Preliminary Evaluation of Various Rapid Influenza Diagnostic Test Methods for the Detection of the Novel Influenza A (H1N1) in Universiti Kebangsaan Malaysia Medical Centre

Z R Zetti, MPath (Medical Microbiology), K K Wong, MSc (Virology), M Haslina, Diploma (DTM), I Ilina, MSc (Medical Microbiology)

Department of Medical Microbiology & Immunology, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, 56000 Kuala Lumpur

SUMMARY

We evaluated the performance of four rapid influenza diagnostic test methods (RIDT) compared to real-time reverse-transcription polymerase chain reaction (rRT-PCR), for the detection of the novel swine-origin influenza A (H1N1) virus (S-OIV) in August 2009. A total of 270 respiratory specimens were tested with rRT-PCR, where 74 of these were tested by BinaxNow® (Inverness), 80 by QuickVue® (Quidel), 37 by Influenza A Antigen Rapid Test (Rockeby Biomed) and 79 by Directigen™ (BD). The sensitivities ranged from 4.4% to 37.0%, specificities 90.9% to 100.0%, positive predictive values 75.0% to 100.0% and negative predictive values 32.3% to 75.0%. RIDT were able to detect S-OIV but the sensitivities were low. The limitations of RIDT must be considered when interpreting results for clinical management.

KEY WORDS:
Novel swine-origin influenza A (H1N1), Detection, Laboratory methods, Diagnostic, Rapid tests, performance

INTRODUCTION

A novel, "non-seasonal" swine-origin influenza A (H1N1) virus (S-OIV), designated as H1N1 A/swine/California/04/2009, emerged in Mexico in early 2009 and caused a worldwide pandemic which continues to expand globally[1,2,3,4,5]. The first two cases of S-OIV in Malaysia were reported on 16th May 2009. As of 20th August 2009, there were more than 4000 reported cases and 68 mortalities in this country[5].

Various laboratory methods are available to detect influenza viruses in respiratory specimens, such as rapid influenza antigen diagnostic tests (RIDT), virus isolation in cell culture and detection of amplified influenza-specific RNA by reverse-transcription polymerase chain reaction (rRT-PCR). These tests vary in terms of sensitivity, specificity, length of time required, and ability to distinguish between influenza A and B, and between influenza A subtypes (seasonal influenza A or novel S-OIV). Confirmation of S-OIV is required for surveillance or epidemiologic purposes and for special circumstances such as those with co-morbidities, severe illness, pregnant patients and healthcare workers with influenza-like illness (ILI). In accordance with the hospital testing policies for suspected cases, respiratory specimens are initially tested with RIDT followed by viral culture. Cases were screened for the purpose of further testing and confirmation with real-time reverse-transcription polymerase chain reaction (rRT-PCR). The criteria used were in accordance with the Ministry of Health Malaysia (MOH) recommendations for patients admitted with ILI[5].

The molecular unit in our microbiology laboratory had set up the Roche LightCycler® 2.0 system, using RealTime Ready Swine Influenza A/H1N1 Detection Set, which is a one-step rRT-PCR based on Taqman probes. The Roche LightCycler® System is mentioned in WHO diagnostic recommendation for influenza A (H1N1) on 21st May 2009[6,7]. Training and trial runs in our laboratory were completed by early August, and the unit was ready to perform routine testing for S-OIV. Emergency Use Authorization (EUA) study for this system was completed by the company in September 2009 and EUA was issued by the Food and Drug Administration (FDA) in November 2009[8].

RIDT has the advantage of being a simple technique with fast results within a clinically relevant time frame. Various RIDT can detect and distinguish between influenza A and B viruses (eg QuickVue® Influenza A+B, BinaxNow® Influenza A&B, Directigen™ EZ Flu A+B, ESPLINE™ Influenza A&B and Clearview Exact® Influenza A&B), detect both but is unable to distinguish between influenza A and B (eg QuickVue® Influenza Test), or detect influenza A only (eg Rockeby Biomed). None of the various RIDT is able to distinguish between the influenza A virus subtypes. RIDT were widely used for the detection of S-OIV during the 2009 pandemic, with variable reported performance[9,10,11,12]. Analytical studies indicate that some RIDT are reactive with the nucleoprotein of S-OIV[9], and data is available on the performance of RIDT compared to RT-PCR for detection of S-OIV in clinical specimens[10]. Seasonal influenza strains continue to co-circulate with S-OIV, but may be variably detected by RIDT. The sensitivity and specificity of the various diagnostic tests available should be continuously evaluated in view of the drift and shift that occur in influenza viruses. We made a

