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Comparison of a Rapid Antigen Test with Nucleic Acid Testing during Cocirculation of Pandemic Influenza A/H1N1 2009 and Seasonal Influenza A/H3N2[▽]

Jen Kok,* Christopher C. Blyth, Hong Foo, Jason Patterson, Janette Taylor, Kenneth McPhie, V. Mala Ratnamohan, Jonathan R. Iredell, and Dominic E. Dwyer

Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, New South Wales, Australia

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The rapid diagnosis of influenza is critical in optimizing clinical management. Rapid antigen tests have decreased sensitivity in detecting pandemic influenza A/H1N1 2009 virus compared to seasonal influenza A subtypes (53.4% versus 74.2%, $P < 0.001$). Nucleic acid tests should be used to detect pandemic influenza virus when rapid antigen tests are negative.

The new pandemic influenza virus A/H1N1 2009 (hereafter referred to as H1N1 09) emerged in March 2009, and a pandemic was declared the following June. As of 11 October 2009, 399,232 laboratory-confirmed cases with more than 4,735 deaths had been reported worldwide (http://www.who.int/csr/don/2009_10_16/en/index.html), although this significantly underestimates the community attack rate. Clinical management of influenza is optimized by the use of rapid diagnostic tests, which facilitate early antiviral use and implementation of infection control measures while obviating the need for further unnecessary investigations and treatment.

Rapid antigen tests (RAT) in a point-of-care format allow a diagnosis of influenza virus infection to be made in patients with influenza-like illness (ILI) within 15 to 30 min. Previous experience has demonstrated high specificities of 94 to 100% but variable sensitivities of 39 to 80% compared to viral culture (1, 3, 5, 10). Furthermore, RAT do not distinguish the different influenza A virus subtypes. Although viral culture remains the "gold standard" for the diagnosis of influenza, nucleic acid testing (NAT) using reverse transcriptase PCR (RT-PCR) has become the method of choice in many laboratories due to its increased sensitivity (2, 6) and its ability to subtype using a multiplex platform. However, both RT-PCR and viral culture have longer turnaround times than RAT and require specialized equipment and technical expertise.

We compared the QuickVue Influenza A+B test (hereafter QuickVue; Quidel Corp., San Diego, CA) to two NAT methods during the 2009 Australian winter, where there was high-level cocirculation of the H1N1 09 and A/H3N2 influenza viruses (seasonal influenza A/H1N1 and B viruses were uncommon). Nose and/or throat swabs collected from patients with ILI were placed in viral transport medium, transported to the laboratory within 24 h, and processed within 72 h. Swab tips were then combined in 1 ml of Hanks' solution, vortexed for 10 s, and divided into two aliquots for RAT and NAT. Nucleic acid extraction was performed by either manual (High Pure PCR product purification kit [Roche Diagnostics GmbH, Mannheim, Germany]) or automated techniques (Qiagen bioROBOT EZ [Qiagen, Valencia, CA] or Abbott M2000 SP [Abbott Molecular Inc., Des Plaines, IL]).

The QuickVue RAT, directed against influenza A and B nucleoprotein, was performed according to the manufacturer's instructions (7). NAT was also performed by either (i) a two-step real-time in-house RT-PCR (hereafter RT-PCR) using primers and a TaqMan probe (Applied Biosystems, Foster City, CA) targeting the H1N1 09 hemagglutinin region and derived from published GenBank sequences (http://www.ncbi.nih.gov/genomes/FLU/SwineFlu.html) (forward primer, 5′-GGCATTCACCATCCATCTACT-3′; reverse primer, 5′-TTCTTGATCCCTCACTTTGG-3′; probe, 5′–6-carboxyfluorescein–CTTCTTGCTGTATCTTGATGWCCCCAC–Black Hole Quencher 1–3′) and primers targeting the matrix (M) region of influenza A virus (9) and/or (ii) the AusDiagnostics Easy-Plex Influenza profile 6 assay (AusDiagnostics, Sydney, Australia), a commercial nested multiplexed tandem PCR (hereafter MT-PCR) with primers targeting hemagglutinin H1 for seasonal influenza A/H1N1 virus, hemagglutinin H3 for influenza A/H3N2 virus, influenza A virus matrix protein for all influenza A virus subtypes, influenza B virus nucleoprotein, and H1N1 virus nucleoprotein specific for pandemic H1N1 09. Additional subtyping was performed if the RT-PCR was negative for H1N1 09 but positive for the M gene using previously described methods (9) or the MT-PCR assay.

