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Comparison of Cepheid® Xpert Flu and Roche RealTime Ready Influenza A/H1N1 Detection Set for detection of influenza A/H1N1

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ABSTRACT

Objective: To compare two influenza polymerase chain reaction (PCR) methods.
Methods: A total of 749 suspected MERS-CoV patients presenting at Johns Hopkins Aramco Healthcare, Saudi Arabia, each submitted a clinical sample for influenza A reflex testing using the on-site Cepheid® Xpert Flu assay and at the Ministry of Health laboratory by the Roche PCR assay.
Results: There was 92.12% overall agreement between the two methods. Specificity of the Cepheid® Xpert Flu was 95.8% for H1N1 and 94.4% for total influenza A. Cepheid® Xpert Flu sensitivity for influenza A was 100% for younger patients (0–19-year age group) but significantly lower both for older patients (68.2% for 60–79-year and 50% for ≥80-year age groups) and overall for males compared to females (72.6% and 94.0%, respectively).
Conclusions: Specificity of the Cepheid® Xpert Flu test was high; however, sensitivity for total influenza A was lower particularly in males and older patients.

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1. Introduction

Both A and B seasonal influenza virus types cause outbreaks and epidemics, while only type A has been known to cause pandemics (WHO, 2016). The pandemic influenza A (H1N1) pdm09 virus was first identified in humans in March/April 2009 and spread worldwide, including to the Kingdom of Saudi Arabia (KSA) (Al-Tawfiq et al., 2011; Balkhy et al., 2010; Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team et al., 2009; Uthman et al., 2014).

The KSA Ministry of Health (MOH) gold standard method for influenza A/H1N1 detection is the RealTime Ready Influenza A/H1N1 Detection Set (Roche Diagnostics GmbH, Germany) (Barbás et al., 2012; Choi et al., 2010; thi Tham et al., 2012; Wenzel et al., 2010). This kit has high specificity but variable sensitivity (Barbás et al., 2012; Choi et al., 2010; thi Tham et al., 2012; Wenzel et al., 2010). In our laboratory, we use the Cepheid® Xpert Flu Assay multiplex real-time PCR (Cepheid) for differential, qualitative detection of influenza A, influenza B, and influenza A (H1N1) pdm09. It has high specificity and sensitivity in nasopharyngeal samples (Novak-Weekley et al., 2012; Popowitch et al., 2011; Salez et al., 2012). Overall, 99% agreement was observed between the two kits in a study on 102 clinical samples (Sohn et al., 2013). Our study was designed to test the sensitivity and specificity of our Xpert Flu Assay with respect to the MOH-approved RealTime Ready Influenza A/H1N1 Detection Set method on a larger series of 749 clinical samples.

2. Methods

2.1. Patient Population and Specimens

Influenza A/influenza A (H1N1) pdm09 testing was carried out on clinical specimens from 749 suspected MERS-CoV patients presenting to Johns Hopkins Aramco Healthcare facilities in the Eastern Province of KSA between April 2015 and February 2016. Table 1 shows the patient and sample characteristics (gender, age group, sample type, and location where sample collected). This testing is indicated by KSA MOH guidelines for patients who meet Category I [acute respiratory illness with clinical and/or radiological evidence of pulmonary parenchymal disease (pneumonia or acute respiratory distress syndrome)] or Category II (hospitalized patient with healthcare-associated pneumonia based on clinical and radiological evidence) criteria for possible MERS-CoV infection (Kingdom of Saudi Arabia Ministry of Health, 2017). The guidelines state that such patients should be simultaneously tested for other common viral and bacterial causes of community-acquired pneumonia (Kingdom of Saudi Arabia Ministry of Health, 2017). Patient age ranged from 1 to 108 years (median 63 years).

Tests were carried out at the Johns Hopkins Aramco Healthcare Centre in Dhahran City using the Cepheid® Xpert Flu Assay multiplex

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Table 1
Patient and sample characteristics.

Patient and sample characteristics	
Characteristic	Number (percentage)
Gender	
Male	390 (52.1%)
Female	359 (47.9%)
Age group (y)	
0–19	66 (8.8%)
20–39	104 (13.9%)
40–59	158 (21.1%)
60–79	248 (33.1%)
≥80	173 (23.1%)
Sample type	
Nasopharyngeal swab (NASPH)	677 (90.4%)
Expectorate deep cough sputum (SPUEX)	32 (4.3%)
Induced sputum (SPUIN)	17 (2.3%)
Tracheal aspirate (TRAC)	23 (3.1%)
Location	
Abqiq City (AB)	23 (3.1%)
Dhahran City (DH)	614 (82.0%)
Al-Hasa (AH)	50 (6.7%)
Ras Tanura City (RT)	53 (7.1%)
Udhailya City (UC)	1 (0.1%)
Unknown	8 (1.1%)

real-time PCR (Cepheid) and by the MOH in Dammam using the RealTime Ready Influenza A/H1N1 Detection Set real-time PCR (Roche Diagnostics). Samples were collected following the user institution's standard procedures and placed into Viral Transport Medium (VTM) tubes (Cepheid). Two samples were collected per patient. One was processed immediately upon receipt in Johns Hopkins Aramco Healthcare Centre. As per MOH regulations, the second sample was kept at 2–8 °C for a maximum of 8 h until transportation to the MOH Dammam regional laboratory. Samples were transferred on a daily basis.

