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Original Article

Detection of 2009 pandemic influenza A(H1N1) virus Infection in different age groups by using rapid influenza diagnostic tests

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Background The performance of rapid influenza diagnostic tests (RIDTs) in detecting influenza A(H1N1) 2009 has varied widely. Evaluations of RIDTs among infected individuals across all age groups have not been described in depth.

Objectives Determine RIDT clinical sensitivity in comparison with influenza detection using real-time RT-PCR among patients infected with influenza A(H1N1) 2009 across all age groups.

Study design This study analyzed respiratory specimens received by the New Hampshire Public Health Laboratories (NHPHL) from September 1, 2009, through December 31, 2009. RIDT performance was evaluated among different age groups of patients determined to be infected with influenza A (H1N1) 2009, and the association between age and RIDT sensitivity was determined.

Results Of 1373 specimens examined, 269 tested positive for influenza A(H1N1) 2009 by real-time RT-PCR (rRT-PCR) and had RIDT results available. Overall clinical sensitivity and specificity of RIDTs were 53·9 and 98·5%, respectively. By age group, clinical sensitivity was 85·7% in patients <2 years old, 60·3% in patients between 2- and 39 years old, and 33·3% in patients aged 40 and older. Logistic regression analysis indicated that increasing age was negatively associated with RIDT performance.

Conclusion Rapid influenza diagnostic test sensitivity decreased significantly with increasing age. Findings from this study may impact a clinician's interpretation of RIDT test results and ultimately have implications in clinical decision-making.

Keywords Influenza, 2009 Pandemic Influenza A (H1N1), rapid influenza diagnostic test, sensitivity.

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Introduction

Background

Rapid influenza diagnostic tests (RIDTs) that detect influenza viral antigens are often used at the point of care to obtain quick diagnostic test results. While these tests were developed and introduced prior to the emergence of influenza A(H1N1) 2009, clinicians have utilized these tests routinely to identify patients infected with influenza A(H1N1) 2009 and to make treatment decisions for those patients. Although the specificity of RIDTs as compared to real-time RT-PCR has been shown to be high (90–100%), substantial variation in testing sensitivity (17·8–76·7%) in detecting influenza A(H1N1) 2009 has been reported.[1–5]

Previous studies have shown that RIDT sensitivity was correlated with increasing viral titer[2,6,7] and that higher RIDT sensitivity was found in younger age groups than older groups.[3,5,8,9] However, these studies were conducted either in pediatric patients or with a small sample of older patients.

Objective

The purpose of this study was to evaluate RIDT performance for the detection of influenza A(H1N1) 2009 in patients by age group, and to determine the association of patient age with RIDT performance.

Study design

Specimens

In response to the influenza A(H1N1) 2009 pandemic, the New Hampshire Public Health Laboratories (NHPHL) received 2078 specimens for influenza testing between September 1, 2009, and December 31, 2009. While acceptable specimen types included nasopharyngeal (NP) swab, nasal aspirate, combined NP swab with oropharyngeal

swab, and posterior-pharyngeal (throat) swabs, 98% of specimens submitted were collected from the nasopharynx. Following collection, RIDT was performed at the hospital laboratory. Specimens were then placed in viral transport medium and transported to NHPHL on cold packs. For the purpose of this study, RIDT positives were defined as those specimens that were positive for influenza A (negative for influenza B) or positive for both influenza A and B by RIDT testing.

Rapid influenza diagnostic tests

Twelve hospital-associated laboratories performed RIDT and submitted influenza specimens to the NHPHL as part of this study. Two of the laboratories used the BD Directigen™ (BD, Franklin Lakes, NJ, USA) EZ Flu A+B rapid kit, three used the Remel X/pect™ (Remel, Lenexa, KS, USA) Flu A & B rapid kit, and seven used the BinaxNOW® (Remel) Influenza A & B rapid test kit. All of these tests are chromatographic immunoassays that utilize membrane bound, anti-influenza monoclonal antibodies for the detection and differentiation of influenza A and B viral antigens.

