



Short communication

Performance of rapid-test kits for the detection of the pandemic influenza A/H1N1 virus

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The early detection of pandemic influenza strains is a key factor for clinicians in treatment decisions and infection control practices. The aims of this study were to determine the analytical sensitivity and clinical performance of the commercially available influenza rapid tests in Taiwan. Four rapid tests for influenza virus (BinaxNow test, QuickVue test, TRU test, and Formosa Rapid test) were evaluated for their detection limit against four influenza viruses (the 2009 pandemic influenza A virus H1N1, seasonal influenza virus H1N1, H3N2, and influenza B virus) circulating in Taiwan. The viral load of these isolates were quantified by rtRT-PCR and then diluted 2-fold serially for the comparison. The lowest detectable viral load of the pandemic influenza A virus H1N1 by the Formosa Rapid test, QuickVue test, TRU test, and Binax Now test was 5.3×10^4 , 1.0×10^5 , 1.0×10^5 , and 4.2×10^5 copies/ μ L, respectively. Of these four tests, the two most sensitive tests (the QuickVue test and the Formosa Rapid test) were chosen to evaluate 62 nasopharyngeal specimens from patients who were suspected of infection with pandemic influenza A virus H1N1. The positive rate for the Formosa Rapid test and the QuickVue test were 53.2% (33/62) and 45.2% (28/62) (McNemar's test, $P=0.125$), respectively. In conclusion, the Formosa Rapid test was the most sensitive test in the present study for the detection of influenza antigens and its clinical performance was similar to that of the QuickVue test ($Kappa=0.776$). This suggests that the Formosa Rapid test could be used to aid clinical decision making in primary health care settings during outbreaks of influenza.

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1. Introduction

A novel influenza A (H1N1) virus, originating in swine and resulting from the recombination of human, avian, and swine influenza viruses, was identified as the cause of a 2009 pandemic outbreak of febrile respiratory infection (Baden et al., 2009; Dawood et al., 2009). Influenza-like illness is characterized by clinical symptoms including fever above 38 °C, coughing, sore throat, rhinitis, myalgia, fatigue, and vomiting (Nicholson, 1992). The rapid diagnosis of patients possibly infected with swine influenza viruses, especially those at high risk of influenza-related complications, is essential not only for the initiation of antiviral treatment but also for surveillance of the infection.

When diagnosing influenza infection, both the accuracy and the time frame in which a result is obtained are important. The

rate of clinical diagnosis of pandemic influenza A virus H1N1 have been reported to range from 63% to 86% in comparison to laboratory diagnosis by reverse transcription-polymerase chain reaction (RT-PCR) or viral culture (Boivin et al., 2000; Monto et al., 2000; Ruest et al., 2003). Currently, laboratory identification of influenza-specific RNA by RT-PCR is the reference method for the diagnosis of influenza virus infection. However, due to the requirement for specialized equipment, RT-PCR cannot be conducted readily in primary health care settings. The pandemic 2009 outbreak has highlighted the need for a rapid influenza diagnostic test to facilitate early treatment of infected individuals because treatment has been beneficial only when antiviral drugs are administered within 48 h of the appearance of symptoms (Treanor et al., 2000). In the clinic, several rapid influenza diagnostic tests that can provide results in a clinically relevant time frame to assist clinical judgment have become available. Recent studies evaluating the accuracy of rapid influenza diagnostic test results using clinical specimens have shown a broad sensitivity range from 10% to 69% (Chan et al., 2009; Faix et al., 2009; Ginocchio et al., 2009; Vasoo et al., 2009). However, little is known about the sensitivity and the specificity of rapid tests for

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Table 1
Comparison of the detection limits of different commercially available influenza virus rapid detection kits.

