The role of rapid testing and clinical decision in the diagnosis of human influenza A H1N1 infection

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ABSTRACT

الأهداف: تقييم دور الاختبار السريع (RIDT) و المعايير الإكلينيكية في تشخيص الانفلونزا (H1N1). وقد كان (RT–PCR) هو المعيار الذهبي للمقارنة.

الطريقة: خلال الفترة من نوفمبر 2009م تم فحص 290 من المرضى المشتبه بإصابتهم بالأنفلونزا أثناء تفشى المرض بالرياض المملكة العربية السعودية وتم حساب مؤشرات صحة الاختبار السريع وأيضا صحة معايير التشخيص الإكلينيكية. تم تحليل مسحات البلعوم باستخدام «Directigen EZ Flu A+B kit» (بيكتون و ديكنسون، الولايات المتحدة الأمريكية)، وكذلك باستخدام الزمن الحقيقي لتفاعل البلمرة التسلسلى (RT–PCR) (روش، ألمانيا). واستخدمت عناصر إيجابية وسلبية في كل تشغيل للعينات.

النتائج: كانت حساسية الاختبار السريع لتشخيص الأنفلونزا 40.5% و (فاصل الثقة 95% =33.0-48.5) وخصوصية الاختبار السريع 94.5% (فاصل الثقة 95% =88.6-97.6) على التوالي. و كانت حساسية التشخيص الإكلينيكي 66.3% (فاصل الثقة 95% =58.4-73.4) وكانت خصوصية التشخيص الإكلينيكي 65.4% (فاصل الثقة 95% =56.3-73.4) وكانت حساسية التشخيص الإكلينيكي أعلى في الحالات المشاهدة في وقت مبكر من المرض 79.2% (فاصل الثقة 95% =57.3-92.1) بينما كانت حساسية الاختبار السريع لتشخيص الأنفلونزا أعلى في المرضى الأصغر سنا 48.4% (فاصل الثقة 95% =35.7-61.3). كانت القيمة التنبؤية الإيجابية للاختبار السريع لتشخيص الأنفلونزا (PPV) 90.4% (فاصل الثقة 95% =57.3–92.1) بينما كانت (PPV) القيمة التنبؤية الإيجابية للتشخيص الإكلينيكي 71.1% (فاصل الثقة 95% =57.3–92.1) كانت القيمة التنبؤية الإيجابية للاختبار السريع لتشخيص الأنفلونزا (PPV) أعلى في الأكبر سناً 94.7% (فاصل الثقة 95% =80.9-99.1) وفى الحالات المشاهدة في وقت متأخر من المرض 90.7% (فاصل الثقة 95% =76.9-97.0) وكانت نسبة الأرجحية المصححة للتشخيص الإكلينيكي ذات دلالة معنوية مع السعال، والصداع والارهاق وليست ذات دلالة معنوية مع العطس وتاريخ عائلي إيجابي للمرض.

خاتمة: يعتبر استخدام الاختبار السريع لتشخيص الأنفلونزا مفيدا في حالات الأوبئة والأماكن عالية الانتشار بينما يكون التشخيص الإكلينيكي والناسخ العكسي–تفاعل البلمرة هما المتممان لتشخيص الأنفلونزا H1N1 في أي ظروف.

Objectives: To evaluate the role of the rapid influenza diagnostic test (RIDT) and clinical decision in the diagnosis of H1N1.

Methods: In November 2009, 290 suspected influenza patients were examined for H1N1 during an outbreak in Riyadh, Saudi Arabia. Nasopharyngeal swabs were analyzed using Directigen EZ Flu A+B kit. Monoclonal anti-human influenza A/B and reverse transcription-polymerase chain reaction (RT-PCR) were used. Positive and negative controls were used in each run of specimens. Validity indices were calculated for RIDT and clinical diagnostic criteria.

