influenza A (H1N1) 2009 virus prevalence.

The emergence of the influenza A (H1N1) 2009 virus in April 2009 (3) has refocused attention on the clinical diagnostic performance of rapid influenza virus antigen tests (RIATs) (2, 6). These tests are widely used in physician offices and hospital laboratories because of their simplicity and speed. RIATs are well known to have poor sensitivity, including for the influenza A (H1N1) 2009 virus (1, 4, 5, 7–10). Few studies have reported poor specificity of RIATs (8), and the widely held view is that the specificity and positive predictive value (PPV) of these tests are relatively high when they are performed during periods of high disease prevalence.

Our institution performed the QuickVue Influenza and Influenza A+B RIATs (Quidel Corp., San Diego, CA) during the influenza A (H1N1) 2009 virus pandemic. The QuickVue Influenza test detects influenza A and B viruses but does not distinguish between them, while the QuickVue Influenza A+B test detects and distinguishes influenza A and B viruses. During routine laboratory testing between April and November 2009, we observed a number of QuickVue-positive specimens that were negative by real-time reverse transcription (RT)-PCR. We also observed QuickVue A+B-positive specimens that had either weak A or weak A and B bands. We evaluated the positive accuracy of the QuickVue RIATs performed during the influenza A (H1N1) 2009 virus pandemic and factors associated with false-positive results and weak bands.

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This study employed a known convenience sample of RIAT-positive specimens. Specimens included nasal, nasopharyngeal, and throat swabs in virus transport medium (VTM) (Multitrans [Starplex Scientific Inc., Etobicoke, Ontario, Canada] or universal virus transport medium [Becton Dickinson, Franklin Lakes, NJ]) and nasal washes. Patients were both pediatric and adult. For specimens with positive QuickVue Influenza or Influenza A+B results that were available to the University of Texas Medical Branch Clinical Microbiology Laboratory (CML) and possessed sufficient volume, an aliquot was removed and stored at −70°C.

The QuickVue Influenza test was performed on saline nasal washes or dry swabs at point-of-care (POC) locations in our institution starting at the beginning of the influenza A (H1N1) 2009 virus pandemic. If confirmatory testing was warranted, a second swab was collected immediately or remaining nasal wash was sent to the CML. Early in the pandemic, most QuickVue-positive specimens were shipped to the state public health laboratory for confirmatory testing or subtyping using the CDC real-time RT-PCR assay. The QuickVue A+B test was performed on swab specimens in VTM or saline nasal washes in the CML starting in September 2009. The intensity of QuickVue A+B bands was noted as positive or weakly positive. The intensity of QuickVue Influenza bands at POC clinics was not noted. The ProFlu+ real-time RT-PCR test (Gen-Probe Prodesse Inc., Waukesha, WI) was performed on all specimens available for this study. Specimens positive for influenza A by the ProFlu+ test were then tested by subtype-specific RT-PCR (ProFlu-ST; Gen-Probe Prodesse). In addition, the CDC influenza virus subtyping RT-PCR was performed at the state public health laboratory on specimens collected early in the outbreak, prior to availability of RT-PCR on site. Each ProFlu+ RT-PCR run included negative and positive controls that were subjected to all steps of the procedure. QuickVue results were considered to be false positive if the RT-PCR result was negative. Odds ratios were calculated using 2×2 contingency tables comparing true and false positives for each specimen type. The Fisher exact test was used to compare proportions in contingency tables. For comparison between two groups, data were analyzed using the Mann-Whitney test. A P value of <0.05 was considered statistically significant.

Thirty specimens positive by the QuickVue Influenza test, collected between April and July 2009, were available for evaluation. Twelve (40%) of these were negative for influenza A and B viruses by RT-PCR. All 12 were negative by the ProFlu+ test. Additionally, five of these specimens were also evaluated by the CDC RT-PCR test, which yielded negative results. Eighteen specimens produced at least one positive RT-PCR test result. Of these, three specimens were evaluated with the ProFlu+/ProFlu-ST tests only (two were positive for influenza A/H1N1 2009 virus, and one was positive for influenza B virus). The ProFlu and CDC RT-PCR tests were both

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TABLE 1. Distribution of specimen types by QuickVue result[a]

Specimen type	No. (%) of specimens with QuickVue result		Odds ratio (95% CI)	*P* value[b]
	True positive	False positive		
Nasal swab	20 (74.1)	6 (35.3)	5.238 (1.441–19.057)	0.015
NP swab	1 (3.7)	0		
Throat swab	3 (11.1)	5 (29.4)	0.30 (0.067–1.359)	0.227
Nasal wash	3 (11.1)	6 (35.3)	0.229 (0.053–1.017)	0.068
Total	27	17		

[a] Not included for statistical analysis: one QuickVue true-positive specimen with unspecified source. NP, nasopharyngeal.
[b] Fisher exact test, two-tailed.

performed on 15 specimens with positive QuickVue Influenza results. Eleven of these had concordant positive influenza A/H1N1 2009 virus results, one had concordant positive influenza B virus results, and three had discordant ProFlu+ and CDC RT-PCR results. Of the specimens with discordant positive RT-PCR results, two were positive for influenza B virus by ProFlu+ and negative by CDC RT-PCR, and one was positive for influenza A/H1N1 2009 virus by CDC RT-PCR and negative by ProFlu+. Fifteen specimens positive by the QuickVue A+B test, collected between September and November 2009, were available for evaluation. Only the ProFlu+/ProFlu-ST RT-PCR tests were performed on these specimens. Five (33.3%) QuickVue A+B-positive specimens were negative by RT-PCR. Ten specimens were positive for influenza A/H1N1 2009 virus by ProFlu+/ProFlu-ST. Combined, the false-positive rate of both QuickVue RIATs was 17 of 45 (37.8%).

