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

Ali A. Rabaan [a,*], Ali M. Bazzi [b], Sana A. Alshaikh [c]

[a] Molecular Diagnostic Laboratory, Johns Hopkins Aramco Healthcare, Dhahran 31311, Saudi Arabia
[b] Microbiology Laboratory, Johns Hopkins Aramco Healthcare, Dhahran 31311, Saudi Arabia
[c] Molecular Diagnostic laboratory, Dammam Regional Laboratory, Ministry of Health, Dammam, 31675, Saudi Arabia

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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.

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

* Corresponding author. Tel.: +966-13-877-6670; fax: +966-13-877-6741.
E-mail addresses: arabaan@gmail.com, ali.rabaan@jhah.com (A.A. Rabaan).

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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

compare mean ± SD for Ct values for which assays gave discordant versus concordant results; $P \leq 0.05$ was accepted as significant.

3. Results

3.1. Agreement Between Cepheid® Xpert Flu Assay and Roche RealTime Ready Influenza A/H1N1 Detection Set

There was overall 92.1% 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) for detection of presence or absence of total FluA ± H1N1 pdm09 in the 749 samples (Table 2). The Kappa statistic was 0.710 (SE = 0.035; 95% confidence interval: 0.641–0.778), indicating substantial overall agreement. Eighty-four samples (11.2%) from the whole group were positive for both FluA and FluA (H1N1) pdm09 by both tests, i.e., true positives for FluA (H1N1) pdm09 (Table 2).

The lowest concordance between the two tests was for detection of total FluA only (not FluA (H1N1) pdm09). Of the 11 samples scored FluA+/H1N1− by the RealTime Ready Influenza A/H1N1 Detection Set, 4 were scored identically by Cepheid® Xpert Flu Assay (Table 2) (36.4% percentage agreement).

3.2. Sensitivity and Specificity of the Cepheid® Xpert Flu Assay Method

Taking the MOH- approved RealTime Ready Influenza A/H1N1 Detection Set as the gold standard, sensitivity (true-positive rate) of H1N1 detection by the Cepheid® Xpert Flu Assay was 84%, i.e., of the 100 samples scored H1N1+ by the RealTime Ready Influenza A/H1N1 Detection Set, 84 were also scored positive by Cepheid® Xpert Flu Assay multiplex real-time PCR (Table 2). The specificity (true-negative rate) for H1N1 detection was 95.8%, i.e., of 649 samples scored negative for H1N1 by the RealTime Ready Influenza A/H1N1 Detection Set (either FluA only or NEG), 622 were also scored H1N1 negative by Cepheid® Xpert Flu Assay (either FluA−/H1N1− or FluA+/H1N1−) (Table 2). Overall, 111 samples were detected as total FluA positive by the MOH gold standard method (FluA or POS), of which 90 were also scored positive by the Cepheid® Xpert Flu Assay (FluA+/H1N1+ or FluA+H1N1−), giving an overall sensitivity of 81.1%. Six hundred thirty-eight samples were scored as FluA negative (FluA−) by the gold standard method, of which 602 were also scored negative by the Cepheid® Xpert Flu Assay (FluA−H1N1−), giving an overall specificity of 94.4%.

There was no significant difference in the median H1N1 or total FluA C_t values for the H1N1-positive Cepheid® Xpert Flu Assay results for samples which were concordant with the MOH gold standard method (median C_t: H1N1 = 24.05, FluA = 22.15) and those that were discordant with the MOH gold standard method (median C_t: H1N1 = 24.40, FluA = 23.20). There was also no significant difference in the C_t values for samples scored POS by the MOH gold standard method which were concordant with the result obtained with the Cepheid® Xpert Flu Assay compared to those which were discordant (C_t = 28.82 ± 3.44 and 29.89 ± 2.43 respectively; $P = 0.250$).

Table 2
Overall concordance between Cepheid® Xpert Flu Assay (Cepheid) and RealTime Ready Influenza A/H1N1 Detection Set real-time PCR kits.

Cepheid® Xpert Flu Assay	RealTime Ready Influenza A/H1N1 Detection Set			Total
	POS (FluA+/H1N1+)	FluA (FluA+/H1N1−)	NEG (FluA−/H1N1−)	
FluA+/H1N1+	84	2	25	111
FluA+/H1N1−	0	4	11	15
FluA−/H1N1−	16	5	602	623
Total	100	11	638	**749**

Number of observed agreements: 690 (92.1% of the observations); number of agreements expected by chance: 545.7 (72.9% of the observations).

