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Full Length Research Paper

Comparison of the Cepheid Xpert FluA/H1N1 screening test with real time polymerase chain reaction (PCR) in detection of 2009 H1N1 Influenza A Pandemic

S. M. Al Johani* and J. Akhter

Division of Microbiology, Department of Pathology and Laboratory Medicine, King Abdulaziz Medical City, Riyadh 11426, Saudi Arabia.

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Although infections with the novel pandemic 2009 influenza A (H1N1) virus (A/H1N1/2009) appear to be relatively mild during the summer months of circulation ('off season'), there has been significant morbidity and hospitalization and several fatal cases. Thus, rapid detection of A/H1N1/2009 is crucial. In contrast to seasonal influenza, where point-of-care (POC) rapid antigen tests and direct fluorescent antibody (DFA) staining ensure rapid detection, diagnosis of A/H1N1/2009 has so far been based mainly on RT-PCR due to lack of sensitivity of the other non-molecular methods. This study is aimed to evaluate the Xpert FluA/H1N1 test (Cepheid®), rapid molecular test for influenza A virus including A/H1N1/2009 for the detection of the recently emerged swine influenza A (H1N1) and compare it with RT-PCR. A total of 386 respiratory samples were tested in parallel using Cepheid Xpert FluA and compared with RT-PCR. We determined the analytical performance characteristics (sensitivity, specificity) of the xpert test using RT-PCR as the gold standard. RESULTS: Xpert Flu A Panel detected A(H1N1) seasonal and 2009 pandemic, A(H3N2), A(H5N2), A(H5N1) and A(H7N7) viruses and correctly subtyped A(H1N1) 2009 virus. Of 386 samples, 53 samples were positive by the two methods, RT-PCR detected 2 samples that were negative by Xpert Flu. Analytical sensitivity was comparable to RT-PCR. The Xpert Flu A Panel is the first commercially available rapid molecular test for detection of influenza A and B viruses and at the same time, it can determine H1 2009 subtype. The test has comparable sensitivity compared with RT-PCR and high specificity. Therefore, it represents a useful rapid test for molecular detection of Flu A and B viruses and can also rule out H1N1.

Key words: Xpert fluA, 2009 H1N1, rapid Influenza diagnostics.

INTRODUCTION

In April 2009, a novel influenza A (H1N1) virus was detected in the US, which developed in to pandemic proportions. Pandemic H1N1 2009 (pdmH1) has a broad clinical spectrum. Although, many cases are mild, its pathogenesis is not necessarily low as it often causes serious respiratory disorder in children, and thus early treatment is necessary.

The Pandemic once declared there were major moves to find a rapid, easy, highly sensitive and specific diagnostic method. A novel real-time RT-PCR for (A/H1N1/2009) was set up in a very short time and validated following industry-standard criteria (Panning et al., 2009; Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, 2009).

As for the infectiousness, based on higher household transmission from parents to children in comparison to seasonal influenza A (FluA), as well as a high prevalence among primary school children, pandemic H1N1 appears more likely to affect children, especially school children. In addition, since pandemic H1N1 has a long incubation period, which can facilitate latent viral transmission,

*Corresponding author. E-mail: johanis@ngha.med.sa. Tel: +9661 2520088 ext. 12817. Fax: +966-1-2520130 or 11264

wearing a mask is useful during the epidemic period. A judgment of recovery should be carefully made especially in children, because the virus remains for a long time even after resolution of fever.