Influenza virus subtype Viral load (copies/ μ L)	Detection limit (titer/viral load)			
	Formosa One Sure Flu A/B Rapid test	QuickVue Influenza A + B test	TRU Flu A/B test	BinaxNow influenza A&B test
swH1N1 3.4×10^6	1:64/5.3 $\times 10^4$	1:32/1.0 $\times 10^5$	1:32/1.0 $\times 10^5$	1:8/4.2 $\times 10^5$
H1N1 1.5×10^7	1:256/5.9 $\times 10^4$	1:64/2.4 $\times 10^5$	1:64/2.4 $\times 10^5$	1:16/9.5 $\times 10^5$
H3N2 3.0×10^6	1:36/9.4 $\times 10^4$	1:16/1.8 $\times 10^5$	1:8/3.8 $\times 10^5$	1:2/1.5 $\times 10^6$
Influenza B 1.7×10^7	1:8/2.1 $\times 10^6$	1:16/1.0 $\times 10^6$	1:8/2.0 $\times 10^6$	1:8/2.0 $\times 10^6$

the detection of the new pandemic influenza A virus. This uncertainty in the clinical performance of rapid influenza diagnostic tests for the detection of the H1N1 strain in patients with influenza-like illnesses during outbreaks prompted this study.

This study aimed to (1) determine the analytical capability of rapid tests to detect the new pandemic influenza A by comparison with seasonal influenza A viruses and (2) conduct a head-to-head comparison of two selected rapid influenza diagnostic tests for their diagnostic performance for detecting pandemic influenza A virus H1N1 in clinical specimens.

Four seasonal Influenza virus strains which circulated in Taiwan, pandemic H1N1 virus A/California/04/2009 (swH1N1), seasonal influenza virus A/Brisbane/59/2007 (H1N1), A/Brisbane/10/2007 (H3N2), and B/Brisbane/60/2008, were isolated and amplified in Madin–Darby canine kidney epithelial cells to prepare viral stocks. After 3 days incubation, the viral supernatant was harvested, and the viral load was quantified using a standard real-time RT-PCR assay (rtRT-PCR) (Ward et al., 2004), and then diluted serially for the comparison. The limit of detection of each rapid test is denoted as the lowest viral load (copies/ μ L) detectable.

Sixty-two nasopharyngeal swabs were obtained from patients at the Chang-Gung Memorial Hospital (Taoyuan, Taiwan) suspected of infection with the pandemic influenza A virus H1N1 in August, 2009. All specimens were collected within 1–2 days of the development of symptoms, kept at 4 °C in the vial transport media and sent to the laboratory immediately. After the extraction process, 120 μ L and 340 μ L aliquots of extracts were applied for the Formosa Rapid test and QuickVue rapid tests, respectively. All tests were processed within 12 h of reception in the laboratory.

Four rapid tests for influenza virus, Binax Now Influenza A&B (Binax; Portland, USA), QuickVue Influenza A + B test (Quidel; San Diego, USA), TRU Flu A/B (Meridian Biosciences; Cincinnati, USA), and Formosa One Sure Flu A/B Rapid test (Formosa Biomedical Technology Corp.; Yilan, Taiwan), were compared for their ability to detect influenza antigens following each of the manufacturer's specific protocols. For evaluation of clinical diagnostic efficacy, the two relatively more sensitive tests (the well studied QuickVue test and the Formosa Rapid test produced in Taiwan) were selected for detection of pandemic influenza A virus H1N1 in clinical specimens collected from patients with an influenza-like illness by a trained technician who was blinded to the other test results.

As shown in Table 1, the detection limits ranged from 10^4 to 10^6 copies/ μ L between the different commercial rapid influenza diagnostic tests. The sensitivity also varied between the different influenza subtypes. All of the rapid influenza diagnostic tests had greater sensitivity for detecting influenza A virus (10^4 to 10^5 copies/ μ L) than influenza B virus (10^6 copies/ μ L). For the detection of pandemic influenza A virus H1N1 2009, the detection limit of the Formosa Rapid test, QuickVue test, TRU test, and Binax Now test was 5.3×10^4 , 1.0×10^5 , 1.0×10^5 , and 4.2×10^5 copies/ μ L, respectively. The clinical diagnostic efficacy of the two tests selected is shown in Table 2. The Formosa Rapid test and the QuickVue test had a positive rate of 53.2% (33/62) and 45.2% (28/62), respectively (McNemar's test, $P=0.125$). Six specimens (6/62; 9.7%) that are not detected by the QuickVue test demonstrated positive results by the Formosa Rapid test.

