

Performance of a Rapid Influenza Test in Children During the H1N1 2009 Influenza A Outbreak

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KEY WORDS

H1N1 influenza, rapid viral testing, children

ABBREVIATIONS

rRT-PCR—real-time reverse-transcriptase polymerase chain reaction

RIDT—rapid influenza diagnostic test

LR—likelihood ratio

CI—confidence interval

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WHAT'S KNOWN ON THIS SUBJECT: Sensitivities of RIDTs have varied widely for the diagnosis of seasonal influenza. Few pediatric-specific data are available on RIDT performance for H1N1 2009 influenza A.



WHAT THIS STUDY ADDS: To the best of our knowledge, this is the largest series reported to date of a comparison of RIDT performance to 2 reference standards, viral culture and rRT-PCR, for the detection of influenza A H1N1 2009 in respiratory specimens from pediatric patients.

abstract

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OBJECTIVE: To evaluate the performance of a rapid influenza diagnostic test (RIDT) in detecting H1N1 2009 influenza A virus in respiratory samples from pediatric patients in comparison to that of real-time reverse-transcriptase polymerase chain reaction (rRT-PCR) and viral culture.

METHODOLOGY. This was a cross-sectional diagnostic-accuracy study conducted at a tertiary care children's hospital. Patients for whom the RIDT (BinaxNOW [Binax, Inc, Portland, ME]), viral culture, and rRT-PCR results were known were included. Sensitivity, specificity, and likelihood ratios (LRs) were calculated.

RESULTS: A total of 3030 specimens had RIDT results paired with both rRT-PCR and viral culture results. With rRT-PCR as the reference, overall test sensitivity was 45% (95% confidence interval [CI]: 43.3%–46.3%) and specificity was 98.6% (95% CI: 98.1%–99%). Positive and negative LRs were 32.9 (95% CI: 22.9–45.4) and 0.56 (95% CI: 0.54–0.58), respectively. RIDT sensitivity was significantly higher in young infants and children younger than 2 years than in older children. Using viral culture as the reference standard, RIDT sensitivity was 55.5% (95% CI: 51.9%–95.6%) and specificity was 95.6% (95% CI: 95%–96.1%). The positive and negative LRs were 12.6 and 0.47, respectively.

CONCLUSIONS: The RIDT had relatively poor sensitivity but excellent specificity in this consecutive series of respiratory specimens obtained from pediatric patients. Although a positive RIDT result was highly accurate in predicting infection with influenza type A H1N1 2009 in children, a negative RIDT result did not preclude a child having H1N1. Therefore, for children at high risk with influenza-like illnesses during high-prevalence periods of influenza, empiric initiation of antiviral therapy should be considered for patients with a negative RIDT result. *Pediatrics* 2010;125:e645–e650

The recent 2009 H1N1 influenza A pandemic^{1–3} reinforced the importance of diagnosing circulating influenza strains in a timely and accurate manner, important because morbidity and mortality have the potential to be ameliorated by judicious use of antiviral medications.^{4,5} The traditional reference standard for the diagnosis of influenza A virus infection has been viral culture. However, the current reference standard for the diagnosis of influenza A, including H1N1 2009, is real-time reverse-transcriptase polymerase chain reaction (rRT-PCR).

Commercially available rapid influenza diagnostic tests (RIDTs) offer the advantages of low cost, ease of use, and rapid turnaround time.⁶ The RIDTs also have shown consistently high specificity for both seasonal (92%–100%) and H1N1 2009 (86%–100%) influenza A virus detection.^{6–15} However, the sensitivity of these assays has varied considerably for both seasonal influenza viruses (49%–95%)^{6–10} and H1N1 2009 influenza A (10%–53%).^{11–15} Furthermore, few data exist on the performance of RIDTs for detection of influenza A H1N1 2009 in children, with sensitivities ranging from 42% to 74%,^{16,17} and few data exist on the performance of these assays in clinical settings. We present here the results of a series of 3030 pediatric respiratory tract specimens in which RIDT, viral culture, and rRT-PCR were performed concurrently during the 2009 H1N1 outbreak. To the best of our knowledge, this is the largest series reported to date of a comparison of RIDT performance to 2 reference standards, viral culture and rRT-PCR, for the detection of influenza A H1N1 2009 in respiratory specimens from pediatric patients.

