Sensitivity Assessment of Rapid Influenza Diagnostic Tests for the Detection of the 2009 Pandemic Influenza A (H1N1) Virus in Clinical Specimens

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Abstract

Background: Influenza antigen detection test kits can provide results in 30 minutes and are used frequently for the detection of seasonal influenza infections. It is very important to determine the efficacy of these tests in the diagnosis of the 2009 pandemic influenza A (H1N1) virus due to the global spread of this new influenza virus.

Methods: We evaluated 4 rapid influenza diagnostic tests (Binax Now Influenza A&B, BD Directigen EZ Flu A+B, Fujirebio Espline Influenza A&B-N, and BioTracer Influenza A&B Test) for their ability to detect the 2009 pandemic influenza A (H1N1) virus and compared these results with the diagnostic sensitivity obtained following real-time polymerase chain reaction (PCR) analysis of the same samples.

Results: Reverse transcriptase polymerase chain reaction (RT-PCR) analysis of 252 specimens identified 176 influenza A (H1N1) positive samples. Of these, between 29 and 62 also tested positive using the 4 rapid tests examined, demonstrating the sensitivity of the respective tests was 16.5%-35.2% with specificities of 100%. However, the 4 rapid tests were 77.3%-100% sensitive when samples contained high influenza A (H1N1) virus titers, suggesting the sensitivity of these detection kits was dependent on viral loads. Finally, the sensitivity of the 4 detection kits tested was higher when samples from children were examined compared with samples from adults.

Conclusions: Data presented in this report indicated the sensitivity of the respective influenza antigen detection test kits examined was low compared to the sensitivity observed following RT-PCR. Nonetheless, they are useful tools during early outbreak investigations of influenza-like illnesses, particularly in the screening of critically ill patients and pediatric patients. However, negative results could be confirmed using other tests (eg, RT-PCR if necessary).

Keywords: pandemic influenza A (H1N1), rapid influenza diagnostic tests, sensitivity, specificity, viral titer

Materials and Methods

Clinical Specimens

Nasopharyngeal aspirate specimens (n=252) were collected from patients with influenza-like illness at the Affiliated Hospital of Yangtze University, Jingzhou HuBei, China, between September 2009 and January 2010. The patients’
ages ranged from 3 months to 70 years of age (52% male and 48% female). Specimens were placed in viral transport media, maintained at 4°C, and transported to the laboratory within 24 hours of collection. Seasonal influenza A cases were not considered in the study.

### Rapid Influenza Diagnostic Tests

Four influenza rapid tests: Binax Now Influenza A&B (Binax, Cranefield, AZ), BD Directigen EZ Flu A+B (BD, San Jose, CA), Fujirebio Esoline Influenza A&B-N (Fujirebio, Tokyo, Japan), and the BioTracer Influenza A&B Test (Bio Focus, Gyeonggi-do, Korea) were evaluated for influenza antigen detection sensitivity. All tests were performed according to the manufacturer’s instructions without knowledge of the RT-PCR results.

### Real-Time Quantitative RT-PCR

Total viral RNA was extracted using the QIAamp Viral RNA kit (Qiagen, Valencia, CA) following the manufacturer’s instructions. Viral RNA was extracted from 200 μL of each sample and RNA was eluted from respective columns with 50 μL RNase-free water and used immediately. Reverse transcriptase polymerase chain reaction was carried out using a commercial kit (Da An Gene, Guangdong, China) containing pre-mixed primers and dual-labeled hydrolysis Taqman probes for detection of pandemic influenza A (H1N1) virus. The IVA H1 primer and probe set were designed to specifically detect the hemagglutinin (HA) gene, and the IVA N1 primer and probe set were designed to specifically detect the neuraminidase (NA) gene, respectively. Reaction conditions were as follows: reverse transcription was carried out at 50°C for 15 minutes. Polymerase chain reaction conditions were as follows: a Taq inhibitor activation at 94°C for 3 minutes, followed by 40 cycles at 95°C for 15 seconds and 55°C for 45 seconds. Fluorescent data were collected during the 55°C annealing/extension step. Cycle threshold (Ct) values served as indicators of the amount of virus present in specimens, and Ct values of ≤35 were considered RT-PCR positive.

### Statistical Analysis

Specimens positive for influenza A (H1N1) following RT-PCR analysis were regarded as true positives. The sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) of the 4 influenza rapid test results were compared to the RT-PCR assay data and calculated using standard formulas. SPSS 16 software (SPSS, Chicago, IL) was used for statistical analysis of the data.

