Evaluation of New Rapid Antigen Test for the Detection of Pandemic Influenza A/H1N1 2009 Virus

Young Jin Choi¹, Hwi Jun Kim¹, Joon Soo Park², Myung Ho Oh², Hae Seon Nam³, Yong Bae Kim⁴, Byung Ki Cho⁵, Mi Jung Ji⁵, and Jin Sik Oh⁶

Department of ¹Laboratory Medicine, ²Pediatrics, ³Parasitology, and ⁴Preventive Medicine, College of Medicine, Soonchunhyang University, Cheonan, Korea

⁵Standard Diagnostics, Inc, Yongin-si, Korea

⁶BioNote, Inc, Hwasung-si, Korea

Running title: New Rapid Antigen Test for Pandemic A/H1N1 2009

Corresponding author:

Young Jin Choi, M. D.

Department of Laboratory Medicine, Soonchunhyang University Cheonan Hospital

23-20, Bongmyung-dong, Cheonan-si, Choongchunam-do, Republic of Korea

82-41-570-3562 Office

82-41-572-2316 Fax

E-mail: clinpath@sch.ac.kr

Word count: 997

Key Words: influenza, pandemic, rapid antigen test, H1N1, SD Bioline
Abstract

We evaluated the SD Bioline Influenza Ag A/B/A(H1N1) Pandemic test kit and compared it with real-time RT-PCR for its ability to detect H1N1 2009. The sensitivity and specificity of the test kit for H1N1 2009 were 77% and 100%, respectively.
In March and early April of 2009, the pandemic influenza A/H1N1 2009 virus (H1N1 2009) was detected in Mexico and the United States, followed by a rapid worldwide person-to-person spread (1,6). The detection of influenza-specific RNA via reverse transcriptase PCR (RT-PCR) is the current method of choice for the detection of influenza (3,5). Due to the requirement for specialized equipment and the long turnaround times, RT-PCR for the detection of H1N1 2009 is currently available in limited clinical settings. Rapid antigen tests (RAT) have been conducted in a variety of clinical settings for the detection of H1N1 2009, due to the rapid results and ready availability of those tests. Recently, a new RAT (SD Bioline Influenza Ag A/B/A(H1N1) Pandemic, Standard Diagnostics, Inc, Yongin-si, Korea) was developed for the specific detection of H1N1 2009. We evaluated the new RAT in comparison with the real-time RT-PCR assay in terms of its ability to detect H1N1 2009.

In an effort to compare the new RAT with real-time RT-PCR, the nasopharyngeal swab specimens were collected using flocked swabs (Copan Diagnostics, Murrieta, CA) from suspected H1N1 2009 patients in November 2009. The age of the patients ranged between 2 weeks and 83 years (average 13.5 years). The clinical specimens on 561 paired swabs (2 per patient) were each placed into pairs of test tubes—one containing 300 µl of buffer solution for the antigen test, and one containing 1 ml of viral transport medium (VTM) for real-time RT-PCR. 198 unpaired swabs (1 per patient) were inserted into test tubes containing 1 ml of VTM for RAT and real-time RT-PCR. In an effort to evaluate the cross-reactivity of RAT, 16 different influenza virus suspensions (influenza A-15 subtypes, influenza B-1 subtype) and 117 virus positive clinical specimens of nasopharyngeal aspirates (seasonal influenza A, adenovirus, coronavirus, human rhinovirus A, human metapneumovirus, and parainfluenza virus), were analyzed. 20 seasonal influenza A positive samples (9 H1N1, 10 H3N2, 1 untypeable strain), which were obtained during the 2008-2009 influenza season, were included in the sample of clinical specimens.
The new RAT, which has 4 lines for the detection of H1N1 2009, influenza A, influenza B, and controls, was conducted using approximately 90 µl of samples. The viral RNA was extracted using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) from 140 µl of samples, in accordance with the manufacturer’s instructions. The RNA was eluted from columns with 50 µl of nuclease-free water. Each reaction mixture for real-time RT-PCR was prepared in accordance with the manufacturer’s instructions, and included 5 µl of RNA extraction and PCR reagents (the Influenza A/H1N1 Detection Set; Roche Applied Science, Mannheim, Germany). Amplification and detection were conducted on a LightCycler 480 (Roche Applied Science, Mannheim, Germany).

Among the 561 paired specimens, 241 tested positive via real-time RT-PCR. Among the 241 PCR-confirmed cases, 186 (77%) were positive for H1N1 2009 and 152 (63%) were positive for influenza A by the new RAT. Among them, 37 cases were positive for H1N1 2009 but negative for influenza A on RAT, and 3 cases were negative for H1N1 2009 but positive for influenza A. Among 320 PCR-negative cases, only one was positive for influenza A by RAT. Among the 198 unpaired specimens, 72 tested positive on PCR. Among the PCR-confirmed samples, 55 (76%) tested positive for H1N1 2009 by RAT and 42 (58%) tested positive for influenza A by RAT. 13 samples were positive for H1N1 2009 by RAT and PCR but negative for influenza A by RAT. Among the 126 PCR-negative samples, only one tested positive for H1N1 2009 by RAT. The overall sensitivity of the H1N1 2009 component and influenza A component in the new RAT were 77% and 62%, respectively (Table 1). The crossing-point (Cp) values of the 72 samples that tested positive on real-time RT-PCR but negative on H1N1 2009 RAT ranged between 20.4 and 37.7 (median value 30.3), whereas the Cp values of samples testing positive on H1N1 2009 RAT and PCR ranged between 15.3 and 34.7 (median value 22.5). The sensitivity of the H1N1 2009 RAT for the 0- to 5-year-old group was 79% (55/70), 77% (156/204) in the 5- to 20-year-old group and 77% (30/39) in the
over 20-year-old group.

