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Letters to the Editor

High Sensitivity of a Rapid Immunochromatographic Test for Detection of Influenza A Virus 2009 H1N1 in Nasopharyngeal Aspirates from Young Children[▽]

Recent reports have suggested that immunochromatographic tests (ICTs) have poor sensitivity for influenza A virus 2009 H1N1 (H1N1 09) infection on swabs (2) and nasopharyngeal washes (1), but they do have advantages, including short turnaround time, lack of hardware, and minimal validation requirements (6). We used the QuickVue Influenza A+B ICT test (Quidel Corp., San Diego, CA) during the winter of 2009 at a major pediatric teaching hospital in Australia. Here we provide in-use pediatric ICT performance estimates for H1N1 09, examine the effect of age and specimen type on sensitivity, and discuss the utility of ICT assays in guiding treatment and infection control decisions.

Once H1N1 began circulating, our state public health response had two phases. In the "contain phase" (22 May to 16 June 2009), all patients presenting with influenza-like illness were tested. A specimen aliquot was sent to the state reference laboratory for influenza A virus testing and strain typing by PCR (2). These results took over 48 h, which was too slow for therapeutic or infection control purposes. During the "protect phase" (17 June onwards), only admitted patients and those with underlying medical conditions had specimens collected, and only specimens positive for influenza A virus by local testing were referred for PCR confirmation. Nasopharyngeal aspiration (NPA) was performed with a size 6 or 8 French flexible suction catheter with attached sputum trap. Flocked nasal swabs with universal transport medium (UTM kit; Copan, CA) and a rayon throat swab were combined for processing.

Our laboratory performed the ICT according to the manufacturer's instructions. Direct fluorescent antibody (DFA) testing for respiratory viruses using the Similfluor respiratory screen (Chemicon, CA) was performed on all negative specimens or specimens not tested by ICT. This assay detects influenza A and B virus, respiratory syncytial virus, parainfluenza virus (serotypes 1, 2, and 3), adenovirus, and human metapneumovirus. All specimens negative by DFA were cultured on R-Mix cells (Diagnostic Hybrids, OH) for 3 days and then stained with influenza A virus antibodies (Imagen; DaktoCytomation, Ely, United Kingdom). All specimens positive for influenza A virus by any local test (ICT, DFA, or culture) were referred for confirmatory influenza A virus PCR and strain typing.

During our 2009 influenza season (June to September), 970 children were tested for respiratory viral infection, and 265 cases of PCR-proven H1N1 09 were detected. Of these, 252 presented during the "protect phase." Each patient's first positive specimen was analyzed for test performance. Of 265 positive specimens, 216 (81.5%) had ICT performed, and 171 (79.2%) of those were positive. The sensitivity of the ICT test for H1N1 09 was significantly greater on NPA specimens (84.1%) than on swab specimens (66.2%) ($P = 0.003$). Patient age significantly affected the sensitivity of the ICT on NPAs ($P = 0.003$), but not on swabs ($P = 0.45$) (Table 1). The specificity of the ICT was calculated as the number of patients without influenza A virus with a negative ICT result divided by the number of patients without influenza A virus tested by ICT. The specificity was 100% for swabs, and it was 98.4% for NPAs.

Higher viral shedding in younger children probably explains the high observed sensitivity (90%) of the ICT for the detection of H1N1 09 in NPA specimens from children less than 5 years old. Our ICT sensitivity estimates during the "protect phase" depend on a hierarchical local testing algorithm with viral culture performed on ICT- and DFA-negative specimens. It has been argued (4) that PCR may be a more appropriate reference standard than culture, and certainly comparison to PCR rather than culture would have yielded slightly lower sensitivity estimates. However, PCR-positive, culture-negative specimens may represent false-positive PCR results or may contain influenza A virus RNA but no viable virus (3). It is unknown whether patients with such results benefit from antiviral therapy or pose an infectious risk to others. Since specimens positive by DFA were not set up for viral culture, it is possible that occasional cases of coinfection by respiratory viruses were missed.

The capacity of a negative ICT result to rule out influenza A

TABLE 1. Relationship between age, specimen type, and ICT positivity in children with proven influenza A virus H1N1 09 infection who had the ICT performed[a]

Specimen type and patient age group	No. of patients with ICT performed	No. of patients with positive ICT result for influenza A virus	ICT sensitivity (%) (95% CI)
Respiratory swabs			
All ages	71	47	66.2 (54.0–77.0)
1st age quartile (0–4.0 yr)	17	10	58.8 (32.9–81.6)
2nd age quartile (4.1–6.7 yr)	18	11	61.1 (35.7–82.7)
3rd age quartile (6.8–11.1 yr)	18	13	72.2 (46.5–90.3)
4th age quartile (11.2–18 yr)	18	13	72.2 (46.5–90.3)
Nasopharyngeal aspirates			
All ages	145	122	84.1 (77.2–89.7)
1st age quartile (0–0.70 yr)	36	32	88.9 (73.9–96.9)
2nd age quartile (0.71–1.83 yr)	36	33	89.2 (74.6–97.0)
3rd age quartile (1.84–4.9 yr)	36	33	91.7 (77.5–98.2)
4th age quartile (5.0–18 yr)	36	24	66.7 (49.0–81.4)

[a] There was a relationship between age and ICT positivity for NPAs ($P = 0.003$), but not for respiratory swabs ($P = 0.45$). This was evaluated by significance tests derived from a logistic regression model using Stata 9.0 (StataCorp, College Station, TX) and including age, specimen type, and an interaction term.

virus infection can be expressed by the negative predictive value (NPV). This measures the probability that a patient with a negative test result is truly free of the disease (5). For the whole 2009 influenza season, the NPV of the ICT on an NPA specimen from a child under 5 years old was 97.5%. The NPV of these specimens was also calculated for each of five phases in the season, early, early-mid, mid, late-mid, and late in the season. The prevalence of H1N1 09 ranged from 25/82 (30.5%) midseason down to 33/274 (12.0%) late in the season. The prevalence of any influenza A virus ranged from 32/82 (39.0%) midseason down to 38/274 (13.9%) late in the season. Interestingly, the NPV was lower (38/42 [90.5%]) early in the season (10 to 29 June) than in the subsequent seasonal phases, when it ranged from 94.3% to 99.6%. This reflected a lower ICT sensitivity (5/9 [56%]) in the early season than in subsequent phases when it varied from 88% to 97%. We hypothesize that this low early sensitivity may have been related to inexperience in interpreting the test, particularly after hours when it was performed by nonvirology staff. It was not related to the use of PCR as the comparator during the "contain phase," since no NPA specimens from children under 5 years old with influenza A virus were tested with the ICT during this period. The high NPVs obtained, particularly once staff were familiar with the assay, indicate that clinicians and infection control practitioners may have a reasonable level of confidence that H1N1 09 infection has been excluded by a negative ICT test on an NPA from a young child.

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