H1N1 Testing: A Comparative Study between the Rapid BD™ Directigen EZ Flu A+B test and PCR

Abdulkarim F. Alhetheel[1,2]*, Ahmed M. Albarrag[1,2], Ali M. Somily[1,2] and Saleh A. Eifan[3]

[1] King Khalid University Hospital, Riyadh, Saudi Arabia.
[2] Department of Microbiology/Pathology, College of Medicine, King Saud University, Riyadh, Saudi Arabia.
[3] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia.

The novel strain of influenza A virus (H1N1) causes infection in millions of people, with significant mortality and morbidity. Therefore, this virus must be precisely detected as quickly as possible to obtain a better prognosis. Here, we evaluated the performance of the rapid BD™ Directigen EZ Flu A+B test (BD-RDT) as a screening method for diagnosing H1N1 infection. A total of 2154 samples from suspected cases received between August 2009 and March 2010 were screened for H1N1 using the BD-RDT, and the results were confirmed by a polymerase chain reaction (PCR) technique. We found that 600 (27.85%) samples tested positive by PCR, and the highest detection was observed in November 2009. The overall sensitivity, specificity, positive predictive value, and negative predictive value of the BD-RDT were 20.5%, 99.3%, 92.48%, and 76.39%, respectively. In conclusion, the BD-RDT has a very low sensitivity, so its results must be confirmed by PCR.

Key words: Rapid test, BD™ Directigen EZ Flu A+B test, PCR, H1N1**.**

Influenza A virus is one of the major pathogens of the respiratory tract in humans and animals.[1] This virus belongs to the single-stranded RNA virus family known as Orthomyxoviridae. Structurally, influenza A virus has two glycoprotein spikes, known as hemagglutinin (H) and neuraminidase (N). These glycoproteins play a vital role in the pathogenesis of influenza infection. In addition, influenza A virus is divided into several subtypes based on the rearrangement of these glycoproteins, which commonly occurs during antigenic shift and during pandemics.[1]

H1N1 is a subtype of influenza A virus that has emerged as a novel strain, causing serious respiratory infections and pandemic,[2-5] Over the last few years, this novel strain of H1N1 virus has been the prevalent, widespread strain of influenza in certain areas of the world, resulting in millions of infections and thousands of deaths among humans.[6-7] Therefore, the immediate and accurate detection of H1N1 in clinical specimens will facilitate early diagnosis and treatment and improve patient outcomes. There are several virological testing methods for the diagnosis of H1N1 infection, such as tissue culture,

* To whom all correspondence should be addressed.
Tel.: +96614671523 (Off); Fax: +9661479162;
Mob.: +966560793999;
E-mail: aalhetheel@ksu.edu.sa
          abdulkarimfahad@hotmail.com

immunofluorescence assays, rapid detection tests (RDTs), and polymerase chain reaction (PCR). However, there is a need for rapid detection methods such as an RDT because they provide an easier, faster, and lower-cost alternative to the diagnostic gold standard of qualitative real-time PCR. An RDT is thus ideal as a screening test in critical and pandemic situations. It is well established that a very good screening test should have a high sensitivity rate and a moderate to high specificity rate. Several studies have revealed that the sensitivity of H1N1 RDTs from different manufacturers ranges between 10% and 82%.[8-20] Thus, in the present study, we investigated the value of the BD™ Directigen EZ Flu A+B test (BD-RDT) as a screening test for the H1N1 pandemic by comparing its results with those of a PCR technique during the period from August 2009 to March 2010.

MATERIALS AND METHODS

This retrospective study was conducted on a total of 2154 nasal or throat swab samples collected from patients with respiratory illness and suspected H1N1 infection. These samples were received from August 2009 to March 2010 by our virology laboratory and analyzed using two techniques: the BD-RDT and PCR. The patients included 1195 males and 959 females, and their ages ranged between 2 years and 70 years.

Rapid detection test (RDT)

An RDT was performed using the BD™ Directigen EZ Flu A+B kit (Becton Dickinson, Sparks, MD) according to the manufacturer's instructions. In total, 300 µL of each freshly collected specimen from patients with influenza-like illness was used to perform the BD-RDT. Two positive controls, consisting of known seasonal influenza and H1N1, were included in each run, as well as one known negative control.