Table I: Comparison of results of the various RIDT with rRT-PCR method

rRT-PCR results	BinaxNow® (n=74)		QuickVue® (n=80)		Rockeby (n=37)		Directigen™ (n=79)		Total (n=270)
	positive influenza A	neg	positive influenza A	neg	positive influenza A	neg	positive influenza A	neg	
M2 only positive	0	0	0	1	1	2	1	0	5
M2 and H1 positive	2	43	2	43	3	21	10	17	141
rRT-PCR negative	0	29	0	34	0	10	0	51	124
Total	2	72	2	78	4	33	11	68	270

Table II: Evaluation of the various RIDT for detection of S-OIV and influenza A (including S-OIV)

Rapid influenza diagnostic tests	BinaxNow® (n=74)		QuickVue® (n=80)		Rockeby (n=37)		Directigen™ (n=79)	
	S-OIV	Influenza A (inc S-OIV)	S-OIV	Influenza A (inc S-OIV)	S-OIV	Influenza A (inc S-OIV)	S-OIV	Influenza A (inc S-OIV)
Sensitivity[a]	4.4%	4.4%	4.4%	4.3%	12.5%	14.8%	37.0%	39.3%
Specificity[b]	100.0%	100.0%	100%	100.0%	90.9%	100.0%	98.1%	100.0%
Positive predictive value[c]	100.0%	100.0%	100%	100.0%	75.0%	100.0%	90.9%	100.0%
Negative predictive value[d]	40.3%	40.3%	44.2%	43.6%	32.3%	30.3%	75.0%	75.0%

[a]Sensitivity = $\dfrac{\text{true positives}}{\text{true positives + false negatives}}$ X 100%

[b]Specificity = $\dfrac{\text{true negatives}}{\text{false positives + true negatives}}$ X 100%

[c]Positive predictive value (PPV) = $\dfrac{\text{true positives}}{\text{true positives + false positives}}$ X 100%

[d]Negative predictive value (NPV) = $\dfrac{\text{true negatives}}{\text{true negatives + false negatives}}$ X 100%

preliminary evaluation of the performance of four commercially available RIDT compared to rRT-PCR for detection of S-OIV, and calculated the sensitivity, specificity, positive predictive value and negative predictive value of the RIDT compared to rRT-PCR.

MATERIALS AND METHODS
Study population and specimens
The specimens were those of patients seen at the various units or departments at Universiti Kebangsaan Malaysia Medical Centre (UKMMC) or admitted with suspected S-OIV, including paediatric patients and healthcare workers, who fulfilled the criteria for specimen-taking and laboratory testing. The criteria used to justify laboratory testing included patients from high-risk groups, who were likely to have co-morbidities which contributed to the severity of the disease and had a high risk of influenza complications. Laboratory testing was also warranted for patients who presented with moderate to severe illness, indicated by presence of "Clinical Assessment Tool" criteria as outlined by the MOH[5]. Specimens were taken as soon as possible after the onset of illness or during the acute stage of illness, when viral levels are highest and until resolution of fever. All specimens were collected as part of the standard of care for laboratory diagnosis purposes. The respiratory specimens included throat swabs, nasopharyngeal swabs, nasal aspirate, wash or swabs. The specimens were collected on swabs with a synthetic tip (such as nylon, polyester or Dacron®) and an aluminium or plastic shaft. The appropriate swabs were provided to the wards, since swabs with cotton tips, wooden shafts and swabs made of calcium alginate were not acceptable, as they may interfere with rRT-PCR. Swabs were submitted in Viral Transport Medium (VTM) and placed in ice or cold packs for transport to the laboratory. All specimens were immediately tested or kept at 2-4°C (≤72 hours) or frozen at –70°C until tested. Specimens which did not fulfill the requirements for specimen collection, transport and storage requirements were excluded.

Confirmation of novel influenza A (H1N1)
The rRT-PCR detection of influenza A is based on the detection of influenza A conserved region of the Matrix Protein 2 (M2) gene. The respective subtype identification for S-OIV is based on detection of the haemagglutinin (H1) gene. The primer/probe set for detection of the M2 gene[13] has been recommended by the WHO for bird flu virus detection in 2007[14]. The primer/probe set for detection of the influenza A H1 gene[15] has been recommended by the Robert Koch Institut in Berlin, Germany, May 2009. Extraction process wa done by using QIAamp® Viral RNA Mini Kit. Each specimen RNA extract was tested by separate primer/probe sets, M2 and H1. No template control (NTC) and positive template control (PTC) for all primer/probe sets were included in each run. NTC was prepared by replacing the template ribonucleic acid with PCR-grade water. PTC was prepared from the PTC stock provided with the set and an additional positive control from the first confirmed cases of S-OIV in the country, provided by the Institute of Medical Research, Kuala Lumpur. The PCR program was done according to the procedure provided by Roche. The NTC reactions for probe/primer sets should not exhibit fluorescent amplification curves that cross the threshold line. When all controls met the requirement, a specimen was considered positive for S-OIV if both the M2 and H1 reaction amplification curves cross the threshold line.