Table 2

Comparison of the performance of the Formosa One Sure Flu A/B Rapid test and the QuickVue Influenza A + B test to clinical definitions of influenza-like illness for the detection of pandemic influenza A/H1N1 2009.^{a,b}

	QuickVue Influenza A + B test		Total
	Positive	Negative	
Formosa One Sure Flu A/B Rapid test			
Positive	27	6	33
Negative	1	28	29
Total	28	34	

Difference between the Formosa One Sure Flu A/B Rapid test and the QuickVue Influenza A + B test were not significant.

^a McNemar's test $P=0.125$.

^b Kappa value = 0.776.

According to the surveillance information, the first outbreak of influenza A/H1N1 was in the 36th week of 2009, and was shown to be the major pandemic strain which accounted for nearly 88.2% of all community influenza strains which have been isolated. In clinical practice, based on the clinical treatment guidelines of the Taiwan Center for Disease Control, patients with influenza-like illness and positive by rapid influenza diagnostic test should be treated with antiviral drugs immediately (Chen et al., 2009).

The existing rapid influenza diagnostic tests, produced by using a monoclonal antibody against the influenza nucleoprotein, were designed for detection of seasonal influenza A and B and not the pandemic influenza A virus H1N1 (Yang et al., 2008). The pandemic influenza A virus H1N1 strain is different from the common human influenza A virus because it combines genes from avian, human, and swine influenza viruses (Dawood et al., 2009). Commercial rapid influenza diagnostic tests have been shown to have a broad range in sensitivity for detecting the influenza A virus H1N1 virus (CDC, 2009; Chan et al., 2009; Vasoo et al., 2009). Therefore, it is necessary to determine whether the rapid influenza diagnostic tests can detect pandemic influenza A virus H1N1 as efficiently as they detect seasonal influenza viruses.

The first objective of this study was to evaluate the detection limit of four rapid influenza diagnostic tests against a panel of standard influenza virus strains. Among them, the QuickVue test and the TRU test had a similar detection limit for influenza A virus H1N1 where the viral load was as low as 1.0×10^5 copies/ μ L. However, the BinaxNow test detected only viral loads greater than 4.2×10^5 copies/ μ L. Similar results were reported by Ginocchio et al. (2009) who found that the performance of the QuickVue test was better than the BinaxNow test for the detection of the H1N1 influenza virus.

Compared with the other rapid influenza diagnostic tests, the Formosa Rapid test is the most sensitive kit, with a sensitivity of 10^4 copies/ μ L for all influenza A virus subtypes, including pandemic influenza A virus H1N1, seasonal influenza virus H1N1, and H3N2. Overall, the detection limit of the different commercial rapid influenza diagnostic tests varied between different influenza subtypes and ranged from 10^4 to 10^6 copies/ μ L, which is 10^3 to 10^4 times less sensitive than rtRT-PCR. This may explain why rapid tests could not detect the virus in specimens with a lower viral titer.

The data on the analytical sensitivity for detection of cultured virus strains may not reflect accurately the sensitivity in clinical specimens. Therefore, in order to evaluate the clinical diagnostic efficacy, an extensive head-to-head comparison for detection of pandemic influenza A virus H1N1 in clinical pharyngeal specimens was conducted. The results showed that the diagnostic efficacy of the Formosa Rapid test was comparable to that of the QuickVue test ($\text{Kappa} = 0.776$). At 50%, the positive rate of both rapid influenza diagnostic tests is similar to previous studies which demonstrated that the QuickVue test had a sensitivity of 53–69% and specificity of 100% for pandemic influenza A virus H1N1 (CDC, 2009; Hurt et al., 2007; Vasoo et al., 2009).

In summary, this study demonstrated that the analytical sensitivity of the Formosa Rapid test for the detection of pandemic influenza A virus H1N1 was found to be slightly superior to the other commercial tests. The clinical performance of the Formosa Rapid test was comparable with the QuickVue test. Since the Formosa Rapid test can be completed rapidly and does not require extensive laboratory facilities, it may be helpful in the timely detection of highly contagious pandemic influenza A virus H1N1 infections.

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