An evaluation of 433 clinical specimens from patients with ILI showed that the RT-PCR and MT-PCR assays had comparable sensitivities and specificities for detecting H1N1 09 and non-H1N1 09 viruses. One hundred forty-six samples (33.7%) were positive for influenza virus by either assay. Seventy-seven samples (17.8%) were positive for H1N1 09 by RT-PCR; 68 of the 77 samples (88%) were also MT-PCR positive. Of the nine samples with discrepant results (RT-PCR positive but MT-PCR negative), manual analysis of melting curves identified four which were positive for H1N1 09 but had been misidentified as negative by the automated system. Thus,

* Corresponding author. Mailing address: Centre for Infectious Diseases and Microbiology Laboratory Services, Level 3, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, New South Wales 2145, Australia. Phone: 61 2 9845 6255. Fax: 61 2 9893 8659. E-mail: jen.kok@swahs.health.nsw.gov.au.

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upon manual analysis of the melting curves, the sensitivity of the MT-PCR assay, compared to that of the RT-PCR assay, improved from 88% (68/77) to 93.5% (72/77). Of the remaining five discrepant samples, four were negative for both H1N1 09 and seasonal influenza viruses and one was positive for influenza A/H3N2 virus. Despite the nested format of the MT-PCR assay, it was less sensitive in detecting H1N1 09. This difference may reflect the different gene targets of the assays and/or the possibility of antigenic mutation/shift in the primer or probe binding areas.

Five hundred specimens from patients with ILI were tested with QuickVue RAT and NAT (388 with RT-PCR, 97 with MT-PCR, and 15 with both). There were 163 (33%) influenza A virus-positive and 337 influenza virus-negative RAT results. Influenza virus was detected by either NAT method in 269 samples (53.8%), with 174 samples positive for H1N1 09 (64.7%), 88 positive for influenza A/H3 virus (32.7%), and 9 positive for other viruses (3.3%; 3 positive for seasonal influenza A/H1N1, 6 positive for untypeable virus). The six samples positive for untypeable virus by MT-PCR were RT-PCR influenza A matrix gene positive at low levels. Two samples (0.7%) were coinfected with H1N1 09 and influenza A/H3 virus. Ninety-three of 174 H1N1 09-positive samples, 68 of 88 A/H3-positive samples, and 4 of the 9 remaining samples were identified by RAT. The overall sensitivity of the QuickVue RAT, compared to that of NAT, was 60.6%. This was significantly reduced when H1N1 09 was compared to influenza A/H3 virus (53.4% versus 77.2%; $P = 0.0002$ by Fisher's exact test) and H1N1 09 was compared to non-H1N1 09 viruses (53.4% versus 74.2%; $P < 0.001$). The negative predictive values for H1N1 09, influenza A/H3 virus, and other influenza virus subtypes were 76.2%, 92.0%, and 97.9%, respectively. The specificity and positive predictive values were 100%; no false positives were identified (Table 1).

Performance characteristics of various RAT in the detection of seasonal influenza virus strains (A/H1N1, A/H3N2, and B) have been previously established (4, 8, 10), but their performance in the current H1N1 09 pandemic remains uncertain. The sensitivity of the QuickVue RAT is consistent with previously published data, but a significant reduction in RAT sensitivity was observed when it was applied to H1N1 09-containing samples. This reduced sensitivity in detecting the pandemic strain may be explained by differences between the H1N1 09 and seasonal influenza A nucleoproteins or differences in the shedding of influenza virus from different levels of the respiratory tract. Although a positive RAT result rapidly assists in the acute management of influenza, NAT or culture should be employed in the appropriate clinical setting of suspected H1N1 09 infection when RAT results are negative.

TABLE 1. Performance characteristics of QuickVue RAT compared to those of NAT

Parameter	H1N1 09 ($n = 174$)[a]	A/H3 ($n = 88$)[a]	Non-H1N1 09 ($n = 97$)[b]
No. of samples RAT positive	93[a]	68[a]	72
No. of samples RAT negative	81	20	25
Sensitivity (%)	53.4	77.2	74.2
Specificity (%)	100	100	100
PPV[c] (%)	100	100	100
NPV[d] (%)	76.2	92	90.2

[a] Includes two samples that were coinfected with H1N1 09 and influenza A/H3.
[b] Includes 88 influenza A/H3 and 9 others (3 seasonal influenza A/H1N1 and 6 untypeable).
[c] PPV, positive predictive value.
[d] NPV, negative predictive value.

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