A positive reaction with only M2 indicates influenza A, which could be seasonal influenza. A specimen was considered negative if no fluorescent amplification curves cross the threshold line.

Rapid influenza detection tests (RIDT)
The four commercially available RIDT compared were QuickVue® Influenza A+B (Quidel), BinaxNow® Influenza A&B (Inverness), Influenza A Antigen Rapid Test (Rockeby Biomed) and Directigen™ EZ Flu A+B (BD). These kits are in-vitro immunochromatographic assays for qualitative detection of influenza virus nucleoprotein antigens, using monoclonal antibodies. The test devices are in the form of strips incorporating a control line and two lines for influenza A and B (BinaxNow®, QuickVue® and Directigen™), or a control line and one test line (Rockeby). The kits were supplied with the required materials and reagents. The tests were performed according to the respective manufacturer's instructions and presence of any visible test line was interpreted as positive.

RESULTS
A total of 270 respiratory specimens were tested with rRT-PCR between 3rd and 26th August 2009. Seventy-four were tested by BinaxNow® Influenza A&B, 80 tested by QuickVue® Influenza A+B, 37 tested by Influenza A Antigen Rapid Test (Rockeby Biomed) and 79 tested by Directigen™ EZ Flu A+B. The comparison of results for RIDT with rRT-PCR method is shown in Table I. No influenza B was detected in this population. A total of 141 specimens out of 270 (52.2%) were positive for S-OIV, where both M2 and H1 were positive by rRT-PCR. Five specimens (1.9%) were positive for non-S-OIV or seasonal influenza A, where only M2 was positive by rRT-PCR. The remaining 124 specimens (45.9%) were negative by rRT-PCR. Calculation of the sensitivity, specificity, positive predictive value and negative predictive value was done using standard formulas and are shown in Table II. The sensitivities ranged from 4.4% to 37.0%, specificities 90.9% to 100.0%, positive predictive values 75.0% to 100.0% and negative predictive values 32.3% to 75.0% for S-OIV.

DISCUSSION
The currently available RIDT are manufactured for detection of seasonal influenza. Our findings indicate that the sensitivities of these kits were rather low, compared to rRT-PCR for detection of S-OIV. The sensitivity for the Directigen™ kit was found to be highest at 37.0% for detection of S-OIV. However, the total number of specimens may be too small to be representative of the true performance of these kits. A smaller number of specimens were tested by the Rockeby kit (n=37), due to unavailability of kit by the fourth week of August, when stocks for most types of kits were unavailable. The results could have been analysed separately for each RIDT kit for different types of specimen, whether children or adult population and duration of illness onset. However, due to budget and stock limitations during the pandemic, the specimens were tested serially for the various kits. The distribution of positive and negative S-OIV cases, and presence of seasonal influenza A, was not equal among the four sets of specimen. A better approach for

evaluation purposes would be to run the various RIDT on the same set of specimens.

Uyeki *et al* described the poor sensitivity of the Quidel QuickVue® test (mean 27%, range 19 to 32) for influenza during the 2007-2008 season[16]. RIDT sensitivity for detection of S-OIV ranged from 10% to 70% compared to rRT-PCR[9]. Another evaluation study reported RIDT sensitivity for detection of S-OIV ranging from 40 to 69%, and declining sensitivity with lower viral titres[11]. Diagnostic yield may be influenced by the time of specimen collection or stage of illness, type of specimen, specimen handling and time interval from specimen collection to testing. Levels of virus in the specimen, as indicated by cycle threshold (Ct) values, and analysis of Ct values would be pursued to obtain information on the correlation of sensitivities with viral load. Continuous evaluation of the kits' performance and usefulness is required before local recommendations are made. RIDT has the advantages of being a simple technique with fast results which require minimal lab set-up. A positive result would prompt antiviral therapy regardless of the influenza subtypes. Furthermore, influenza virus can occur in waves during times of pandemic, where S-OIV will continue to co-circulate with seasonal influenza. Further investigations are warranted to see the variations of RIDT sensitivity according to influenza A subtypes.

CONCLUSION
The findings show that these RIDT are able to detect S-OIV in respiratory specimens. A positive RIDT result is helpful in deciding to start antiviral therapy, due to the high specificity and positive predictive value. However, due to the rather low sensitivity and negative predictive value, a negative RIDT does not rule out influenza virus infection. While positive results for influenza A has a high positive predictive value, it cannot distinguish between influenza A virus subtypes, which could be S-OIV or seasonal influenza A viruses. The limitations of RIDT must be considered when interpreting the results for clinical management. Empirical antiviral therapy should be considered based on clinical indications such as severity of illness and the risk of influenza complications, despite negative RIDT results.

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