3.3. Influence of Gender

Sensitivity and specificity of the tests were considered separately for samples from females ($n = 359$) and males ($n = 390$). For the samples from males, of 54 samples scored as positive for H1N1 pdm09 (POS) by the gold standard method, 42 (77.8%) were also scored positive by Cepheid® Xpert Flu Assay multiplex real-time PCR (FluA+/H1N1+) (Table 3). This gave 77.8% sensitivity for H1N1 detection by the Cepheid® Xpert Flu Assay compared to the MOH-approved RealTime Ready Influenza A/H1N1 Detection Set. The sensitivity was higher at 91.3% (for samples from females). However, the difference in the distribution between males and females of true-positive samples versus samples scored H1N1+/FluA+ by the MOH method and H1N1− by Cepheid® Xpert Flu Assay was not statistically significant (Chi-square statistic = 3.38; $P = 0.07$) (Table 3). Specificity of the Cepheid® Xpert Flu Assay was 95.2% (298 out of 313) for females and 96.4% (324 out of 336) for males. The number of observed agreements for samples from males was 366 (93.9%).

Overall sensitivity for total FluA detection (total FluA ±H1N1 pdm09) was 73.8% for males (45 out of 61) and 90.0% for females (45 out of 50), while specificity was 94.8% (312 out of 329) and 93.8% (290 out of 309) respectively (Table 3). The difference between males and females in number of true-positive samples for total FluA ±H1N1 pdm09 versus samples scored total FluA+ (POS or FluA) by the MOH method but negative by Cepheid® Xpert Flu Assay was statistically significant (Chi-square statistic = 4.72; $P = 0.03$).

The age group distribution differed significantly between males and females, with a higher-than-predicted proportion of men and a lower-than-predicted proportion of females being aged ≥80 years (Chi-square statistic = 16.07; $P = 0.003$) (Table 4).

3.4. Influence of Age Group

To determine if there was any variation in sensitivity and specificity of detection according to age of the patient from whom the sample was taken, the group was divided into age groups (Tables 1, 5). The sensitivity of the Cepheid® Xpert Flu Assay for detection of H1N1 by comparison to the gold standard method was 100% for the 0–19-year group. Sensitivity was lower for older age groups (Table 5), but the variation across age groups in distribution of true-positive samples for H1N1 versus samples which were positive for the MOH method and negative for the Xpert method was not statistically significant (Chi-square statistic = 5.76; $P = 0.22$). Specificity for H1N1 detection remained uniformly high across age groups (Table 5).

Sensitivity for overall FluA detection (FluA+/H1N1+ or FluA+/H1N1−) also dropped across the age groups from 100% for the 0–19-year group to 50% for the ≥80-year group (Table 5). The variation across

Table 3
Concordance between Cepheid® Xpert Flu Assay (Cepheid) and RealTime Ready Influenza A/H1N1 Detection Set real-time PCR kits for samples from females versus males.

Cepheid® Xpert Flu Assay	RealTime Ready Influenza A/H1N1 Detection Set			Total
	Females			
	POS (FluA+/H1N1+)	FluA (FluA+/H1N1−)	NEG (FluA−/H1N1−)	
FluA+/H1N1+	42	1	14	57
FluA+/H1N1−	0	2	5	7
FluA−/H1N1−	4	1	290	295
Total	46	4	309	**359**
	Males			
Cepheid® Xpert Flu Assay	POS (FluA+/H1N1+)	FluA (FluA+/H1N1−)	NEG (FluA−/H1N1−)	Total
FluA+/H1N1+	42	1	11	54
FluA+/H1N1−	0	2	6	8
FluA−/H1N1−	12	4	312	328
Total	54	7	329	**390**

Table 4
Age group distribution for females versus males.