### Results

Nasopharyngeal aspirate samples (n=252) were screened for influenza (H1N1) using RT-PCR and 4 different influenza rapid tests (Binax Now Influenza A&B, BD Directigen EZ Flu A+B, Fujirebio Esoline Influenza A&B-N, BioTracer Influenza A&B Test). One hundred and seventy-six samples were positive for influenza A (H1N1) by RT-PCR; however, the rapid tests only identified between 29 and 62 of the 176 positive samples, indicating a sensitivity range between 16.5% and 35.2%, respectively (Table 1). BD Directigen EZ Flu A+B was the most sensitive test examined, and the BioTracer Influenza A&B Test was the least sensitive rapid detection test examined.

Among pediatric samples (n=119, ≤15 years of age) 91 specimens were positive for influenza A (H1N1) by RT-PCR. The rapid tests demonstrated higher sensitivity using specimens from pediatric patients vs adult patients (Figure 1). For example, the sensitivity of the BD Directigen EZ Flu A+B in pediatric patients was 44% compared to 26% when adult samples were screened.

The viral titers of the respective samples screened also significantly affected test sensitivities. That is, of the 22 specimens with highest viral titers (Ct values ≤20), 2 of the rapid tests examined were 100% sensitive, and the other 2 kits identified 17 and 20 positive samples, respectively, demonstrating that the influenza rapid tests had 77.3%-100% sensitivity in detecting influenza A (H1N1) when virus titers were high. However, when the 4 rapid tests were used to screen the 98 specimens with Ct values between 21 and 30, their sensitivity declined significantly (Table 2) and there were few positive results in the 56 specimens with low viral titers (Ct values between 31 and 35).

### Discussion

Different laboratory diagnostic tests can be used for detecting the presence of influenza virus in respiratory specimens, including rapid antigen detection tests, virus isolation from cell culture-based assays, diagnostic microarrays, or RT-PCR. Rapid antigen detection tests require less technical skill (compared to RT-PCR) and can provide results in approximately 30 minutes, making them a frequently used diagnostic approach during disease surveillance or as point-of-care tests. Most rapid antigen detection tests have been designed for detecting seasonal influenza viruses, and the sensitivity of the rapid antigen detection tests for use in the detection of the 2009 pandemic influenza A (H1N1) virus in clinical specimens must be defined.

This study compared 4 rapid influenza diagnostic tests for their respective sensitivities in the detection of the influenza A (H1N1) virus in 252 clinical specimens. The data presented in this report demonstrated that all 4 rapid assays had low sensitivities with respect to identifying H1N1 antigens. The BD Directigen EZ Flu A+B test was the most sensitive of the 4 rapid tests examined (35%) and the BioTracer Influenza A&B Test had the lowest sensitivity (16%). The Binax Now

### Table 1. Diagnostic Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value of the Four Rapid Tests Examined Compared to RT-PCR

| Rapid Test                  | Sensitivity (%) | Specificity (%) | PPV (%)  | NPV (%)  |
|-----------------------------|-----------------|-----------------|----------|----------|
| Binax Now Influenza A&B     | 38/176 (22)     | 76/76 (100)     | 38/38 (100) | 76/214 (36) |
| BD Directigen EZ Flu A+B    | 62/176 (35)     | 76/76 (100)     | 62/62 (100) | 76/190 (40) |
| Fujirebio Esoline Influenza A&B-N | 41/176 (26) | 76/76 (100)     | 41/41 (100) | 76/211 (36) |
| BioTracer Influenza A&B Test | 29/176 (16)     | 76/76 (100)     | 29/29 (100) | 76/223 (34) |

PPV, positive predictive value; NPV, negative predictive value.
Influenza A&B and Espline Influenza A&B-N had sensitivities of 26% and 22%, respectively. These results were comparable to other recent studies reporting sensitivities of 17%-51% for some rapid tests used for the detection of pandemic influenza A (H1N1) in clinical specimens.\textsuperscript{8,9} However, all of the 4 rapid tests examined in this study demonstrated high specificity. The lack of false-positive results may be related to the fact that this study was carried out in the absence of potential background interference from seasonal influenza A and influenza B infections by eliminating a few seasonal influenza cases that could have resulted in the detection of positive responses not due to H1N1 related infections.

Factors influencing the performance of the rapid influenza diagnostic tests include specimen quality, temporal factors relating to the time of specimen collection post-infection, disease severity, nature in which the samples were stored and transported, and the most significant factor, viral titers. Previous studies demonstrated that using nasopharyngeal and/or nasal samples to screen for the presence of viral particles would provide the best chance of detecting viruses because of the higher viral loads present in samples collected from these sites.\textsuperscript{10} In this study, the rapid tests had sensitivities ranging from 77.3% to 100% when specimens containing high viral titers were screened; however, sensitivity plummeted when samples contained medium to low viral concentrations.