Qualitative real-time PCR

Viral RNA was extracted from 400 µL of each nasopharyngeal specimen using the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Diagnostics, Indianapolis, IN) and the MagNA Pure LC Instrument (Roche Applied Science, Indianapolis, IN). The extracted viral RNA was eluted into 60 µL of elution buffer. This step was followed by one-step reverse transcription and

amplification using the RealTime ready Influenza A/H1N1 Detection Set with the RealTime ready RNA Virus Master and LightCycler 2.0 Instrument (Roche Applied Science, Indianapolis, IN). A total of 5 µL of extracted RNA was mixed with 15 µL of master mix containing a primer for influenza A virus, and at the same time, 5 µL of extracted RNA was mixed with another 15 µL of master mix containing a specific primer for swine H1N1. Both samples were run on the LightCycler 2.0 Instrument as recommended by the manufacturer.

Statistical analysis

Data were collected and entered into a Microsoft Office Excel file for ease of handling of different statistical measures and statistical presentation. Percentages, mean values, and Student's t-test were applied to determine significance, where applicable. Agreement was determined using the Kappa value. The significance level was established at $P<0.05$. The sensitivity (Sn), specificity (Sp), positive predictive value (Ppv), and negative predictive value (Npv) were determined by the following formulae: Sn = true positive / (true positive + false negative), Sp = true negative / (true negative + false positive), Ppv = true positive / (true positive + false positive), and Npv = true negative / (true negative + false negative).

RESULTS

During the study period from August 2009 to March 2010, we received 2154 samples that were tested for H1N1. The seasonal analysis showed a trend that can be described as an increase in the number of suspected samples received for H1N1 testing from August 2009 (n=47) to November 2009 (n=815), when the highest number was recorded, as shown in Figure 1. The number of samples then declined from December 2009 (n=269) until March 2010 (n=196) (Figure 1). In a similar trend, the number of positive H1N1 cases identified by the BD-RDT or PCR also increased from August 2009 (n=10 vs 19 for BD-RDT vs PCR), until it reached the highest number of positive cases in November 2009 (n=90 vs 359) (Figures 1 and 2). Similar to the number of clinical samples, the number of positive H1N1 samples also declined from December 2009 (n=8 vs 22), until it reached the lowest number of positive cases,

Table 1. Sensitivity, specificity, and positive and negative predictive
values of the BD-RDT among females and males and overall

Gender	# Cases	PCR+		PCR-		Sn(%)	Sp(%)	Ppv(%)	Npv(%)
		RDT+	RDT-	RDT+	RDT-				
Female	959	60	221	6	672	21.35	99.11	90.91	75.25
Male	1195	63	256	4	872	19.75	99.54	94.03	77.30
Overall	2154	123	477	10	1544	20.50	99.30	92.50	76.40

detected in March 2009 (n=2 vs 5) (Figs. 1 and 2). In this study, we found that among the total of 2154 clinical samples tested, 600 (27.78%) samples were positive for H1N1 by PCR throughout the study period. In total, 319 (53.17%) were samples from males, and 281 (46.83%) were samples from females. We also found that among the positive cases, approximately 123 (5.70%) samples were true positives (positive by the BD-RDT and PCR), whereas 10 (0.46%) samples were false positives (positive by the BD-RDT and negative by PCR), and surprisingly, 477 (22.08%) samples were false negatives (negative by the BD-RDT positive by PCR) (Table 1). The average detection rate of H1N1 was significantly reduced in the BD-RDT compared with PCR (6% vs 22%, $P<0.05$). The overall sensitivity, specificity, positive predictive value, and negative predictive value of the BD-RDT in comparison with the PCR were 20.5%, 99.3%, 92.5%, and 76.4%, respectively. The specific sensitivities of the BD-RDT in females and males were 21.35% and 19.75%, respectively, whereas the specificities were 99.11% and 99.45%, respectively (Table 1).
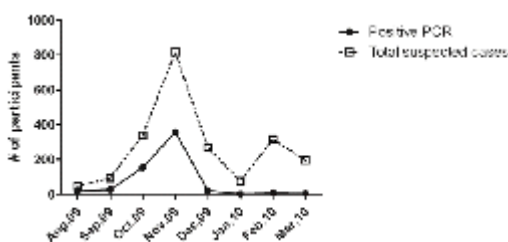
The symbols and line graph show the seasonal analysis of H1N1 in Riyadh. The square symbols with the dotted line represent the total number of suspected cases. The circle with the solid line represents the positive cases detected by PCR.