Age group (y)	Gender	
	Female ($n = 359$)	Male ($n = 390$)
0–19	30 (8.4%)	36 (9.2%)
20–39	49 (13.6%)	55 (14.1%)
40–59	82 (22.8%0	76 (19.5%)
60–79	136 (37.9%)	112 (28.7%)
≥80	62 (17.3%)	111 (28.5%)
Total	359 (100%)	390 (100%)

age groups in number of true-positive samples for total FluA versus samples scored FluA positive for the MOH method (POS or FluA) but negative by the Cepheid method was statistically significant (Chi-square statistic = 13.30; $P = 0.01$). This was primarily attributable to the higher-than-predicted number of samples from patients aged over 80 years for whom there was a positive identification of FluA overall by the MOH method but a negative result for the Cepheid Xpert method.

3.5. Influence of Sample Type

For the nasopharyngeal swab (NASPH) ($n = 677$) samples, sensitivity for HIN1 detection was 84.0%, while sensitivity for overall FluA detection was 81.7%. Specificity was 95.5% and 94.1%, respectively. For other sample types, there were insufficient numbers to draw firm conclusions, particularly with respect to sensitivity (Table 1).

3.6. Influence of Location

It was difficult to draw firm conclusions on the sensitivity of detection in locations other than DH given the smaller numbers of samples from other locations (Table 1), in particular those scored positive by either method. For example, for the AH and AB samples, there were only four and six samples scored FluA+/H1N1+, respectively, or one and zero, respectively, for FluA+/H1N1− by the gold standard method. These low numbers precluded using these data in statistical analyses. Specificity for H1N1 and FluA overall was uniformly high across all locations [AB: 100% (16/16) for both; DH: 95.5% (526/551) and 94.3% (511/543), respectively; AH: 97.8% (44/45) and 91.1% (41/45), respectively; RT: 96.3% (26/27) and 100% (26/26) respectively].

Sensitivity was 82.5% (52 out of 63) and 78.9% (56 out of 71), respectively, for DH samples for H1N1 and FluA, respectively, while specificity was 95.5% (526/551) and 94.3% (511/543), respectively. Sensitivity for RT samples was higher at 96.2% (25 out of 26) and 96.3% (26 out of 27), respectively. However, when the number of true-positive samples versus samples scored as H1N1+/FluA+ by the MOH method and negative by Cepheid® Xpert Flu Assay was compared between RT and DH, there was no statistically significant difference (Chi-square statistic = 2.92; $P = 0.09$).

3.7. Influenza B Detection

Unlike the MOH-approved RealTime Ready Influenza A/H1N1 Detection Set, the Cepheid® Xpert Flu Assay can detect influenza B. In this study, seven samples gave a positive result for influenza B with negative results for influenza A and H1N1. Five of these samples gave a FluA−/H1N1− result with the gold standard kit, while one gave a FluA+/H1N1+ and the other gave a FluA+/H1N1− result.

4. Discussion

Real-time PCR-based tests are the methods of choice for FluA/H1N1 diagnosis as they are rapid and sensitive (Choi et al., 2010; Lam et al., 2010). In this study, 749 samples were taken in various locations in the Eastern Province of KSA from suspected MERS-CoV cases. In the KSA, it is mandatory that such samples be submitted to the MOH for testing and that patients who meet Category I or II status are reflexively tested for other common bacterial and viral causes of community-acquired pneumonia, such as influenza (Kingdom of Saudi Arabia Ministry of Health, 2017). We compared the results for influenza A/influenza A (H1N1) pdm09 identification between the gold standard method of choice of the MOH, the RealTime Ready Influenza A/H1N1 Detection Set, and the test used in our institution, the Cepheid® Xpert Flu Assay multiplex real-time PCR kit.

Overall, concordance was high. However, if the RealTime Ready Influenza A/H1N1 Detection Set was taken as the gold standard, specificity of the Cepheid® Xpert Flu Assay was generally high, while sensitivity was lower. A significant trend towards lower sensitivity of total Influenza A (FluA) detection was observed in samples taken from males versus females and from older patients, although it was not statistically significant for influenza A (H1N1) pdm09 detection specifically. A larger number of samples would be helpful in establishing if there was any significant trend in reduced sensitivity of influenza A (H1N1) pdm09 detection for males or for older patients. Reduced sensitivity of total FluA detection in older patients could be related to decreased viral shedding in older adults or to delayed presentation, which has been shown to influence sensitivity of other types of H1N1 diagnostic tests such as rapid antigen testing (Flamaing et al., 2003; Talbot et al., 2010). In our study, there was a higher-than-predicted proportion of older males compared to females aged ≥80 years, which could be relevant to the significantly reduced sensitivity of detection of FluA in samples from males versus females. Sensitivity of detection of influenza A (H1N1) pdm09 was also lower in males; while this was not statistically significant, the difference was approaching significance, and a larger sample number may allow a determination of whether this represents a real reduction in sensitivity.