Results: The sensitivity and specificity of RIDT were 40.5% (95% confidence interval [CI]: 33.0-48.5), and 94.5% (95% CI: 88.6-97.6). The sensitivity of clinical decision was 66.3% (95% CI: 58.4-73.4), and the specificity was 65.4% (95% CI: 56.3-73.4). The sensitivity of clinical decision was higher in early presenters (79.2%; 95% CI: 57.3-92.1). The RIDT sensitivity was higher in younger patients (48.4%; 95% CI: 35.7-61.3). The positive predictive value (PPV) was 90.4% (95% CI: 80.7-95.7) for RIDT, and 71.1% (95% CI: 63.1-78.0) for clinical decision. The PPV for RIDT was greater for older (94.7%; 95% CI: 80.9-99.1) and late (90.7%; 95% CI: 76.9-97.0) presenters. The adjusted odds ratio for clinical decision was significant for cough, headache, and fatigue.

Conclusions: The RIDT can be useful in epidemics and high prevalence areas, whereas clinical decision, and RT-PCR complement the diagnosis of H1N1 in any setting.

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Influenza is a major global public health threat. During a pandemic, prompt, and accurate diagnoses are critical for successful treatment and prevention of transmission.[1] Compared with viral culture, reverse transcription-polymerase chain reaction (RT-PCR) is reported to have superior sensitivity for detection of influenza infection,[2,3] and is the diagnostic standard for novel influenza A H1N1 infection (H1N1). The RT-PCR was the standard recommended by the World Health Organization (WHO) during the 2009 influenza pandemic.[4] However, both of these methods are expensive, require technical expertise, and the results are not immediately available for the guidance of clinical decisions.[5] The rapid diagnosis of influenza is necessary to implement timely antiviral therapy and infection control measures to reduce virus transmission.[6,7] Additionally, accurate rapid diagnosis will reduce costs and improve patient outcomes, and will reduce unnecessary testing and prescription of antibiotics.[6] Rapid point-of-care diagnostic tests can be performed in less than 15 minutes and improve the management of patients suspected to be infected with H1N1.[7] However, inconsistent performance has been reported for H1N1 rapid influenza kits.[8] During the last H1N1 pandemic of 2009, the US Centers for Disease Control and Prevention (CDC) evaluated 3 widely used commercially available rapid influenza diagnostic tests (RIDT): Inverness Medical BinaxNOW Influenza A&B (Binax Inc., Scarborough, Maine, USA), Becton Dickinson Directigen EZ Flu A+B (Becton, Dickinson and Company, Sparks, Maryland, USA) and Quidel QuickVue Influenza A+B (Quidel Corporation, San Diego, California, USA). They found that the tests had a low sensitivity (40-69%) among all specimens tested. This sensitivity declined substantially as virus levels decreased.[8] The study had some limitations, such as testing relatively few clinical specimens, and shipping in different or unknown transport media. Therefore, it was recommended to interpret the results of the test with caution, and make treatment decisions based on the level of clinical suspicion, underlying medical conditions, severity of illness, and risk for complications, until additional data is available.[8]

The uncertainty regarding the sensitivity of RIDT kits and the role of clinical judgment for the identification of H1N1 prompted us to carry out this investigation. The aim of our study was to evaluate the role of RIDT and clinical decision for the diagnosis of H1N1. The RT-PCR was the gold standard for comparison.

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Methods. Data for the study were collected from November 10 to 25, 2009, from patients who presented at an influenza outpatient clinic at King Khalid University Hospital (KKUH), King Saud University, Riyadh, Saudi Arabia. The outpatient clinical services were provided by the Department of Family Medicine. Only patients who had not received any antiviral treatment for influenza were included in the study. A structured questionnaire was used to collect the following information from each patient: socio-demographic data, such as age and gender, symptoms (for example, history of fever, rigors, headache, muscle ache, nausea, sore throat, sneezing, cough, fatigue) and the duration of symptoms. After taking a complete history, clinicians reviewed the patient questionnaire and performed a clinical examination. These board-certified practicing family physicians were up to date on the WHO case definition for influenza-like illness (ILI; a person with a sudden onset of fever of >38°C and cough or sore throat in the absence of other diagnoses),[4] and were involved in the management of H1N1 cases from the beginning of the pandemic. Based on clinical judgment and experience (and without knowing the RT-PCR or RIDT test results), each treating physician was asked to specify whether they thought the patient was infected with H1N1.