METHODS

Electronic records of respiratory specimen virologic tests were obtained

from Texas Children's Hospital secondary data sources between May 21, 2009, and September 20, 2009. During the study period all upper and lower respiratory tract specimens underwent testing by RIDT, viral culture, and rRT-PCR for influenza A with subtyping for H1N1 2009 and seasonal H1 and H3 influenza A. Samples for which all 3 assays were not performed were excluded from this study.

The performance of a commercially available RIDT, BinaxNOW influenza A and B test (Binax, Inc, Portland, ME), was evaluated by comparing it to the reference standards of rRT-PCR and viral culture. The Binax RIDT is an in vitro immunochromatographic assay that detects influenza nucleoprotein antigens. The RIDT was performed in a laboratory by trained virology technicians according to manufacturer instructions immediately after receipt of the specimen in the laboratory and within 2 hours of specimen collection from the patient. Quality-control measures included using internal-kit positive and negative controls as well as external laboratory controls for each test kit run within a 24-hour period.

After the RIDT was performed, all respiratory specimens were immediately split into 2 aliquots and sent for both rRT-PCR and viral culture. Specimens were inoculated onto 3 cell lines (human foreskin fibroblasts, human lung carcinoma [A549], and rhesus monkey kidney cell culture monolayers) to allow detection of influenza viruses types A and B, adenovirus, cytomegalovirus, herpes simplex virus, parainfluenza viruses, picornaviruses, and respiratory syncytial virus. Cell cultures were examined daily for 14 days under light microscopy. Viruses were identified by the presence of cytopathic effect or temperature-dependent hemadsorption phenomena by using guinea pig red blood cell suspension (performed on days 2, 5, and 14

of incubation). Virus identification was confirmed by using an immunofluorescence assay with commercially available virus-specific monoclonal antibodies.

A 1-step reverse-transcription, multiplex real-time PCR assay using 2 sets of TaqMan hydrolysis primers and probes was developed and validated in the Molecular Microbiology Laboratory at Texas Children's Hospital. The primer and probe set specific to the matrix gene (*M*) detects all subtypes of human influenza A. The primer and probe set specific to the hemagglutinin gene (*swHA*) of H1N1 2009 influenza A was used to subtype influenza A (2009 H1N1). For samples in which the matrix gene was detected and the *swHA* was not detected, further molecular subtyping was performed by using primers and probes specific to the hemagglutinin genes for seasonal H1 and H3 influenza. In brief, the primer and probe designs for H1N1 2009 influenza A were based on the following sequences: A/Texas/04/2009, A/Texas/05/2009, A/California/4/2009, A/California/5/2009, A/California/7/2009, and A/California/9/2009. The H1N1 2009 influenza PCR assay performance was validated by using Centers for Disease Control and Prevention reference standards.¹⁸ rRT-PCR was performed directly on RNA extracted from the original specimen, not on the viral clinical isolate.

Sensitivity, specificity, and likelihood ratios (LRs) (positive and negative) were estimated for the RIDT by using rRT-PCR and viral culture as the reference standards. SPSS 16 (SPSS, Inc, Chicago, IL) was used for statistical analysis. Institutional review board approval to conduct this study was obtained.

RESULTS

From May 21, 2009, to September 20, 2009, virologic results were recorded for 3102 respiratory specimens from 2686 patients. Seventy-two specimens

TABLE 1 Percentage of 2009 H1N1 Influenza A Virus–Positive Specimens by RIDT, Viral Culture, and RT-PCR According to Month, Specimen Source, and Patient Hospital Location

Characteristic	<i>n</i>	% RIDT-Positive	% Viral Culture–Positive	% rRT-PCR–Positive
All specimens	3030	11.3	13.4	22.7
Month ^a				
May	252	9.9	14.7	17.1
June	628	11.5	15.4	19.1
July	447	7.2	10.7	16.3
August	611	7.7	10.0	15.1
September	1092	15.2	15.0	33.1
Specimen source				
Nasal wash	1836	10.0	12.0	20.3
Nasal swab	1016	13.8	16.8	29.4
Tracheal aspirate	127	8.7	7.1	8.7
Sputum	17	17.6	17.6	17.6
Bronchoalveolar lavage	13	15.4	7.7	7.7
Nasopharynx	11	0.0	0.0	9.1
Hospital location				
Emergency department	1875	14.6	18.1	30.3
Critical care unit	406	4.2	2.7	5.2
Inpatient unit (noncritical care)	339	1.5	3.2	8.0
Outpatient clinic	225	16.0	16.9	25.8
Bone marrow transplant unit	175	5.7	4.0	8.0
Operating room	10	10.0	10.0	10.0

^a Study period was May 21 to September 20, 2009.