The relatively small number of tracheal and SPUIN samples prevented drawing firm conclusions on variation between different sample types. Likewise, conclusions on influence of the location were limited by the relatively small numbers of positive samples from locations other than Dhahran city.

The pandemic influenza A (H1N1) pdm09 virus ("swine flu") was first identified in humans in March/April 2009 in Mexico, Canada, and the United States (Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team et al., 2009). Subsequently, it spread worldwide by person-to-person contact, including to the KSA (Al-Tawfiq et al., 2011; Balkhy et al., 2010; Uthman et al., 2014). In June 2009, WHO declared that influenza A (H1N1) pdm09 had reached pandemic level phase 6. In order to prevent and control influenza outbreaks, epidemics, and pandemics and initiate appropriate and timely antiviral treatment, rapid and accurate diagnostic methods are essential. Clinical presentations of different seasonal flu strains, including influenza A (H1N1)

Table 5
Sensitivity and specificity of Cepheid® Xpert Flu Assay (Cepheid) according to age group.

Age group (years)	Percentage of observed agreements	Sensitivity (H1N1 detection)	Specificity (H1N1 detection)	Sensitivity (FluA overall)	Specificity (FluA overall)
0–19 ($n = 66$)	96.97	100% (18/18)	95.8% (46/48)	100% (20/20)	97.8% (45/46)
20–39 ($n = 104$)	94.23	88.9% (16/18)	95.3% (82/86)	88.9% (16/18)	95.3% (82/86)
40–59 ($n = 158$)	87.34	83.3% (30/36)	92.6% (113/122)	84.6% (33/39)	89.1% (106/119)
60–79 ($n = 248$)	92.34	68.2% (15/22)	95.6% (216/226)	68.2% (15/22)	94.7% (214/226)
≥80 ($n = 173$)	93.06	83.3% (5/6)	97.6% (163/167)	50% (6/12)	96.3% (155/161)

pdm09, are indistinguishable both from each other and from other acute respiratory viral infections (Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team et al., 2009). Diagnostic methods of choice are therefore currently based on molecular techniques, in particular, the real-time PCR as in the kits used in this study, which have the advantages of being rapid and sensitive (Choi et al., 2010; Lam et al., 2010).

The RealTime Ready Influenza A/H1N1 Detection Set (Roche Diagnostics GmbH, Germany) incorporates a generic influenza virus A PCR targeting the matrix protein-2 "M2 gene" (M2 PCR) and a specific PCR targeting the variable part of hemagglutinin (HA) of influenza A (H1N1) pdm09 (H1 PCR) (Barbás et al., 2012; Choi et al., 2010; thi Tham et al., 2012; Wenzel et al., 2010). This kit has been shown to have specificity of 97%–100% in detection of both influenza A and of influenza A (H1N1) pdm09 in comparison to the US Centers for Disease Control and Prevention/WHO real-time PCR protocol (Choi et al., 2010; thi Tham et al., 2012). Sensitivity results have been more variable. In one study of 1341 nasopharyngeal (NP) samples collected in Argentina in 2009, sensitivity for seasonal influenza A detection in the presence or absence of influenza A (H1N1) pdm09 was 75.4%, sensitivity for influenza A (H1N1) pdm09 detection was 76.4%, and sensitivity for detection of influenza A only was 53.3% (Barbás et al., 2012). However, other studies suggested that sensitivity of newer generations of the kit was moderate to good, between 85.8% and 88% for influenza A (M2 PCR) and approximately 88.2% for influenza A (H1N1) pdm09 (H1 PCR), and was comparable to other molecular tests (Choi et al., 2010; thi Tham et al., 2012; Wenzel et al., 2010).