Nasopharyngeal swabs were used to collect samples from each patient's naso-pharynx. The samples were blind tested with RIDT and real-time RT-PCR in the molecular biology laboratory at KKUH. We used Microsoft Access software (Microsoft Corporation, Redmond, WA, USA) to enter the data and MedCalc and Epi Info™ software (CDC, Atlanta, GA, USA) for analysis.

RIDT test procedure. Freshly collected specimens (nasopharyngeal swab) were placed in viral transport media (Micro Test M4RT®, Multi Microbe Media, Lenexa, KS, USA),[9] and rapidly transported to the virology laboratory. They were then processed immediately for rapid testing for influenza A and B viral antigens according to the manufacturer's instructions for the Directigen™ EZ Flu A+B kit (Becton Dickinson and Company, Sparks, Maryland, USA). Briefly, the specimen was applied onto the specimen area, where in the presence of Influenza A/B, they migrate and react with colloidal gold conjugated to mouse monoclonal anti-human Influenza A or B virus and moreover, react

with mouse monoclonal anti-human Influenza A or B virus. They were caught in the test line area, where they were visible. The purple-red line indicated the presence of Influenza A or B virus. Simultaneously, purple-red lines were also visible for catching colloidal gold conjugated to rabbit immunoglobulin by anti-rabbit immunoglobulin on the control line, regardless of presence of Influenza A or B. In-house positive controls and in-house negative controls were included in the run of every patch of specimens. Suspected results were repeated for confirmation.

Real-time RT-PCR. For extraction of nucleic acids from the specimens, high pure viral nucleic acid kits (Roche, Mannheim, Germany) were used. Viruses were lysed by detergent and proteinase K to release total viral nucleic acid (NA). Then, with the presence of a chaotropic salt (guanidine HCL), viral NA binds selectively to the glass fiber fleece in a special centrifuge tube. The NA remained bound while contaminating cellular components were removed during a series of rapid "wash-and-spin" steps. The viral NA was removed by the use of low salt elusion. Precipitation, organic solvent extraction, or extensive handling of viral NA was not required. The A/H1N1 RNA virus detection was performed with a real-time Ready Influenza A/H1N1 Detection Kit on a Light Cycler 1.2 (Roche, Mannheim, Germany) according to the manufacturer's instructions. Nucleic acids were stored at -70°C until tested.[10] Following the extraction of the viral genome, the detection procedure took around 6 hours to complete. The amplification and detection of viral NA procedure included the usage of positive and negative controls in each run of specimens.

Statistical methods. Real-time RT-PCR was used as the gold standard test. Sensitivity and specificity values (validity testing) and kappa statistics (reliability testing) were calculated for clinical decision and RIDT results. Positive and negative predictive values and corresponding 95% confidence intervals (CIs) were also estimated. Histograms were used to confirm that the continuous variables (for example, age, day of presenting symptoms) were normally distributed. The Student's t-test was used to test for statistical significance. Log transformation of the data was attempted if it was not normally distributed. Proportions were calculated for categorical variables. Demographic and clinical characteristics were compared using the 3 diagnostic criteria (PCR, clinical decision, and RIDT). The Chi-square test was used to test for statistical significance (α=0.05). Logistic regression modeling was performed to evaluate demographic (age and gender), general (family history, day of presentation), and clinical (fever, headache, muscle aches, fatigue, nausea, sore throat, sneezing, and cough) characteristics for their utility as diagnostic criteria.

After the study purpose and protocol were explained, an informed consent was obtained from each patient. The study was approved by the King Saud University institutional review board and conducted in accordance with the Helsinki Declaration.

Results. Two hundred and ninety patients from all age and gender groups enrolled in the H1N1 clinics. The mean ± standard deviation (SD) age was 25.18±12.1 years, and 56.9% (165/290) were male. The RT-PCR test results indicated that 51.2% (163/290) of patients were infected with H1N1. Patients with positive RT-PCR results were significantly younger than non-H1N1 ILI cases (RT-PCR negative; p=0.025). Overall 16% of the patients presented within 2 days of ILI. In contrast, 20% of the H1N1 positive cases (diagnosed by RT-PCR) presented within 2 days of ILI. The RIDT detected 66 of the 163 H1N1 cases (RIDT sensitivity was 40.5%, 95% CI: 33.0-48.5).