(2%) were excluded from the analysis: 48 had RIDT only, 1 had rRT-PCR only, 3 RIDT results were indeterminate, 3 rRT-PCR results were indeterminate, 2 were rRT-PCR–positive for seasonal influenza virus, and 15 influenza A iso-

lates were nonsubtypeable. There were 3030 specimens available for analysis: 342 (11.3%) tested positive for influenza A by RIDT, 407 (13.4%) tested positive for influenza A by viral culture, and 689 (22.7%) tested posi-

tive for 2009 H1N1 influenza A by rRT-PCR (Table 1). Using rRT-PCR and viral culture as the reference standards, the performance of the RIDT is shown in Tables 2 and 3. Using rRT-PCR as the reference standard, overall sensitivity and specificity were 45% and 98.6%, respectively, and positive and negative LR were 32.9 and 0.56, respectively. Given a pretest probability of 22.7%, the posttest probability of infection was 91% (95% confidence interval [CI]: 87%–93%) with a positive RIDT result and 14% (95% CI: 13%–15%) with a negative RIDT result. There were 10 specimens that tested positive by culture but negative by rRT-PCR, whereas there were 292 specimens that tested negative by culture but positive by rRT-PCR.

Viral cultures were positive for at least 1 virus in 650 instances (21.5%). The most common virus isolated was influenza A (407 [13.4%]), which accounted for 63% of all positive culture results during the study period. Other viruses isolated included parainfluenza viruses (101 [3.3%]), adenovirus (63 [2.1%]), rhinovirus (37 [1.2%]), entero-

TABLE 2 Performance of RIDT Compared With rRT-PCR for the Diagnosis of 2009 H1N1 Influenza A Virus Infection in Pediatric Patients for 3030 Specimens

Characteristic	<i>n</i>	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Overall	3030	45.0 (43.3–46.3)	98.6 (98.1–99.0)	32.9 (23.4–46.7)	0.56 (0.54–0.58)
Upper airway ^a	2880	44.6 (43.0–45.7)	98.9 (98.4–99.2)	39.3 (26.7–58.4)	0.56 (0.55–0.58)
Nasal wash	1836	43.5 (41.1–45.4)	98.6 (97.9–99.0)	30.4 (19.9–46.9)	0.57 (0.55–0.60)
Nasal swab	1016	45.5 (43.6–46.3)	99.4 (98.6–99.8)	81.5 (32.2–211.3)	0.55 (0.54–0.57)
Sputum	17	100.0 (55.8–100.0)	100.0 (90.5–100.0)	—	0.00 (0.00–0.49)
Lower airway	140	58.3 (35.0–77.2)	95.3 (93.1–97.1)	12.4 (5.1–26.5)	0.44 (0.24–0.70)
Tracheal aspirate	127	54.5 (30.9–74.1)	95.7 (93.4–97.5)	12.7 (4.7–30.1)	0.48 (0.27–0.74)
Bronchoalveolar lavage	13	100.0 (23.0–100.0)	91.7 (85.2–91.7)	12.0 (1.5–12.0)	0.00 (0.00–0.91)

^a Nasopharynx (*n* = 11) was not reported in this subcategory, because there were no positive results with the RIDT.

TABLE 3 Performance of RIDT Compared With Viral Culture for the Diagnosis of 2009 H1N1 Influenza A Virus Infection in Pediatric Patients for 3030 Specimens