Sample processing and RNA extraction

Upon arrival at NHPHL, specimens were processed in preparation for nucleic acid extraction. All nucleic acid extractions were performed following the protocols supplied with either the Roche MagNA Pure LC Total Nucleic Acid Isolation kit (Roche Diagnostics, Mannheim, Germany) or the QIAmp® Viral RNA Mini kit (Qiagen, Valencia, CA, USA).

Real-time PCR testing

Following nucleic acid extraction, specimens were tested for the presence of influenza A and B viruses using the Centers for Disease Control and Prevention (CDC) Real-time RT-PCR (rRT-PCR) Flu Panel. Specimens that exhibited fluorescence growth curves that crossed the threshold line within 37 PCR cycles were determined to be positive for the presence of influenza virus. If a specimen tested positive for influenza A, further testing was performed to characterize the subtype of influenza virus using both CDC rRT-PCR Flu Panel and rRT-PCR Swine Panel. These subtyping panels consist of primers and probes specific to hemagglutinin gene from seasonal A/H1 influenza, seasonal A/H3 influenza, A(H1N1) 2009 virus and a fourth set of primers and probes to the nucleoprotein gene of A(H1N1) 2009 virus.

Comparative and statistical analysis

Of the 2078 specimens, 1414 had undergone RIDT at a healthcare facility before being submitted to NHPHL. Forty-one of these specimens were excluded from this study because the internal control failed to amplify, thus signifying inadequate specimen collection or loss of specimen integrity. The remaining 1373 specimens with known RIDT results and valid rRT-PCR results were used to evaluate the performance of RIDT. Analysis and graphics were conducted using R statistical software, version 2.12.1.[10] Confidence intervals for sensitivities were computed using the method of Agresti and Coull.[11]

Results

Overall RIDT sensitivity and specificity

The mean age of patients with RIDT results was 44·9 years, ranging from 6 to 87 years with a median of 48 years. Of 1373 specimens with both RIDT and rRT-PCR results, 269 were positive for influenza A(H1N1) 2009 by RT-PCR, with 145 RIDT positives and 124 RIDT negatives, resulting in an overall clinical sensitivity of 53·9%. Of 1104 specimens testing negative for influenza A(H1N1) 2009 by RT-PCR, 17 had been reported as positive for influenza A by RIDT, producing an overall specificity of 98·5%.

RIDT sensitivity and specificity by patient age

Patients were divided into 10-year age groups, except for patients younger than 2 years of age. This cohort was grouped separately because previous reports [5,9] indicate that RIDTs performed on children younger than 2 years exhibit higher sensitivity than RIDTs performed on older patients. The resulting eight age groups were <2 years, followed by 2–9, 10–19, 20–29, 30–39, 40–49, 50–59, and 60 years or older.

Table 1 shows RIDT sensitivity and specificity by age group. Specificity was high (94·3–99·6%) in all groups. Sensitivity of RIDT varied substantially; the highest RIDT sensitivity (85·7%) was observed in patients younger than two years, while the lowest was observed in the 40-49 years age group (25·7%, Figure 1), followed by 60+ years (34·8%) and 50–59 years (40·6%). A Cochran-Armitage test for trend in sensitivity, using the midpoints of the age intervals as scores, was highly significant ($P < 0{\cdot}001$).

Based on the observed results, ages were then collapsed into younger than 2 years, 2–39 years, and over 39 years. Respective sensitivities were 85·7%, 60·3%, and 33·3%.

Logistic regression, using positive RIDT as outcome, indicated that the odds ratio (OR) for a positive RIDT in patients under 2 years of age was 11·25 (95% confidence interval [CI] = 2·88–43·95) compared to those aged 60 and older. Odds ratios for groups younger than 40 were significant or nearly so (Table 2).

Another logistic regression, with ungrouped ages as covariate, showed that age was significantly negatively associated with a positive RIDT result ($P < 0{\cdot}001$), with each year increase in age reducing the odds of a positive RIDT by 2·6%. Sensitivity of 50% occurred at 33·8 years. Results thus indicated a significant decrease in sensitivity with increasing age.