Fig. 1. Seasonal analysis of H1N1 swine flu in Riyadh between August 2009 and March 2010
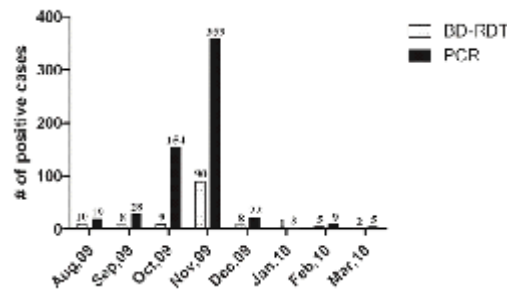
The bar graph shows the positive samples detected by the BD-RDT and PCR. The white, dotted bars represent the positive cases detected by the BD-RDT. The black bars represent the positive cases detected by PCR

Fig. 2. Number of H1N1-positive cases detected by the BD-RDT and PCR from August 2009 to March 2010

DISCUSSION

This is the first report from a large tertiary care university hospital in Saudi Arabia on the detection and analysis of H1N1 infection covering eight months of flu season in 2009. Our data suggested that the second wave of the outbreak of H1N1 infection most likely started with 19 positive cases in August 2009. The wave then reached a peak of 359 positive cases in November 2009 and ended with 5 positive cases in March 2010. Reports from Saudi Arabia have confirmed that the appearance of H1N1 infection was observed between May and June 2009, which was

perhaps the first wave.[21-23] These data are consistent with the findings of other studies from different regions, which have also reported waves of the outbreak of H1N1 infection between June and November 2009.[24-25]

In the present study, we found that the trends of the BD-RDT and PCR in the detection of H1N1 were similar, but the detection rate was significantly reduced in the BD-RDT compared with PCR, especially during the peak of the outbreak. This result was confirmed using a measurement of agreement (Kappa = 0.261, $P<0.0001$) (Figure 2). The overall sensitivity, specificity, positive predictive value, and negative predictive value of the BD-RDT in comparison with PCR were 20.5%, 99.3%, 92.5%, and 76.4%, respectively. It is known that sensitivity carries a greater significance than specificity by being responsible for controlling the rate of false-negative cases. The low sensitivity that appeared in this study implied a possible loss of 477 of 2156 false-negative cases if they had not undergone PCR testing (Table 1). In contrast, the importance of specificity being high (99.4%) lies in the fact that it ensures a low number of false positives, for example, only 10 of 2156 in the current study (Table 1). Our results were similar to those of a study conducted by Al-Johani et al. in the same region in October 2009. Although the study was performed on a relatively low number of samples in comparison with the current work, the study showed that among 143 nasal swabs and nasopharyngeal aspirates tested for H1N1, approximately 34 (23.8%) were positive by PCR, and the sensitivity, specificity, positive predictive value, and negative predictive value of the BD-RDT compared with PCR were 20.6%, 99%, 87.5%, and 80%, respectively.[8] Other reports from different countries have revealed that the sensitivity of the same BD-RDT kit in comparison with PCR or tissue culture is between 43.8% and 82%.[9,19, 26-28] The disagreement between these reports suggests that the BD-RDT is a very fragile technique and that many factors may influence its result. In fact, it has been reported that the sensitivity and specificity of H1N1 RDTs may vary due to several factors, including product type, sample type, inappropriate sample collection, the time of sample collection, the patient's age, and other factors.[29] A study conducted in the United States of America showed that the overall sensitivity and specificity of a rapid

test were 53.9% and 98.5%, respectively, and the sensitivity obtained was significantly reduced with increasing age.[27] Another study revealed that the sensitivity of a rapid test increased significantly in patient samples that had been collected within two days of onset.[30] In the present study, we also explored whether gender affected the sensitivity and specificity of the BD-RDT, and based on our data, it appeared that gender had no significant effects on the sensitivity or specificity of the BD-RDT, confirming others' findings.[30] Several of the limitations in this report included that the time of sample collection after onset was unknown and that the time between sample collection and performing the test was also unknown. In addition, our data were not analyzed on the basis of the patient's age. Thus, all of these factors may have had significant effects on the sensitivity of the RDT.[29]

CONCLUSIONS

The overall sensitivity of the BD-RDT was very low (20.5%), which could be related to the heterogeneity of the population tested and/or to differences in the time of sample collection; a definite explanation is not readily available. In contrast, the specificity and the positive predictive value were relatively high. Thus, using PCR to confirm negative BD-RDT more often is recommended, especially during the peak of the season, to minimize false-negative cases.

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