Characteristic	<i>n</i>	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Overall	3030	55.5 (51.9–58.9)	95.6 (95.0–96.1)	12.6 (10.4–15.1)	0.47 (0.43–0.51)
Upper airway ^a	2880	55.2 (51.6–58.6)	95.7 (95.1–96.2)	12.7 (10.5–15.4)	0.47 (0.43–0.51)
Nasal wash	1836	54.3 (49.4–58.8)	96.1 (95.4–96.7)	13.9 (10.8–17.9)	0.48 (0.43–0.53)
Nasal swab	1016	55.6 (50.0–60.5)	94.7 (93.6–95.7)	10.4 (7.7–14.0)	0.47 (0.41–0.53)
Sputum	17	100.0 (55.8–100.0)	100.0 (90.5–100.0)	—	0.00 (0.00–0.49)
Lower airway	140	60.0 (33.8–81.1)	94.6 (92.6–96.2)	11.1 (4.6–21.5)	0.42 (0.20–0.72)
Tracheal aspirate	127	55.6 (28.9–78.3)	94.9 (92.9–96.7)	10.9 (4.1–23.4)	0.47 (0.22–0.77)
Bronchoalveolar lavage	13	100.0 (23.0–100.0)	91.7 (85.2–91.7)	12.0 (1.5–12.0)	0.00 (0.00–0.91)

^a Nasopharynx (*n* = 11) was not reported in this subcategory, because there were no positive results with the RIDT.

TABLE 4 Diagnostic Accuracy of RIDT Compared With rRT-PCR for 2009 Novel H1N1 Influenza A Infection in 2809 Unique Patient Visits

Characteristic	<i>n</i>	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Positive LR (95% CI)	Negative LR (95% CI)
Overall	2809	45.3 (43.7–46.5)	98.7 (98.2–99.1)	36.0 (24.8–52.7)	0.55 (0.54–0.57)
Patient age, mo					
<3	222	62.5 (47.6–62.5)	100.0 (98.8–100.0)	—	0.38 (0.38–0.53)
3–23	738	56.6 (49.6–61.0)	98.9 (98.1–99.5)	53.5 (26.6–112.0)	0.44 (0.39–0.51)
24–59	600	43.1 (37.5–47.0)	98.0 (96.7–98.8)	21.2 (11.4–40.1)	0.58 (0.54–0.65)
≥60	1249	43.4 (41.8–44.4)	98.7 (97.8–99.3)	34.1 (18.7–63.2)	0.57 (0.56–0.60)
Patient location ^a					
Emergency department	1832	45.8 (44.2–46.8)	98.9 (98.2–99.3)	41.7 (25.0–70.4)	0.55 (0.54–0.57)
Critical care	321	33.3 (17.7–49.3)	97.7 (96.8–98.6)	14.4 (5.5–36.1)	0.68 (0.51–0.85)
Inpatient (not ICU)	296	13.0 (5.3–16.6)	99.6 (99.0–99.9)	35.6 (5.3–246.7)	0.87 (0.84–0.96)
Outpatient	216	56.4 (48.7–59.9)	98.1 (95.5–99.3)	30.2 (10.9–90.8)	0.45 (0.40–0.54)
Bone marrow transplant unit	134	50.0 (29.5–61.6)	98.4 (96.3–99.5)	30.5 (8.1–122.7)	0.51 (0.39–0.73)

^a Ten specimens were obtained in the operating rooms; 1 tested positive with the RIDT, rRT-PCR, and viral culture.

virus (17 [0.6%]), cytomegalovirus (16, 0.5%), herpes simplex viruses (5 [0.2%]), and respiratory syncytial virus (4 [0.1%]). The presence of a virus other than influenza A H1N1 2009 in the respiratory sample did not influence performance of the RIDT.

There were 2809 unique patient visits during which specimens were tested (Table 4); median age of the patients was 4 years, and 43.9% were female. RIDT sensitivity compared with rRT-PCR was significantly higher in young infants and children younger than 2 years (sensitivity: 57.6% [95% CI: 51.6%–61.3%]) than in children aged 2 and older (sensitivity: 43.4% [95% CI: 41.7%–44.5%]). There were 67 unique patient visits for neonates (aged 0–28 days); 2 (2.9%) tested positive for influenza A with the RIDT, 2 (2.9%) tested positive for influenza A with viral culture, and 3 (4.3%) tested positive for 2009 H1N1 influenza A with rRT-PCR. In neonates, the RIDT sensitivity was 66.7% (95% CI: 25.9%–66.7%) and specificity was 100.0% (95% CI: 98.1%–100.0%). RIDT sensitivity was significantly lower for patients tested in the inpatient units (excluding the bone marrow transplant units) than any other hospital or outpatient unit.