The median age of patients with true-positive QuickVue RIAT results was 12 years, compared to 20 years for patients with false-positive results ($P = 0.58$). The distribution of specimen types by RIAT result status is shown in Table 1. Nasal washes represented a higher proportion of specimens with false-positive RIAT results (35.3%) than specimens with true-positive results (11.1%), although this difference did not reach significance ($P = 0.068$).

A review of laboratory records from September through November 2009 showed that of 35 positive QuickVue A+B results (out of 281 total tests performed), 15 (42.9%) were noted to have weak bands (14 results were reported as weak A band present; one result was reported as both weak A and weak B bands present). Of the 15 total QuickVue A+B-positive specimens available for further RT-PCR analysis (which includes specimens with strong bands and specimens with weak bands), four of the five (80%) RT-PCR negative specimens had weak A ($n = 3$) or weak A and B ($n = 1$) bands and 8 of the 10 (80%) RT-PCR-positive specimens had weak A ($n = 7$) or weak A and B ($n = 1$) bands. Specimens with false-positive QuickVue A+B results were no more likely to have weak bands than specimens with true-positive QuickVue A+B results. ProFlu+ crossing threshold (C_T) values of specimens with weak QuickVue A+B bands ($n = 8$) were compared to specimens with strong QuickVue A+B bands ($n = 2$). The mean C_T of specimens with weak QuickVue A+B bands (25.6; 95% confidence interval, 24.5 to 26.78) was higher than that of specimens with strong QuickVue A+B bands (21.7; 95% confidence interval, 21.11 to 22.29) ($P = 0.044$), suggesting that weak bands in the QuickVue A+B test may be associated with low virus titers in specimens.

Most studies have reported relatively high QuickVue RIAT specificities of 84 to 100% and PPV of 84 to 97% for influenza A viruses, including the H1N1 2009 pandemic subtype (4, 7, 9, 10). One recent study reported QuickVue specificity of 46.4% during the influenza A (H1N1) 2009 pandemic, but according to the authors, data may have been skewed by testing algorithms (8). The rate of false-positive QuickVue RIAT results (37.8%) observed in this study was unexpected, given the high disease prevalence during the study period (average monthly culture positivity rate through October 2009 on a different sample set was 27.5%, decreasing to 9.1% in November 2009). As stated previously, separate swabs were used to perform the QuickVue Influenza RIAT at POC locations and RT-PCR. Sampling error could account for some of the negative RT-PCR results. However, the QuickVue A+B test and RT-PCR were performed with the same swab and produced similar RIAT false-positive rates. For this study, we defined false-positive QuickVue specimens as those negative by Food and Drug Administration-cleared, real-time RT-PCR assays. RT-PCR has become a standard of care for influenza virus diagnostics; a positive result represents a laboratory-confirmed case, according to the current CDC case definition (http://www.cdc.gov/h1n1flu/specimencollection.htm). QuickVue RIATs were performed on fresh specimens shortly after collection, while RT-PCR was carried out on frozen specimens. Both CDC and ProFlu+ RT-PCR assays were performed on a subset of 20 specimens, producing 17 (85%) concordant results. Discordant RT-PCR results included two ProFlu+ influenza B-positive/CDC-negative results and one CDC influenza A H1N1 2009 virus-positive/ProFlu+-negative result. The discordant influenza B virus results were obtained early in the study, when influenza B virus was circulating in the local area, and both patients had signs and symptoms consistent with influenza. The specimen with discordant influenza A (H1N1) 2009 virus results was further tested by RT-PCR at Gen-Probe Prodesse using alternate primers and failed to yield positive results. We speculate that RNA may have degraded in this specimen, perhaps during the freeze/thaw process.

While patients with false-positive QuickVue RIAT results were older than patients with true-positive results (median age, 20 years versus 12 years), this difference in these relatively small sample sets was not significant. Interestingly, nasal washes represented a higher proportion of QuickVue false-positive specimens than true-positive specimens (35.3% versus 11.1%) ($P = 0.068$). Nasal wash is an indicated specimen type for the QuickVue RIATs. We should note that some false-positive QuickVue results were produced from throat swabs, a specimen type for which the manufacturer has no performance claims. Another limitation of this retrospective study is that we employed a known convenience sample of specimens that were positive by the QuickVue RIATs. As such, we could not calculate the specificity of the QuickVue RIATs because we did not determine the true infection status of all specimens. We were not able to perform RT-PCR on every QuickVue RIAT-positive specimen for several reasons, including specimen volume and availability of personnel to locate, catalogue, and aliquot samples. We believe that our data from this subset of

RIAT-positive specimens reflect the performance that would be observed in all positive specimens in our patients. RT-PCR confirmation/subtyping of positive QuickVue RIAT specimens was performed on a diverse patient population: pediatric and adult, outpatients (clinics and emergency department), and hospitalized patients. Specimens that were included in the study were selected solely based on a positive RIAT result without knowledge of source, patient information, or clinical history.

Weak influenza virus bands in the QuickVue A+B test were frequently observed (nearly 43% of all positive results). False-positive QuickVue A+B results were no more likely to have weak bands than true-positive results. Specimens with weak bands had higher RT-PCR C_T values, suggesting that low virus titers may be associated with weak bands.

In conclusion, we observed an unexpectedly high rate of false-positive QuickVue RIAT results during a period of high influenza A (H1N1) 2009 virus prevalence. Patient age and specimen source may be associated with false-positive QuickVue RIAT results, although additional studies are required. Weak bands were frequently observed with the QuickVue A+B RIAT but were not associated with false-positive results.

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