Table 1 - Diagnostic performance of the rapid influenza diagnostic test and clinical decision for detection of H1N1 infection in comparison with reverse transcription-polymerase chain reaction test (Gold Standard) in a study at King Khalid University Hospital, Riyadh, Saudi Arabia.

Variables	Positive (n)	Negative (n)	Reverse transcription-polymerase chain reaction								Kappa (*P*-value)
			Sensitivity		Specificity		PPV		NPV		
			(%)	(95% CI)	(%)	(95% CI)	(%)	(95% CI)	(%)	(95% CI)	
Rapid test											0.324 (0.001)
Positive	66	7	(40.5)	(33.0, 48.5)	(94.5)	(88.6, 97.6)	(90.4)	(80.7, 95.7)	(55.3)	(48.4, 62.0)	
Negative	97	120	-	-	-	-	-	-	-	-	
Clinical decision											0.313 (0.001)
Positive	108	44	(66.3)	(58.4, 73.4)	(65.4)	(56.3, 73.4)	(71.1)	(63.1, 78.0)	(60.1)	(51.4, 68.3)	
Negative	55	83	-	-	-	-	-	-	-	-	

PPV - positive predictive value, NPV - negative predictive value, CI - confidence interval

Table 2 - Diagnostic performance of the rapid influenza diagnostic test and clinical decision for the detection of human influenza A H1N1 infection in comparison to reverse transcription polymerase chain reaction (RT-PCR) test (gold standard) by age and day of presentation of patients.

Variables	RT-PCR										Kappa (*P*-value)
	Positive	Negative	Sensitivity		Specificity		PPV		NPV		
			(%)	(95% CI)	(%)	(95% CI)	(%)	(95% CI)	(%)	(95% CI)	
Rapid test, years											0.315 (<0.001)
<20											
Positive	30	5	(48.4)	(35.7, 61.3)	(87.2)	(71.8, 95.2)	(85.7)	(69.0, 94.6)	(51.5)	(39.0, 63.9)	
Negative	32	34	-	-	-	-	-	-	-	-	
≥20											0.319 (<0.001)
Positive	36	2	(35.6)	(26.5, 45.9)	(97.7)	(91.3, 99.6)	(94.7)	(80.9, 99.1)	(57.0)	(48.7, 64.9)	
Negative	65	86	-	-	-	-	-	-	-	-	
Clinical decision											
<20											0.245 (0.013)
Positive	41	16	(66.1)	(52.9, 77.4)	(59.0)	(42.2, 74.0)	(71.9)	(58.3, 82.6)	(52.3)	(36.9, 67.3)	
Negative	21	23	-	-	-	-	-	-	-	-	
≥20											0.344 (<0.001)
Positive	67	28	(66.3)	(56.2, 75.2)	(68.2)	(57.3, 77.5)	(70.5)	(60.2, 79.2)	(63.8)	(53.2, 73.3)	
Negative	34	60	-	-	-	-	-	-	-	-	
Rapid test											
Within 2 days											0.368 (0.004)
Positive	11	2	(45.8)	(26.2, 66.8)	(91.3)	(70.5, 98.5)	(84.6)	(53.7, 97.3)	(61.8)	(43.6, 77.3)	
Negative	13	21	-	-	-	-	-	-	-	-	
Later than 2 days											0.340 (<0.001)
Positive	39	4	(40.6)	(30.9, 51.1)	(95.1)	(87.2, 98.4)	(90.7)	(76.9, 97.0)	(57.5)	(48.6, 65.9)	
Negative	57	77	-	-	-	-	-	-	-	-	
Clinical decision											
Within 2 days											0.402 (0.005)
Positive	19	9	(79.2)	(57.3, 92.1)	(60.9)	(38.8, 79.5)	(67.9)	(47.6, 83.4)	(73.7)	(48.6, 89.9)	
Negative	5	14	-	-	-	-	-	-	-	-	
Later than 2 days											0.364 (<0.001)
Positive	67	27	(69.8)	(59.4, 78.5)	(66.7)	(55.2, 76.5)	(71.3)	(60.9, 79.9)	(65.1)	(53.7, 75.0)	
Negative	29	54	-	-	-	-	-	-	-	-	