Direct antigen detection (DFA), typing, and/or subtyping of influenza can be rapidly carried out by several methods, such as nucleic acid techniques, immunofluorescence assay, or enzyme-linked immunosorbent assay. Benefits of DFA include identification of good quality specimens based on the presence of epithelial cells, and the ability to test individual specimens without batching, thus improving the turn-around time compared with most PCR-based assays for low numbers of samples. In contrast to RT-PCR-based methodologies, DFA suffers from a lack of sensitivity and issues of false-positives have been identified especially during times of low prevalence or due to misreading of slides because of the subjective nature of the test. Culture-based methods have been traditionally used for the detection and characterization of influenza; however, they have a lower sensitivity compared with most molecular tests for the detection of influenza. Tissue culture methods utilize shell vial (for example, mixed monolayers of human adenocarcinoma cells and mink lung cells on a glass slide) or traditional tube or flask culture techniques (for example, rhesus monkey kidney cells or Madine Darby canine kidney cells) to support the growth of influenza. Key limitations are based on the fact that, tissue culture requires special skills for identification of cytopathic effect, which is labor intensive, and uses paired immunofluorescent methodologies to identify the virus. Emerging strains of influenza that are more pathogenic may require highly controlled environments. By contrast, PCR-based methods often involve the use of lysis buffer for viral inactivation and release of nucleic acid, thus making it feasible for laboratories with Biosafety Level 2 capabilities to handle these viruses (Pabbaraju et al., 2011).

Rapid diagnosis of influenza can facilitate timely clinical management decisions and applications of infection control precautions. Antigen detection tests for Flu A were less sensitive in comparison to culture and RT-PCR in detecting (A/H1N1/2009) with sensitivity ranging between 50-80% in several studies (Panning et al., 2009; Loeffelholz, 2008; Al Johani, 2009; Cheng et al., 2009).

In this study, we evaluated the performance of a new Molecular Point-Of-Care (POC) GeneXpert flu A /H1N1 test from Cepheid® for rapid detection of different Influanza A virus including (A/H1N1/2009) virus and compared it to RT-PCR results at King Abdulaziz Medical City, Riyadh, Saudi Arabia.

MATERIALS AND METHODS

A total of 186 respiratory patient samples were collected from patient's with influenza like illness (ILI) attending the King Abdulaziz Medical City, Riyadh. This is a 1,000 bed tertiary care facility. Patient specimens were tested in parallel using a real-time reverse-transcriptase PCR (RT-PCR) (Roche Diagnostics GmbH, Mannheim, Germany®) kits and GeneXpert FluA/ H1N1 kit from Cepheid®. All specimens were collected from patients with influenza-like illness who met the World Health Organization and Centre of Disease Control (CDC's) guidelines for screening (CDC, 2009).

Polymerase chain reaction (PCR) method

A/H1N1/2009 was detected by Reverse-Transcription Polymerase Chain Reaction (RT-PCR) based assay that begins with isolating of viral RNA from patient's nasopharyngeal aspiration specimen.

Briefly, viral RNA was extracted on the QIA symphony®SP system from 400-µL nasopharyngeal aspirates using the QIAsymphony Virus/Bacteria Kits (Qiagen, Hamburg, Germany®). The RNA was reverse-transcribed to cDNA using Transcriptor First Strand cDNA Synthesis kit (Roche Diagnostics GmbH, Mannheim, Germany®), following the manufacturer recommendations. The resultant cDNA is then amplified and detected by specific primers and probes for H1N1 using LightMix InfA swine H1 kit (TIB MOLBIOL GmbH, Berlin, Germany) and following the manufacture recommendation.

The amplification and the presence of H1N1 genotype is confirmed by the melting curve analysis using the Roche lightCycler 2.0 instruments.

GeneXpert FluA test

The Cepheid GeneXpert instrument is a cartridge-based PCR system for performing nucleic acid extraction, PCR amplification, and real-time detection automatically without intermediate sample-handling steps. GeneXpert FluA test were used according to the manufacturer's instructions on 100 µL of original sample from patients with ILI.

The GeneXpert Dx Systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence in simple or complex samples using real-time RT-PCR and PCR assays. The system consists of an instrument, personal computer, and preloaded software for running tests and viewing the results. The systems require the use of single-use disposable GeneXpert cartridges that hold the RT-PCR and PCR reagents and host the RT-PCR and PCR processes. Because the cartridges are self-contained, cross-contamination between samples is minimized. For a full description of the systems, refer to the appropriate *GeneXpert Dx System Operator Manual*.