DISCUSSION

The RIDT evaluated in this series of respiratory specimens obtained from pediatric patients showed suboptimal

sensitivity but excellent specificity for the detection of H1N1 2009 influenza A virus infection. When compared with the performance of RIDTs in previous years for the detection of seasonal influenza A,^{11–15} the sensitivity of the RIDT for H1N1 2009 influenza A virus was lower. These results, in the largest pediatric population yet studied, concur with the results of few existing studies of H1N1 diagnostics in children.^{16,17} Similar to reports of evaluations of the performance of RIDTs in previous influenza seasons, the highest sensitivity was seen for infants and children younger than 2 years of age, a group also known to be at higher risk of influenza complications.^{19,20} However, given the risk of concomitant serious bacterial infection, such as occult urinary tract infection^{21,22} or secondary bacterial pneumonia or bacteremia complicating influenza virus infection,²³ a positive influenza RIDT result should not preclude evaluation and treatment for other etiologies of fever in the young infant. Conversely, a negative RIDT result should not preclude initiation of empiric antiviral therapy in children at high risk.

RIDT sensitivity was higher for patients evaluated in the emergency department compared with those in most inpatient units (excluding bone marrow transplant unit), with performance being lowest in critical care unit patients. Viral

load in respiratory secretions varies on the basis of where a patient is in the natural history of influenza infection,²⁴ and emergency department patients may have presented early in the clinical course, at a time when they had higher viral loads. In contrast, inpatients may have been screened later into their clinical presentation.

There was no significant difference noted in RIDT performance for specimens obtained via nasal swabs versus nasal washes. Because obtaining a nasal swab requires much less time and technical expertise and produces less potential for aerosol formation, it can be considered a viable alternative to nasal washes for the collection of respiratory specimens for detection of influenza A H1N1 2009 by RIDT, rRT-PCR, or viral culture. Care must be taken to obtain an adequate sample. The RIDT seemed to be more sensitive in lower respiratory tract isolates, but the small sample size precluded statistical evaluation.

Finally, we evaluated RIDT performance by using both rRT-PCR and viral culture as reference standards. Historically, the US Food and Drug Administration has recommended viral culture as the reference standard for evaluation of new RIDT kits, whereas for H1N1 2009 influenza A, the Centers for Disease Control and Prevention recommended rRT-PCR as the confirmatory test performed when available.²⁵

When RIDT performance was compared with viral culture, the performance of the RIDT for detection of influenza A H1N1 2009 seemed comparable to detection of seasonal influenza A reported in past studies.^{6–10} rRT-PCR offers several advantages over RIDT, including the ability to simultaneously identify influenza A and subtype the virus, a relatively rapid turnaround time (results are typically available within 24–48 hours), and increased sensitivity of detection. Molecular detection of viral RNA does not depend on viable virions and will amplify genetic material of viruses that have fastidious growth requirements. In addition, multiplex PCR has the ability to detect viral coinfections. However, viral culture offers other advantages: the potential to detect other viral infections for which RIDTs do not exist and targets are not included in PCR assays and to help elucidate the role of dual viral infections in clinical presentation.

There were limitations to this retrospective study. It was not possible to determine why some children had

RIDTs requested and others did not. Furthermore, the guidance for clinical criteria for selection of patients for evaluation by RIDT evolved during the study period as national recommendations for screening and treatment changed.²⁶ Therefore, selection bias resulting from sample ascertainment is possible, and it is unclear what impact the selection bias would have made on the overall estimation of RIDT accuracy. However, it is possible that differences in testing patterns could have influenced the diagnostic accuracy between various patient locations. It also was not possible to determine how long children had had symptoms before obtaining specimens for RIDT, viral culture, and rRT-PCR. Potentially, children with <24 or 48 hours of symptoms might have increased viral antigen levels in respiratory tract secretions, increasing the sensitivity of the RIDT.

CONCLUSIONS

The RIDT displayed excellent specificity but relatively poor sensitivity for the

diagnosis of 2009 H1N1 influenza A virus infection in pediatric patients. Treatment of children at high risk who experience influenza-like illness during periods of high prevalence of influenza in the community should not rely solely on the results of RIDT. Empiric antiviral therapy and careful clinical evaluation should be considered for these patients, and confirmatory testing with rRT-PCR or viral culture should be performed, when available, especially if the patient is severely ill, requires hospitalization, or does not respond as anticipated.

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