PPV - positive predictive value, NPV - negative predictive value, CI - confidence interval

The attending clinicians correctly diagnosed 108 cases (clinician sensitivity was 66.3%, 95% CI: 58.4-73.4) (**Table 1**). Of the 127 H1N1 RT-PCR negative cases, RIDT falsely classified 7 patients as positive for H1N1 infection (RIDT specificity was 94.5%, 95% CI: 88.6-97.6). Clinicians misclassified 44 cases as positive for H1N1 (clinician specificity was 65.4%, 95% CI: 56.3-73.4) (**Table 1**). Using RT-PCR as a reference, the RIDT and clinical decision kappa tests for agreement were 0.324 (p<0.001) and 0.313 (p<0.001).

Compared with RT-PCR as the gold standard, RIDT sensitivity and specificity were 48.4% (95% CI: 35.7-61.3) and 87.2% (95% CI: 71.8- 95.2) for patients <20 years old. The RIDT sensitivity was 35.6% (95% CI: 26.5-45.9) for patients ≥20 years old. Values for diagnostic sensitivity and specificity for clinical decision by KKUH attending physicians did not vary by age of patient (**Table 2**).

Overall positive predictive value (PPV) of RIDT was 90.4% (95% CI: 80.7-95.7) and 71.1% (95% CI: 63.1-78.0) for clinical decision (**Table 2**). The RIDT PPV was greater for older (94.7%; 95% CI: 80.9-99.1) than for younger (85.7%; 95% CI: 69.0-94.6) individuals. However, the PPV for clinical decision was similar across age groups (**Table 2**). The PPVs for RIDT were 90.7% (95% CI: 76.9-97.0) and 84.6% (95% CI: 53.7-97.3) for patients who presented later than 2 days and within 2 days after the onset of ILI symptoms. The PPV values for clinical decision were similar for day of presentation (**Table 2**). Then negative predictive value (NPV) was generally high for clinical decision compared with RIDT (**Table 2**).

Cough was the most prevalent symptom reported by RT-PCR positive patients (93%), but was also reported by 65.3% of ILI non-H1N1 cases. Sore throat (91.7% in true cases versus 81.1% in non-cases), fatigue (81.3% in cases, and 67.8% in non-cases), headache (75.9% in cases, and 67.2% in non-cases), sneezing (66.4% in cases, and 51.6% in non-cases), fever (61% in cases, and 30.8% in non-cases), muscle aches (58.8% in cases,

Table 3 - Demographic and clinical features of patients according to H1N1 diagnosis made using reverse transcription-polymerase chain reaction.

Variables	PCR +ve*		PCR -ve†		*P*-value
	n	(%)	n	(%)	
Age					0.11
<20 years	75	(46.0)	46	(36.2)	
≥20 years	88	(54.0)	81	(63.8)	
Gender					0.02
Male	83	(50.9)	82	(64.6)	
Female	80	(49.1)	45	(35.4)	
Family history					1.00
Positive	46	(33.1)	36	(32.7)	
Negative	93	(66.9)	74	(67.3)	
Presentation					0.40
Within 2 days	24	(20.0)	23	(22.1)	
Later than 2 days	96	(80.0)	81	(77.9)	
Fever					<0.001
Present	72	(61.0)	28	(30.8)	
Not present	46	(39.0)	63	(69.2)	
Cough					<0.001
Present	147	(93.0)	81	(65.3)	
Not present	11	(07.0)	43	(34.7)	
Headache					0.11
Present	120	(75.9)	84	(67.2)	
Not present	38	(24.1)	41	(32.8)	
Muscle aches					0.07
Present	124	(58.8)	87	(41.2)	
Not present	34	(21.5)	39	(31.0)	
Fatigue					0.01
Present	122	(81.3)	82	(67.8)	
Not present	28	(18.7)	39	(32.2)	
Nausea					0.01
Present	68	(44.4)	35	(28.7)	
Not present	85	(55.6)	87	(71.3)	
Sore throat					0.01
Present	137	(91.7)	103	(81.1)	
Not present	13	(08.3)	24	(18.9)	
Sneezing					0.01
Present	101	(66.4)	65	(51.6)	
Not present	51	(33.6)	61	(48.4)	