The Xpert Flu Assay includes reagents for the detection and differentiation of Influenza A, Influenza B and Influenza A subtype 2009 H1N1 directly from nasal aspirates/washes (NA/W) and nasopharyngeal (NP) swab specimens of patients suspected of having influenza. A Sample Processing Control (SPC) and a Probe Check Control (PCC) are also included in the cartridge. The SPC is present to control for adequate processing of the target viruses and to monitor the presence of inhibitors in the PCR reaction. The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability.

RESULTS

A total of 186 patients with symptoms of influenza were tested in parallel by using two different methods GeneXpert FluA and RT-PCR. Of 186 samples, 53

Table 1. Comparing of Xpert Flu A with Gold standard test.

		CDC RT-PCR		
		+	-	Total
Xpert Flu A	+	53	0	53
	-	2	131	133
	Total	55	131	186

patient samples were positive by the two methods, RT-PCR detected 2 patient samples that were negative by Xpert Flu. Results were classified as true positive, true negative, false positive, or false negative Xpert Flu A detected A(H1N1) seasonal and 2009 pandemic, A(H3N2), A(H5N2), A(H5N1) and A(H7N7) viruses and correctly subtyped A(H1N1) 2009 virus. Analytical sensitivity was comparable to RT-PCR. Xpert Flu A sensitivity and specificity were 96.36 and 100% respectively when compared to RT-PCR (Table 1).

Sensitivity = 53/55 x 100 = 96.36%

Specificity = 131/131 x 100 = 100%

Positive Predictive Value = 53/ (53 + 2) = 96.36%

Negative Predictive Value = 131 (0 + 131) = 100%

DISCUSSION

In January 2010, Cepheid granted Emergency Use Authorization (EUA) from the U.S. Food and Drug Administration (FDA) for its Xpert® Flu A Panel test. The test, which runs on Cepheid's GeneXpert® System, identifies Influenza A, Influenza B, and identified the 2009 H1N1 influenza virus in less than one hour. The FDA has authorized Cepheid's Xpert Flu A Panel to be used in laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) to perform "moderate complexity" (not waived) testing, enabling the test to be performed in hospital near-patient settings.

Molecular testing is now recognized as the new gold standard for detection of influenza virus infection, test availability for 2009, H1N1 has so far been limited to high-complexity laboratories and results are not typically available around the clock. Xpert Flu A Panel combines the convenience and ease-of-use of rapid testing with the performance of PCR, in a test format that maximizes medical value by providing results when they are most needed.

All other rapid Influenza tests currently on the market are designed to detect influenza type A, type B, or both. They can distinguish influenza A from influenza B, but cannot distinguish (A/H1N1/2009) it from seasonal strains of flu (Wilde, 2009).

Routine testing for (A/H1N1/2009) using rapid Enzyme Immunoassay (EIA) tests is not recommended by the CDC because the sensitivities of the currently available rapid tests for the detection of (A/H1N1/2009) are quite poor. Various studies have shown detection rates between 11 and 70% (CDC 2009a; Faix et al., 2009; CDC, 2009b; Jenny et al., 2010; Sambol et al., 2010). This means that the rapid test may fail to detect (A/H1N1/2009) in 30-90% of cases (Wilde, 2009). Xpert Flu A, on the other hand, combines the rapid detection time, on demand use and high sensitivity and specificity, which are comparable to RT-PCR (Jenny et al., 2010; Sambol et al., 2010).

The Xpert Flu A Panel is the first commercially available POC molecular test for detection of influenza A virus and determination of the H1 2009 subtype and is comparably sensitive compared with RT-PCR and highly specific and therefore it represent an excellent alternative to antigenic POC tests, but it is by far more expensive than routine Point Of Care testing.

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