In our laboratory, we use an alternative real-time PCR method, the Cepheid® Xpert Flu Assay multiplex real-time PCR (Cepheid). It allows differential, qualitative detection of viral RNA from influenza A, influenza B, and influenza A (H1N1) pdm09. In this study, we nominated the MOH-approved RealTime Ready Influenza A/H1N1 Detection Set as the gold standard. However, there is some evidence to suggest that the Cepheid® Xpert Flu Assay may be more sensitive than the RealTime Ready Influenza A/H1N1 Detection Set in comparison to other molecular tests or to culture detection (Barbás et al., 2012; Choi et al., 2010; Novak-Weekley et al., 2012; Popowitch et al., 2011; Salez et al., 2012; Sohn et al., 2013; thi Tham et al., 2012;Wenzel et al., 2010). The Xpert assay has been previously shown to be 100% specific for detection of seasonal influenza A and influenza A (H1N1) pdm09 in NP samples compared both to other molecular tests and to culture testing (Novak-Weekley et al., 2012; Popowitch et al., 2011; Salez et al., 2012). Furthermore, its sensitivity in comparison to other molecular tests was 100% for seasonal influenza A and between 90% and 98.4% for influenza A (H1N1) pdm09, 90%–100% for seasonal influenza A in comparison to culture detection, and 100% for influenza A (H1N1) pdm09 in comparison to culture detection (Novak-Weekley et al., 2012; Popowitch et al., 2011; Salez et al., 2012). In a small comparison study of 102 clinical samples in Korea, overall, 99% agreement was observed between the Cepheid® Xpert Flu Assay multiplex real-time PCR kit and the RealTime Ready Influenza A/H1N1 Detection Set (Sohn et al., 2013). In our larger study, we observed 92.1% agreement. The Xpert kit requires less time for test performance, including less hands-on time (Sohn et al., 2013). Another important advantage is that the Xpert kit can also detect influenza B. While influenza B infections cause epidemics more rarely than the A strains, they nevertheless do cause human illness and are incorporated in the seasonal influenza vaccines (WHO, 2016).

However, recent studies have suggested that caution is needed in interpretation of automatic results generated by the Cepheid® Xpert Flu Assay which result in false-negatives (Engelmann et al., 2017). Careful examination of amplification curves and endpoints is recommended to avoid reporting of such false-negatives. In our study, we observed no significant difference in median and endpoint C_t values for Cepheid® Xpert Flu Assay which were concordant with the Roche assay compared to those which were discordant. Nevertheless, this remains a concern with use of the Cepheid® Xpert Flu Assay (Engelmann et al., 2017). Sensitivity of the Cepheid® Xpert Flu Assay can also vary according to genetic drift resulting in different seasonal influenza strains (Stellrecht et al., 2017). Sensitivity of the Cepheid® Xpert Flu Assay was 85.2%, with different limits of detection depending on strain. Thus, it is recommended that strain-associated variances should be carefully monitored for the Cepheid® Xpert Flu Assay (Stellrecht et al., 2017).

Limitations of our study included the relatively small number of samples from sites other than Dhahran City and of sample types other than NP swabs. Also, available clinical information was limited to age, sex, areas, and sample types. We had no information concerning underlying or subsequent conditions of the patients. Another limitation was that duplicate samples were taken from patients and the two assays were not performed on the same sample; thus, there is the possibility that there may be differences in sample quality which could contribute to discordance between assays. The fact that C_t values did not differ for either assay between discordant and concordant samples suggests that this is unlikely, but it remains a possible limitation.

Over 200,000 respiratory deaths are estimated to have occurred globally during the 2009/2010 influenza A (H1N1) pdm09 pandemic, with 80% of deaths in people younger than 65 years (Dawood et al., 2012). In the Eastern Province of KSA, 587 cases were detected from samples sent to the MOH by the Saudi Aramco Medical Services Organization (SAMSO) between June and October 2009, an incidence of 3.5 per 1000 (Herzallah et al., 2011). Infection was particularly common among younger people. It is therefore vital to be vigilant in monitoring potential outbreaks of this disease in the KSA. Testing samples both on site in the Johns Hopkins Aramco Healthcare Centre using the Cepheid® Xpert Flu Assay and in the MOH laboratory using the RealTime Ready Influenza A/H1N1 Detection Set may help expedite detection of cases and improve reliability of detection in the Eastern Province. This should help with prevention and control of outbreaks, and with initiation of appropriate and timely antiviral treatment. However, the lower agreement between the tests in terms of positive results should be borne in mind, in particular for males and for older patients.

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