PCR - polymerase chain reaction - *positive, †negative

and 41.2% in non-cases), and nausea/vomiting (44.4% in cases, and 28.7% in non-cases) were also reported (**Table 3**). Sore throat was the most prevalent clinical feature among H1N1 RT-PCR negative cases, followed by fatigue, headache, cough, and sneezing (**Table 3**).

More males than females were RT-PCR negative (p=0.023), but more females presented with fever (61% versus 31%). Sneezing, cough, sore throat, fatigue, and fever were present in a greater proportion in RT-PCR positive patients. The RT-PCR positive patients were more likely to be younger. There was no relationship between RT-PCR results and family history or day of presentation. Using the variables positive family history, presence of headache, muscle aches, fatigue, nausea, fever, and respiratory symptoms of cough and sore throat, but not sneezing, clinical decision was more likely to correctly identify younger patients as H1N1 positive.

The unadjusted relationship between clinical decision, patient history, and symptoms was statistically significant for fever, cough, headache, fatigue, sore throat, muscle aches, nausea/vomiting, and family history of H1N1 (**Table 4**). Results for adjusted odds ratios (AORs) from multivariable modeling of history and symptoms related to clinical decision were higher for cough (AOR 3.5, 95% CI: 1.48-8.27) than no cough, headache (AOR 2.34, 95% CI: 1.06-5.14) than no headache, fatigue (AOR 2.31, 95% CI: 1.05-5.08) than without fatigue, not sneezing (AOR 1.98, 95% CI: 1.02-3.84) than sneezing, and positive family history (AOR 2.59, 95% CI: 1.34-5.01) compared with no family history of ILI.

Discussion. Early detection of H1N1 is a key component of effective infection-control practices and management of epidemics. The RIDTs require little technical skill, have a fast turnaround, and can be performed in an emergency department setting. However, assay sensitivity and specificity are low enough to make them of questionable value.[8] Our RIDT and clinician decision (H1N1 presentation and patient characteristics) results are consistent with the results for validity measures reported by other studies.[11,12] Compared with RT-PCR, our study found that RIDT had a low sensitivity (40.5%) when used for the detection of H1N1 in patients that presented at the KKUH outpatient clinic (median age 22 years). A study conducted by the CDC revealed that the sensitivity of an RIDT, which was the same as in our study (Directigen™ EZ Flu A+B kit [Becton Dickinson and Company, Sparks, Maryland, USA]), was 49% for novel H1N1 influenza.[8] Ganzenmueller et al[13] used RT-PCR as the gold standard for the detection of H1N1 virus in German patients and reported RIDT sensitivity as low as 18.2% (median age 24 years). Velascoa et al[14] reported an RIDT sensitivity of 63% among patients that presented at a general hospital in the Philippines (median age 13.7 years). A recent meta-analysis of 17 studies conducted in 2009 and 2010 with a total of 1879 cases and 3477 non-cases reported an overall RIDT sensitivity of 51% (95% CI: 41.0-60.0), and a specificity of 98% (95% CI: 94.0-99.0).[15]

Several factors may influence the ability of the RIDT to detect the H1N1 virus. Study-to-study variability

Table 4 - Demographic and clinical features of patients according to H1N1 diagnosis made using clinical decision.