Table 1. Rapid influenza diagnostic test (RIDT) sensitivity and specificity by age groups

		PCR positive					PCR negative		
Age	*n*	RIDT+	RIDT−	Sensitivity	Lower 95% CI	Upper 95% CI	RIDT+	RIDT−	Specificity
<2	115	24	4	85·7	68·5	94·3	5	82	94·3
2–39	446	91	60	60·3	52·3	67·8	6	289	98·0
2–9	140	30	21	58·8	45·2	71·2	1	88	98·9
10–19	89	25	16	61·0	45·7	74·3	2	46	95·8
20–29	112	19	13	59·4	42·2	74·4	2	78	97·5
30–39	105	17	10	63·0	44·2	78·4	1	77	98·7
40+	812	30	60	33·3	24·4	43·6	6	716	99·2
40–49	149	9	26	25·7	14·2	42·1	3	111	97·4
50–59	156	13	19	40·6	25·5	57·7	1	123	99·2
60+	507	8	15	34·8	18·9	55·1	2	482	99·6
Total	1373	145	124	53·9	47·9	59·8	17	1087	98·5

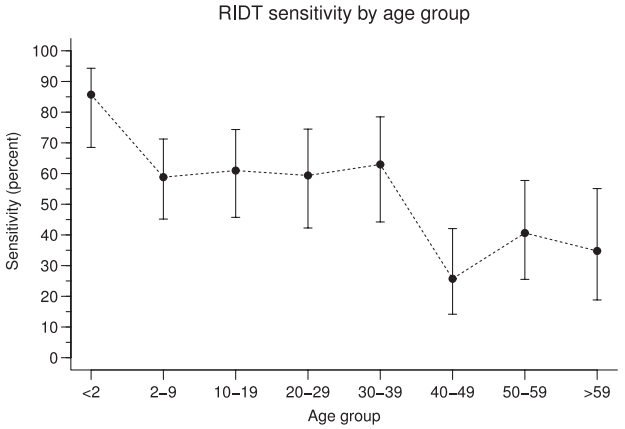

Figure 1. Rapid influenza diagnostic test sensitivity by age group.

Sensitivity and specificity by RIDT manufacturer

Table 3 presents the sensitivity and specificity of three RIDT kits including BinaxNOW® Influenza A & B (Binax), Remel Xpect™ Flu A & B (Xpect) and BD Directigen™ EZ Flu A+B (BD) compared to real-time RT-PCR for the detection in influenza A/H1N1 (2009) based on age group. Specificity was high (92·2–99·4%) in all age groups by all the three RIDT kits. Overall sensitivity was 56·6%, 48·8%, and 59·1% for Binax, Xpect, and BD, respectively. In patients younger than 2 years, sensitivity for Binax, Xpect, and BD was 85·7%, 100%, and 80%, respectively. The sensitivity for Binax, Xpect, and BD was 66·7%, 64·7%, and 58·1% respectively in 2–39 age group; and 33·3%, 36%, and 46·2% respectively in the age 40 and older group.

Discussion

This study analyzed respiratory specimens received by NHPHL during the influenza A(H1N1) 2009 pandemic. RIDT performance in different age groups was evaluated, and the association of age with RIDT sensitivity was determined.

Table 2. Odds ratios for positive rapid influenza diagnostic test (RIDT) by age group, compared to ages 60 and older

	RIDT+ (*n* = 145)		RIDT− (*n* = 124)				
Age group	*n*	Pct.	*n*	Pct.	Odds ratio	Lower 95% CI	Upper 95% CI
<2	24	85·7	4	14·3	11·25	2·88	43·95
2–9	30	58·8	21	41·2	2·68	0·96	7·45
10–19	25	61·0	16	39·0	2·93	1·01	8·48
20–29	19	59·4	13	40·6	2·74	0·90	8·32
30–39	17	63·0	10	37·0	3·19	1·00	10·17
40–49	9	25·7	26	74·3	0·65	0·21	2·04
50–59	13	40·6	19	59·4	1·28	0·42	3·90
60+	8	34·8	15	65·2	Referent	–	–

Table 3. Comparison of BinaxNOW® influenza A & B, Remel X/pect™ Flu A & B and BD Directigen™ EZ Flu A+B to real-time PCR for the detection of influenza A/H1N1 (2009)