In the cross-reactivity test using the viral suspensions, A/Korea/01/2009(H1N1) was strongly positive for H1N1 2009 by RAT and A/Swine/Korea/GC0503/2005 (H1N1) was weakly positive. The others were negative for H1N1 2009 (Fig. 1). Among the 117 clinical specimens, only one specimen (seasonal influenza A H1N1) was weakly positive for H1N1 2009 by RAT and the others were negative.

The RAT for the detection of H1N1 2009 revealed a broad range of sensitivity and specificity (9.6~75% and 80~100%, respectively) (4,5,7,8). Because of its low sensitivity, the clinical utility of RAT remains a subject of debate (2,8,9). Our results show that the new RAT features relatively high sensitivity for the detection of H1N1 2009. We presume that the difference of antibody affinity and targeting sites (hemagglutinin and nucleoprotein) of detection components may affect different sensitivity. Additionally, in the majority of cases, the new RAT can distinguish between seasonal influenza and H1N1 2009, although our results are preliminary due to the very limited number of samples and further specificity testing of the RAT with more seasonal influenza viruses may be required. Because more than 99% of the current seasonal H1 strains are resistant to oseltamivir, diagnostic tests to distinguish the pandemic strain from seasonal influenza may be important for clinical management (9). Also, RAT has the advantage of providing rapid results. In our laboratory, the average turnaround times of RAT and real-time RT-PCR were 0.9 and 14.9 hours, respectively.

We suggest that SD Bioline Influenza Ag A/B/A(H1N1) Pandemic may be a useful diagnostic tool for the detection of H1N1 2009 in appropriate clinical settings, although a negative RAT may require more sensitive confirmatory assays. Additionally, further studies may be necessary to validate the assay using other samples types and to determine the sensitivity of the assay for pandemic H1N1 2009, using titered viral stocks.
References


FIG. 1. The cross-reactivity test of the new RAT to 16 different influenza viruses.
TABLE 1. Performance of the new RAT\textsuperscript{a} compared to real-time RT-PCR for the detection of Pandemic Influenza A/H1N1 2009 virus

<table>
<thead>
<tr>
<th>Detection component of the new RAT</th>
<th>No. of cases</th>
<th>Total</th>
<th>PCR+</th>
<th>PCR-</th>
<th>PCR+</th>
<th>PCR-</th>
<th>% Sensitivity (95% CI)</th>
<th>% Specificity (95% CI)</th>
<th>PPV\textsuperscript{b} (%)</th>
<th>NPV\textsuperscript{c} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection component for 2009 H1N1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paired samples\textsuperscript{d}</td>
<td>561</td>
<td>186</td>
<td>55</td>
<td>0</td>
<td>320</td>
<td>77</td>
<td>(72-82)</td>
<td>100 (99-100)</td>
<td>100</td>
<td>85</td>
</tr>
<tr>
<td>Unpaired sample\textsuperscript{e}</td>
<td>198</td>
<td>55</td>
<td>17</td>
<td>1</td>
<td>125</td>
<td>76</td>
<td>(65-84)</td>
<td>99 (96-100)</td>
<td>98</td>
<td>88</td>
</tr>
<tr>
<td>Overall</td>
<td>759</td>
<td>241</td>
<td>72</td>
<td>1</td>
<td>445</td>
<td>77</td>
<td>(72-81)</td>
<td>100 (99-100)</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td>Detection component for Influenza A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paired samples</td>
<td>561</td>
<td>152</td>
<td>89</td>
<td>1</td>
<td>319</td>
<td>63</td>
<td>(57-69)</td>
<td>100 (98-100)</td>
<td>99</td>
<td>78</td>
</tr>
<tr>
<td>Unpaired sample</td>
<td>198</td>
<td>42</td>
<td>30</td>
<td>0</td>
<td>126</td>
<td>58</td>
<td>(47-69)</td>
<td>100 (97-100)</td>
<td>100</td>
<td>81</td>
</tr>
<tr>
<td>Overall</td>
<td>759</td>
<td>194</td>
<td>119</td>
<td>1</td>
<td>445</td>
<td>62</td>
<td>(57-67)</td>
<td>100 (99-100)</td>
<td>100</td>
<td>79</td>
</tr>
</tbody>
</table>

\textsuperscript{a} As determined by SD Bioline Influenza Ag A/B/A(H1N1) Pandemic

\textsuperscript{b} PPV = Positive Predictive Value

\textsuperscript{c} NPV = Negative Predictive Value

\textsuperscript{d} Two nasopharyngeal swabs (placing in 2 test tubes—one containing buffer solution and one containing viral transport medium) from the same patient at the same time were prepared for RAT and real-time RT-PCR

\textsuperscript{e} One nasopharyngeal swab (placing in a tube containing viral transport medium) was used for RAT and real-time RT-PCR