Variables	Clinical Dx +ve*		Clinical Dx -ve†		OR (95% CI)	Adjusted OR (95% CI)
	n	(%)	n	(%)		
Age					1.09 (0.68-1.74)	-
<20 years	65	(42.8)	56	(40.6)		
≥20 years	87	(57.2)	82	(59.4)	-	
Gender					1.21 (0.76-1.94)	-
Male	83	(54.6)	82	(59.4)		
Female	69	(45.4)	56	(40.6)		
Family history					3.42 (1.92-6.09)	2.59 (1.34-5.01)
Positive	60	(44.8)	22	(19.1)		
Negative	74	(55.2)	93	(80.9)	-	-
Presentation					1.30 (0.67-2.50)	-
Within 2 days	24	(20.0)	23	(22.1)		
Later than 2 days	96	(80.0)	81	(77.9)		
Fever					2.11 (1.20-3.68)	-
Present	65	(56.0)	35	(37.6)		
Not present	51	(44.0)	58	(62.4)		
Cough					3.44 (1.79-6.61)	3.50 (1.48-8.27)
Present	130	(89.7)	98	(71.5)		
Not present	15	(10.3)	39	(28.5)		
Headache					2.75 (1.60-4.73)	2.34 (1.06-5.14)
Present	120	(81.6)	84	(61.8)		
Not present	27	(18.4)	52	(38.2)		
Muscle aches						
Present	125	(85.0)	86	(62.8)	3.36 (1.90-5.96)	-
Not present	22	(21.5)	39	(31.0)		
Fatigue						
Present	119	(84.4)	85	(65.4)	2.86 (1.60-5.12)	2.31 (1.05-5.08)
Not present	22	(15.0)	39	(32.2)		
Nausea						
Present	66	(47.5)	37	(27.2)	2.42 (1.46-4.00)	-
Not present	73	(52.5)	99	(72.8)		
Sore throat					3.39 (1.57-7.31)	-
Present	137	(93.2)	109	(80.1)		
Not present	10	(6.8)	27	(19.9)		
Sneezing					1.11 (0.68-1.79)	1.98 (1.02-3.84)
Present	83	(58.5)	83	(61.0)		
Not present	59	(41.5)	53	(39.0)		

Dx - diagnosis, *positive, †negative, OR - odds ratio, CI - confidence interval

in rapid flu test performance can be attributed to differences between the tests used, the type of specimen, and the influenza type/subtype. Patient age, delay in presentation since symptom onset, and the "gold standard" used for validation (for example, viral culture, PCR, immunoassay, or serology) can also affect conclusions on test performance. In our study, rapid test performance varied with patient age. The RIDT sensitivity was higher for patients <20 years of age (48.4%) than for patients ≥20 years of age (35.5%). These results are similar to the results of Rouleau et al[16] who reported higher RIDT sensitivity for younger adults, which was reduced by approximately half for patients ≥40 years of age (23-25% versus 10-12%). Similarly, Fernandez et al[17] reported the highest positive test results in patients aged 0 to 24 years. Block et al[18] suggested that children might shed more virus particles (and for a longer duration) than adults do, which may explain this age-related pattern. However, although the test sensitivity was greater in the younger age group, in our study the PPV was greater in the older age group. The PPV increases as true prevalence increases, and in our study the prevalence of infection was higher among the older patients who presented at the clinic.

The RIDT sensitivity was significantly associated with timing of specimen collection. Sensitivity

decreased with longer delay between symptom onset and collection of the specimen. Sensitivity was higher within 2 days after onset of symptoms (45.8%) than it was for patients who presented later than 2 days after onset of symptoms (40.6%). Velasco et al[14] reported similar results at 0-2 days (range of 62-75%) than on succeeding days (3 to ≥5 days; range 36-50%).

In a study that used volunteers, Carrat et al[19] examined the dynamics of viral shedding and symptoms following influenza virus infection. They found that viral shedding increased sharply between 0.5 and one day, and consistently peaked at 2 days, after challenge. The duration of viral shedding was 4.8 days, and the peak preceded the peak in symptoms of all influenza types and subtypes by one day.

