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High Sensitivity of a Rapid Immunochromatographic Test for the Detection of Influenza A Virus 2009 H1N1 on Nasopharyngeal Aspirates from Young Children

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Recent reports have suggested that immunochomatographic tests (ICT's) 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 2009 Australian winter at a major pediatric teaching hospital. 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" (22nd May to 16th June 2009) all patients presenting with influenza-like illness were tested. A specimen aliquot was sent to the state reference laboratory for Influenza A testing and strain typing by PCR[2.]. These results took over 48 hours which was too slow for therapeutic or infection control purposes. During the "protect phase" (17th June onwards), only admitted patients and those with underlying medical conditions had specimens collected, and only specimens positive for Influenza A by local testing were referred for PCR confirmation. Nasopharyngeal aspiration (NPA) was performed with a 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 specimens negative or not tested by the ICT. This assay detects Influenza Virus A&B, Respiratory Syncytial Virus, Parainfluenza Virus 1,2 &3, Adenovirus, and Human Metapneumovirus. All specimens negative by DFA were cultured on R-Mix cells (Diagnostic Hybrids, Ohio) for 3 days then

stained with Influenza A antibodies (Imagen, DaktoCytomation, Ely, UK). All specimens positive for Influenza A by any local test (ICT, DFA or culture) were referred for confirmatory Influenza A PCR and strain typing.

During our 2009 influenza season (June-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 the 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 NPA's (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 in whom the ICT was negative, divided by the number of patients without Influenza A in whom the ICT was performed. This was 100% on swabs and 98.4% on NPA's.

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 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 co-infection by respiratory viruses were missed.

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49 The capacity of a negative ICT to rule out Influenza A infection can be expressed by the negative
50 predictive value (NPV). This measures the probability that a patient with a negative test result is truly
51 free of the disease.[5] For the whole 2009 Influenza season, the NPV of the ICT on an NPA specimen from
52 a child under 5 was 97.5%. The NPV of these specimens was also calculated for each of 5 seasonal
53 phases: early, early-mid, mid, late-mid, and late season. The prevalence of H1N1 09 ranged from 25/82
54 (30.5%) mid-season down to 33/274 (12.0%) late-season. The prevalence of any Influenza A ranged from
55 32/82 (39.0%) mid-season down to 38/274 (13.9%) late-season. Interestingly, the NPV was lower (38/42,
56 90.5%) in the early season (10 – 29 June) than in the subsequent seasonal phases when it ranged from
57 94.3% to 99.6%. This reflected a lower ICT sensitivity (5/9, 56%) in the early season than in subsequent
58 phases when it varied from 88% to 97%. We hypothesise that this low early sensitivity may have been
59 related to inexperience in interpreting the test, particularly after hours when it was performed by non-
60 virology staff. It was not related to the use of PCR as the comparator during the "contain" phase, since
61 no NPA specimens from children under 5 with influenza A were tested with the ICT during this period.
62 The high NPV's obtained, particularly once staff were familiar with the assay, indicate that clinicians and
63 infection control practitioners may have a reasonable level of confidence that H1N1 09 infection has
64 been excluded by a negative ICT test on a NPA from a young child.

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70 **References**

71 **1. Karre T, Maguire HF, Butcher D, Graepler A, Weed D, and Wilson ML**. 2010. Comparison of Becton
72 Dickinson Directigen EZ Flu A+B Test against the CDC real-time PCR assay for detection of 2009
73 pandemic Influenza A/H1N1 virus. J. Clin. Microbiol. **48**: 343-344
74 **2. Kok J, Blyth CC, Foo H, Patterson J, Taylor J, McPhie K, Ratnamohan VM, Iredell JR, and Dwyer DE**.
75 2010. Comparison of a rapid antigen test with nucleic acid testing during cocirculation of pandemic
76 Influenza A/H1N1 2009 and seasonal Influenza A/H3N2. J. Clin. Microbiol. **48**: 290-291
77 **3. Mehlmann M, Bonner AB, Williams JV, Dankbar DM, Moore CL, Kuchta RD, Podsiad AB, Tamerius**
78 **JD, Dawson ED, and Rowlen KL.** 2007. Comparison of the MChip to viral culture, reverse transcription-
79 PCR, and the QuickVue Influenza A+B test for rapid diagnosis of influenza. J. Clin. Microbiol. **45**: 1234-
80 1237
81 **4. Ruest A, Michaud S, Deslandes S, and Frost EH**. 2003. Comparison of the Directigen Flu A+B test, the
82 QuickVue Influenza Test, and Clinical Case Definition to viral culture and reverse-transcription PCR for
83 rapid diagnosis of influenza virus infection. J, Clin. Microbiol. **41**: 3487-93
84 **5. DL Sackett, RB Haynes, GH Guyatt, and P. Tugwell**. 1991. Clinical epidemiology. A basic science for
85 clinical medicine, 2nd ed., Little, Brown and Co., Boston, MA.
86 **6. Welch DF, and Ginocchio CC**. 2010. Role of rapid immunochomatographic antigen testing in diagnosis
87 of Influenza A Virus 2009 H1N1 infection. J. Clin. Microbiol. **48**: 22-25
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Patient age	Patients with ICT performed	Patients with positive ICT for Influenza A	ICT Sensitivity (95% CI)	Significance Test
Respiratory Swabs				
All ages	71	47	66.2% (54.0 – 77.0%)	p = 0.45 for relationship between age and ICT positivity of respiratory swabs*
1st age quartile (0 to 4.0 years)	17	10	58.8% (32.9 – 81.6%)	
2nd age quartile (4.1 – 6.7 years)	18	11	61.1% (35.7 – 82.7%)	
3rd age quartile (6.8 – 11.1 years)	18	13	72.2% (46.5 – 90.3%)	
4th age quartile (11.2 – 18 years)	18	13	72.2% (46.5- 90.3%)	
Nasopharyngeal Aspirates				
All ages	145	122	84.1% (77.2 – 89.7%)	p = 0.003 for relationship between age and ICT positivity of NPA's*
1st age quartile (0 – 0.70 years)	36	32	88.9% (73.9 – 96.9%)	
2nd age quartile (0.71 – 1.83 years)	36	33	89.2% (74.6 – 97.0%)	
3rd age quartile (1.84 – 4.9 years)	36	33	91.7% (77.5 – 98.2%)	
4th age quartile (5.0 – 18 years)	36	24	66.7% (49.0 – 81.4%)	

* significance tests derived from a logistic regression model using Stata 9.0 (StataCorp, College Station, TX) including age, specimen type, and an interaction term

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