Development and Evaluation of a Rapid Influenza Diagnostic Test for the Pandemic (H1N1) 2009 Influenza Virus

Donghyok Kwon, Kyeongcheol Shin, Mihwa Kwon, Hee-Bok Oh, Chun Kang, and Joo-Yeon Lee*

Division of Influenza Virus, Center for Infectious Diseases, National Institute of Health, Korea Centers for Disease Control and Prevention, Seoul, Republic of Korea

Received 12 August 2010/Returned for modification 7 October 2010/Accepted 15 October 2010

We evaluated a new rapid influenza diagnostic test for the pandemic (H1N1) 2009 influenza virus by using real-time reverse transcription-PCR (rRT-PCR) and viral culture. The sensitivities were 68.5% and 64.5%, and the specificities were 98.4% and 97.6%, respectively. This kit should be used with caution, and negative results should be verified by a confirmative test.

In April 2009, a mixed-origin H1N1 influenza virus was recognized as a causative agent of influenza-like illnesses (ILI) in humans. Since its emergence, the virus has spread rapidly throughout the world, causing a pandemic.

Most commercial rapid influenza diagnostic tests (RIDTs) can detect only the influenza virus or can distinguish influenza A and B but cannot distinguish the pandemic (H1N1) 2009 influenza virus from seasonal influenza. Recent studies have indicated that the poor performance of the RIDT approach and nonspecific detection of the pandemic (H1N1) 2009 virus were the main obstacles to their widespread use in private clinics (2, 7).

With the need for a new rapid kit with reasonable sensitivity and specificity for the pandemic (H1N1) 2009 virus, we developed a new lateral-flow RIDT kit [SD Bioline Influenza Ag A/B/A(H1N1) Pandemic] in collaboration with the company Standard Diagnostics, Inc. (Yongin-si, Gyonggi, Republic of Korea). A monoclonal antibody (MAb) against the hemagglutinin (HA) protein of the pandemic (H1N1) 2009 virus was developed using the Korean isolate, A/Korea/01/2009, and applied to a new RIDT kit (1), together with a MAb that had been developed previously against the nucleoprotein (NP) of seasonal influenza A and B viruses (8). This made it possible to detect and distinguish the pandemic and seasonal influenza viruses. We evaluated the new kit for the application of potential clinical use in pandemic and seasonal epidemics.

From December 10 to December 30, 2009, 432 clinical specimens from patients with ILI were tested using the new RIDT, and the results were compared with those of real-time reverse transcription-PCR (rRT-PCR) and virus isolation in MDCK cell cultures to determine the sensitivity and specificity for the diagnosis of pandemic (H1N1) 2009 influenza. We also determined the detection limit of the kit using serial dilutions of the Korean pandemic virus isolate.

For evaluation, we supplied the new kits to 11 sentinel clinics of six provinces in the Republic of Korea that participate in influenza laboratory surveillance (4). The ages of the 432 patients providing the samples ranged from 0 to 83 years (median, 20 years), and 232 (53.7%) were female. The median time from onset to visiting clinics was 1 day (range, a few hours to 12 days). Sentinel doctors applied the RIDT to nasal swab specimens obtained using flocked swabs (Copan Diagnostics, Murrieta, CA). Clinicians collected another specimen by using a sterile swab applicator and sent the specimen in universal viral transport medium (Becton, Dickinson and Company, Sparks Glencoe, MD) to each provincial Research Institute of Health and Environment (RIHE) with recorded information (demographic, symptom, and RIDT results). At the RIHE, specimens were split into two aliquots, and one was tested by rRT-PCR according to the standard protocols developed by the U.S. Centers for Disease Control and Prevention (CDC). The other aliquot of each specimen was transferred to the Division of Influenza Virus, Korea CDC, for viral culture. Specimens were inoculated onto MDCK cells and monitored daily for the presence of a cytopathic effect. Virus identification was confirmed by rRT-PCR.

Among 432 specimens, 178 tested positive by rRT-PCR and 186 tested positive by viral culture. Among the 178 rRT-PCR-confirmed cases, 122 were positive, and among the 186 viral culture confirmed cases, 120 were positive with the new RIDT. Using rRT-PCR as the reference standard, the overall sensitivity was 68.5% and the specificity was 98.4%. With viral culture as the reference, the RIDT sensitivity and specificity values were 64.5% and 97.6%, respectively. The negative predictive values for rRT-PCR and viral culture were 81.7% and 78.4%, and the positive predictive values were 96.8% and 95.2%, respectively. To evaluate the agreement between the RIDT and two confirmative methods, percent agreement and kappa statistic were calculated (6) and showed moderate agreements (percent agreement, 86.1% for rRT-PCR and 83.3% for viral culture; kappa, 0.70 for rRT-PCR and 0.65 for viral culture).

Among 340 patients who had a record of their symptom onset and sample collection date, 86 (25.3%) visited the clinic on the day of symptom onset, and 158 (46.5%) visited 1 day later. When the RIDT performance was evaluated by day of onset, the sensitivity was lower at 3 or more days after the onset of symptoms; however, the sensitivity was highest at day
The detection limit of the new kit against the HA protein of the A/Korea/01/2009 virus was confirmed to be $10^4$ PFU/ml. In contrast, the detection limit against the NP protein was $10^3$ PFU. However, when the kit was applied to clinical specimens, no difference between the two targets was found. This might reflect a difference in MAb affinity to the targeting sites of each protein.

In one recent study, the sensitivity and specificity of the new RIDT were 77% and 100%, respectively, and the component for pandemic (H1N1) 2009 virus, the HA protein, was detected more sensitively than the component for influenza A virus, the NP protein (1). The sensitivity and specificity of the new RIDT were lower than those of that study. We found that the test performance varied depending on the clinics in which the tests were performed, and this might be attributable to the persons who collected the specimens. Although the clinicians were trained well for collecting specimens, there might be some differences in performance. In this study, we used separate samples for the RIDT and for rRT-PCR and viral culture. The use of the two different sample types might have affected the sensitivity and specificity of the rapid test results against two confirmative methods (3, 5). Although applied to a limited number of samples, 10 influenza vaccine strains of the 2005-2006 to 2009-2010 seasons, the new RIDT could distinguish between seasonal and pandemic (H1N1) 2009 influenza virus specifically.

Thus, we found that this new RIDT had reasonable sensitivity and high specificity compared with those of rRT-PCR and viral culture for detecting the pandemic (H1N1) 2009 virus. Although the negative RIDT results should be confirmed with more sensitive methods, this kit may be useful in sentinel clinics if used with caution.

We thank the staff at Seoul, Incheon, Daejeon, Gwangju, Gangwon, and Jeonbuk provincial RIHEs for their laboratory testing. Additionally, we acknowledge the contributions of participating sentinel doctors for evaluating the new RIDT kit.

This study was supported by a grant from the Korea CDC.

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