In the current study, the clinical impression by treating physicians at KKUH had a higher sensitivity (66.3%) than RIDT (40.5%). Using the clinical prediction rule of fever and cough within 48 hours after onset of symptoms and RT-PCR as the gold standard for diagnosis, Stein et al[20] found a sensitivity of 75% and a specificity of 89% for clinical decision among patients 18-90 years. Monto et al[21] reported sensitivity and specificity values of 64% and 67%, for clinical decision (fever and cough within 48 hours of onset). Compared with the common cold and seasonal flu, headache, fatigue, and absence of sneezing are also much more likely to be present during H1N1 infection. Our logistic regression modeling indicated that fever, cough, and younger age were the most significant predictors when RT-PCR (data not shown) results were used for the diagnosis. However, cough, headache, fatigue,[12,22] no sneezing, and family history were also significant clinical decision indicators.

Younger age[16,17] and earlier day of presentation of symptoms[19] have also been reported as important diagnostic criteria in other settings. Our model for clinical decision did not include fever, which was excluded from the first model we examined. The lack of significance for fever could have resulted from co-linearity between the symptoms of fever and cough. In addition, we did not have data for use of antipyretics by patients. We also encountered missing values for fever (n=81) and day of symptom presentation (n=66), which may have affected the significance of our results. However, the missing data were not biased by age, gender, family history, fever, muscle ache, or fatigue, but on having more cough, more headache, more sneezing, and less nausea. In addition, our final model did not change when it was adjusted for missing values.

In this study, the clinical criteria for diagnosis of H1N1 and the results of PCR and RITD tests were related. Though consistency for such a relationship in the reported literature is assuring that these results are not significantly influenced by selection or other types of biases,[18,23] caution is needed for diagnosing H1N1 in a syndromic way, as ILI may have several types of presentations.[23] However, the syndromic approach is indispensable for an H1N1 pandemic, especially since patients travel to and from epidemic and endemic areas, use over the counter medicines, and vaccination status varies in different settings and communities.

The RIDT specificity exceeds that of unaided clinical decision by 29-31%, and false-positive results occur approximately 8.2 times more frequently by unaided clinical decision than by the RIDTs alone.[12] However, decisions on H1N1 infection status are made in many different settings. For example, prevalence can vary from setting to setting. In areas with low prevalence, patients that are RIDT negative, but have a high clinical suspicion of infection can be recommended for PCR testing. When prevalence in the community is low but patients have travelled to an endemic or epidemic area, decision by clinicians would have significant value given that physicians should be well aware of the predictive value of the rapid test.

In our study, only 47.5% of the cases diagnosed by clinicians were not correctly diagnosed by RIDT. However, this study was conducted during an H1N1 epidemic in November 2009, in Riyadh, Saudi Arabia. With increased prevalence, confirmatory tests (for example, RT-PCR) can be applied to those with symptoms (fever, cough, headache, and fatigue) more predictive of H1N1 infection, especially for younger patients and an earlier day of symptom presentation. In this study, clinical decision considered presence of headache, cough, fatigue, positive family history, younger age, and tended to be positive on the earlier day of patient presentation.

During an H1N1 epidemic, the cost of treatment, unneeded hospitalization, and non-indicated viral therapy that result from false positive results are acceptable when compared with saving lives and reducing the suffering that could occur if cases are missed or treatment is delayed. Therefore, the considerably low sensitivity and the high false negative results reported for RITD are not acceptable. Many cases of H1N1 infection will be misdiagnosed, or treatment will be delayed during the critical early days of an infection. Our findings indicated that a positive RIDT result can be used to make treatment decisions, but a negative result does not rule out infection with

H1N1 virus. Therefore, if there is a strong suspicion of influenza infection clinical judgment should be used for treatment decision.[23,24]

For low and middle-income countries with limited resources and without sophisticated diagnostic facilities and equipment, emphasis should be placed upon the cost-effective and cost-efficient use of RIDT and RT-PCR for the diagnosis of influenza, especially of H1N1. Though there is a need for better rapid tests for diagnosing influenza,[15] the RIDT can be useful in an established epidemic or pandemic situation when there is high prevalence of disease and, therefore, a high PPV. In conclusion, clinical decision is an important tool in diagnosis of H1N1 influenza in any setting, whereas RIDT is more useful in epidemics and high prevalence areas. Negative RITD results should be subject to further testing with RT-PCR for diagnosing H1N1 influenza.

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