The rapid assays demonstrated a higher sensitivity using specimens from pediatric patients (compared to adults), perhaps because the viral loads in this population were higher. Younger children tend to shed greater quantities of influenza virus for prolonged periods compared to adults\textsuperscript{11,12} as a consequence of immunologic immaturity or lack of cross-reactive protective antibodies capable of binding the influenza A (H1N1) virus.\textsuperscript{13,14}

In summary, this study indicated the rapid influenza diagnostic tests evaluated had high specificities and a PPV, but low sensitivities and NPV when used for the detection of pandemic influenza A (H1N1) compared to RT-PCR. Although rapid tests demonstrated low sensitivities, they still serve a purpose when investigating potential outbreaks of influenza-like illnesses, especially in the early diagnosis of critically ill patients or pediatric patients where positive results are indicative of an influenza virus infection. However, if negative results are obtained following the use of a rapid influenza diagnostic test, results should be confirmed using other laboratory tests such as RT-PCR if necessary. \textsuperscript{1m}

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1. World Health Organization. Influenza A (H1N1) update. Available at: http://www.who.int/csr/don/2010_03_05/en/index.html. Accessed 5 March 2010.
2. Centers for Disease Control and Prevention. Swine influenza A (H1N1) infection in two children: Southern California, March–April 2009. MMWR Morb. Mortal. Wkly. Rep. 2009;58:400-402.
3. Garten RJ, Davis CT, Russell CA, et al. Antigenic and Genetic Characteristics of Swine-Origin 2009 A (H1N1) Influenza Viruses Circulating in Humans. Science. 2009;325:197-201.
4. Cazacu AC, Greer J, Taherivand M, Demmler GJ. Comparison of lateral-flow immunoassay and enzyme immunoassay with viral culture for rapid detection of influenza virus in nasal wash specimens from children. *J. Clin. Microbiol.* 2003;41:2132-2134.

4. Liolios L, Jenney A, Spelman D, Kotsimbos T, Caton M, Wesselingh S. Comparison of a multiplex reverse transcription-PCR-envelope hybridization assay with conventional viral culture and immunofluorescence techniques for the detection of seven viral respiratory pathogens. *J. Clin. Microbiol.* 2001;39:2779-2783.

5. Poon LL, Chan KH, Smith GJ, Leung CS, Guan Y, Yuen KY, Peiris JS. Molecular detection of a novel human influenza (H1N1) of pandemic potential by conventional and real-time quantitative RT-PCR assays. *Clin. Chem.* 2009;55:1555-1558.

6. Rahman M, Vandermause MF, Kieke BA, Belongia EA. Performance of Binax NOW Flu A and B and direct fluorescent assay in comparison with a composite of viral culture or reverse transcription polymerase chain reaction for detection of influenza infection during the 2006 to 2007 season. *Diagn. Microbiol. Infect. Dis.* 2008;62:162-166.

7. Hurt AC, Alexander R, Hibbert J, et al. Performance of six influenza rapid tests in detecting human influenza in clinical specimens. *J. Clin. Virol.* 2007;39:132-135.

8. Faix DJ, Sherman SS, Waterman SH. Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus in humans. *N. Engl. J. Med.* 2009;361:728-729.

9. Ginocchio CC, Zhang F, Manji R, et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. *J. Clin. Virol.* 2009;45:191-195.

10. Smit M, Beynon KA, Murdoch DR, Jennings LC. Comparison of the NOW Influenza A & B, NOW Flu A, NOW Flu B, and Directigen Flu A&B assays, and immunofluorescence with viral culture for the detection of influenza A and B viruses. *Diagn. Microbiol. Infect. Dis.* 2007;57:67-70.

11. Cazacu AC, Chung SE, Greer J, et al. Comparison of the Directigen Flu A_B membrane enzyme immunoassay with viral culture for rapid detection of influenza A and B viruses in respiratory specimens. *J. Clin. Microbiol.* 2004;42:3707-3710.

12. Frank AL, Taber LH, Wells CR, et al. Patterns of shedding of myxoviruses and paramyxoviruses in children. *J. Infect. Dis.* 1981;144:433-441.

13. Hancock K, Veggilia V, Lu X, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. *N. Engl. J. Med.* 2009;361:1945-1952.

14. Greenbaum JA, Kotturi MF, Kim Y, et al. Pre-existing immunity against swine-origin H1N1 influenza viruses in the general human population. *Nat. Acad. Sci. USA.* 2009;106:20365-20370. 2.A new figure 1.