RIDT	Patients		PCR positive			PCR negative		
	Age	*n*	RIDT+	RIDT−	Sensitivity (%)	RIDT+	RIDT−	Specificity (%)
Binax	<2	65	12	2	85·7	4	47	92·2
	2–39	271	42	21	66·7	2	164	98·8
	40+	662	15	30	33·3	3	504	99·4
	Total	846	69	53	56·6	9	715	98·8
Xpect	<2	1	1	0	100·0	0	0	–
	2–39	43	11	6	64·7	2	24	92·3
	40+	149	9	16	36·0	1	123	99·2
	Total	193	21	22	48·8	3	147	98·0
BD	<2	39	8	2	80·0	1	28	96·6
	2–39	119	25	18	58·1	2	74	97·4
	40+	71	6	7	46·2	1	57	98·3
	Total	229	39	27	59·1	4	159	97·6

RIDT, rapid influenza diagnostic test.

RIDT sensitivity for the detection of A(H1N1) 2009 decreased with age and was unevenly distributed among age groups. The highest RIDT sensitivity was observed in patients younger than 2 years; by comparison, sensitivity was significantly higher than in patients aged 2–39 years, and patients 40 years and older. Age was significantly negatively associated with a positive RIDT result. This is consistent with other studies[5,9] that found RIDT sensitivity to be significantly higher in infants and children younger than 2 years than in older children. Although higher sensitivity in younger age groups has been observed in several studies,[3,5,8,9] most of the study subjects were children aged 17 years or younger. Our study examined a broad age spectrum of patients and found that a significant decrease in RIDT sensitivity was associated with increasing age. These data demonstrate that RIDT sensitivity is significantly impacted by age of patients. Thus, clinicians should understand the limitation of RIDT[12] and carefully evaluate negative RIDT results, especially for tests performed on older patients.

This study also evaluated the sensitivity and specificity of three RIDT kits including BinaxNOW® Influenza A & B (Binax), Remel Xpect™ Flu A & B (Xpect) and BD Directigen™ EZ Flu A+B (BD) compared to real-time RT-PCR for the detection of influenza A(H1N1) 2009 based on age group. Specificity was high (92·2–99·4%) in all age groups by all RIDTs. RIDT sensitivities were similar within age groups for the three RIDT kits; although sensitivity of the Xpect for younger than two age group appeared to be slightly higher, it was likely due to the small sample size ($n = 1$) included in this group. However, decreased RIDT sensitivity with increasing age was observed in all the three RIDT kits and is thought to be due to decreased levels of viral shedding in older patients.[12,13] These data indicate that our finding that RIDT sensitivity decreased with increasing age is independent of RIDT kit.

Overall sensitivity for Xpect (48·8%) was lower than that of Binax (56·6%) and BD (59·1%) kits. However, this difference is likely because of the small number of specimens ($n = 1$) tested by Xpect from the <2 age group which normally displays the highest RIDT sensitivity. This discrepancy illustrates the importance of utilizing data from similar patient populations when comparing the performance of RIDT kits.

Factors that can impact RIDT performance include specimen type,[7,14] time of specimen collection after onset of illness,[7] duration of specimen storage, and transportation conditions. We were not able to compare RIDT performance among different specimen types because 98% of the specimens included in this study were NP swabs. Although information regarding the time of specimen collection after illness onset was not available, most specimens are outbreak-related and thought to be collected soon after illness onset. While no information regarding specimen storage or transport prior to RIDT testing was available, it is assumed that all specimens included in this study were tested promptly after specimen collection.

This study demonstrated that RIDT sensitivity is not uniform across age groups. Younger age groups displayed a higher RIDT sensitivity than older groups. Findings from this study may impact a clinician's interpretation of RIDT test results and ultimately have implications in clinical decision-making. Thus, it is important to enhance the RIDT-users' awareness of the limitation of RIDTs. Negative

results obtained by RIDT, especially from older patients with influenza-like symptoms, should be carefully evaluated before treatment decisions are made.

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Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the New Hampshire Department of Health and Human Services. The use of trade names and names of commercial sources is for identification only and does not imply endorsement by this agency.

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