2.2. MOH Specimen Type and Processing

One volume of mucoid sample was mixed with 2 vol of bacterial lysis buffer, incubated at room temperature for 5 min, and then centrifuged for 5 min at 15,000 rpm. Supernatant was collected and used for extraction of viral nucleic acid.

2.3. MOH Nucleic Acid Extraction:

Nucleic acid extraction was performed using MagNA Pure 96DNA and viral nucleic acid small volume kit on a Magna Pure 96 instrument (Roche Diagnostics, Indianapolis, IN, USA), according to the manufacturer's instructions. The Pathogen Universal-200 purification protocol was used. A total of 200 μl volume of sample material was used for extraction, and the nucleic acids were eluted into 50 μl of elution buffer.

2.4. MOH Reverse Transcription and DNA Amplification: RealTime Ready Influenza A/H1N1 Detection Set

Detection of the influenza A (H1N1) pdm09 virus was performed by the MOH using RealTime Ready Influenza A/H1N1 Detection Set (Roche Diagnostics GmbH, Germany). Amplification of each target (M2 and H1) was performed as one-step RT-PCR using RealTime Ready RNA Virus Master according to the manufacturer's instructions. Thermal cycling was performed in a LightCycler 2.0 instrument (Roche Diagnostics, Indianapolis, IN, USA) using the following conditions for both PCRs: 58 °C for 8 min; 95 °C for 30 s; followed by 45 cycles of 95 °C for 1 s, 60 °C for 20 s, and 72 °C for 1 s; and cooling to 40 °C for 30 s. With the first PCR (M2 PCR), two sets of probes and primers (targeting human nucleic acid and influenza A/M2 gene) and four controls were used. Controls included extracted control for human nucleic acid (internal sample control), commercial positive plasmid control for the whole PCR, negative extracted control (water), and no template negative control. Three controls were used for the second PCR (H1 PCR): commercial positive plasmid control for the whole PCR, negative extracted control (water), and no template negative control. In addition, only one set of probes and primers targeting influenza A/H1 gene was used.

Results were validated after evaluating the results of all controls. Results were interpreted as positive if the crossing point (Cp) value was ≤40, with the presence of a sigmoid curve. Negative results were reported if no value or Cp value was >40, with the absence of a sigmoid curve. To report a sample as positive for influenza A/H1N1 (POS), the result had to be positive for both M2 PCR and H1 PCR. Samples positive for only M2 PCR were considered influenza A M2 positive and negative for influenza A subtype H1N1 (FluA). Samples negative for the first PCR (M2 PCR) did not undergo the second PCR (H1 PCR) and were considered negative for influenza A (NEG).

2.5. Cepheid® Xpert Flu Assay Procedure

For the Cepheid® Xpert Flu Assay procedure, samples were processed and tests carried out and interpreted according to the manufacturer's instructions (Cepheid). Briefly, samples were mixed/diluted in appropriate volumes with Universal Transport Medium, and 300 μl was transferred into the Xpert Flu Assay cartridge. Samples were tested on the GeneXpert Dx instrument according to the manufacturer's instructions. Results and amplification curves for samples and endogenous controls were reviewed. Internal controls included Sample Processing Control (SPC) (Cepheid). Each sample also included a Probe Check Control. New reagent kits were validated by retesting at least one known positive and one known negative patient sample. Failed controls were reviewed and repeated using fresh cartridges. Possible failed results included invalid [control SPC failed OR FluA target RNA not detected/influenza A (H1N1) pdm09 target RNA is detected OR the sample was not properly processed OR PCR was inhibited], error (assay aborted), or no result (insufficient data collected). Possible valid results included FluA+/H1N1+, FluA+/H1N1− or FluA−/H1N1−.

2.6. Statistical Methods

We calculated the percentage agreement between the Cepheid® Xpert Flu Assay multiplex real-time PCR (Cepheid) and the RealTime Ready Influenza A/H1N1 Detection Set real-time PCR (Roche Diagnostics) using the Kappa statistic. Results were interpreted based on the guidelines that negative Cohen's kappa means no agreement between methods, 0–0.20 is slight, 0.21–0.40 is fair, 0.41–0.60 is moderate, 0.61–0.80 is substantial, and 0.81–1 is almost perfect agreement (Altman, 1991; Landis and Koch, 1977). We measured the sensitivity and specificity of the Cepheid® Xpert Flu Assay multiplex real-time PCR by taking the MOH-approved RealTime Ready Influenza A/H1N1 Detection Set real-time method as the gold standard. True positives were defined as samples scored positive by the MOH method and also identified as positive by the Cepheid® Xpert Flu Assay for total FluA ± H1N1 pdm09 as appropriate. Sensitivity (true-positive rate) was calculated as the percentage of infections positively identified by both methods compared to the gold standard method only. Specificity (true-negative rate) was calculated as the percentage of samples identified as negative by both methods compared to the gold standard method only. Chi squared analysis was used to compare distribution of true-positive samples versus samples scored positive by the MOH method (total FluA ± H1N1 pdm09 as appropriate) but negative by the Cepheid® Xpert Flu Assay according to gender, age group, or location; $P \leq 0.05$ was accepted as significant. Student's t test was used to

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