Unique Finding of a 2009 H1N1 Influenza Virus Positive Clinical Sample Suggests Matrix Gene Sequence Variation

Key words: 2009 H1N1 influenza, matrix gene, variant, RT-PCR
The 2009 H1N1 influenza virus has rapidly spread all over the world. At this time, regions in the northern hemisphere are at the beginning of the typical annual respiratory season although the 2009 H1N1 virus has been highly prevalent for the last several months. This year, it is likely that both 2009 H1N1 and seasonal influenza viruses will coexist for a period of time. Rapid and accurate laboratory diagnosis of influenza virus and its subtype is very important for selection of appropriate antiviral therapy and initiation of infection control measures for hospitalized patients. However, with limited useful assays available, this is a challenging task. While quick and easy to perform, rapid influenza antigen detection assays are known to suffer from low sensitivity for the 2009 H1N1 (1, 2). Compared to virus culture, RT-PCR is not only sensitive, but also much more rapid and therefore, is widely used (3). Here we report our finding of a highly unusual case of 2009 H1N1 influenza.

The patient is a 4 year old girl who presented with persistent wheezing for a week. On Oct. 25, she was admitted through ER because of 1 day of fever, cough, rhinorrhea, and labored breathing. The patient was treated with Oseltamivir and supportive therapy. Her condition substantially improved and she was discharged 1 day after admission. While the history was unremarkable, the laboratory testing results of viral assays were very interesting. The nasopharyngeal (NP) aspirate/wash as well as NP swab collected at admission tested positive for influenza A virus by the Binax rapid antigen detection assay (Inverness Medical, Princeton, NJ). However, RT-PCR on the same specimens with the ProFlu+ assay (Gen-Probe Prodesse, Inc., Waukesha, WI) was negative for influenza A
and B and RSV viruses. To investigate this further, influenza A virus subtyping by PCR as well as virus culture were conducted. Surprisingly, the same nucleic acid extract that had been negative for influenza virus by the ProFlu+ assay was strongly positive for 2009 H1N1 influenza using the ProFlu-ST assay (Gen-Probe Prodesse, Inc., Waukesha, WI) with Cycle Threshold (Ct) = 17. Both shell viral culture with R-Mix cells (Diagnostic Hybrids, Athens, OH) and tube culture with the Rhesus monkey kidney cells stained positive for influenza A virus by Bartels Viral Respiratory Screening and Identification kit (Trinity Biotech, Carlsbad, CA). On repeated RT-PCR assays with the cell culture supernatant, results were the same as those obtained by testing the initial NP specimen, i.e., ProFlu+ assay negative for influenza A and B, but ProFlu-ST assay positive for 2009 H1N1 influenza. Split samples from the same cell culture supernatant were also run independently in two other institutions, and the results remained the same. The same cell culture supernatant was referred to Illinois Department of Public Health (IDPH) laboratories for testing using the CDC RT-PCR assay. This sample was positive for novel influenza A (H1N1) RNA (all three reactions of InfA, SW InfA, and SW H1 were positive). This same sample tested positive for influenza A by using MultiCode®-RTx Influenza A/B reagents (EraGen Biosciences, Madison, WI), and was also identified as 2009 H1N1 influenza by using a laboratory-developed assay for subtyping performed at Evanston Hospital of the NorthShore University Health Systems (4). A summary of laboratory viral test results are listed in Table 1.

Although more study of this 2009 H1N1 influenza virus isolate which was not detected by the ProFlu+ assay is ongoing, one possible cause may be sequence variation
in the primer and/or probe binding regions in the virus matrix gene. It is interesting that performance of some other PCR assays targeting the same gene were not affected, indicating that the hypothesized sequence variation might be limited. Previous experience with the ProFlu+ and ProFlu-ST assays has demonstrated excellent correlation between the two assays with only few discordant cases (ProFlu+ positive and ProFlu-ST negative) and no previous cases of ProFlu+ negative and ProFlu-ST positive found. The ProFlu+ assay does not differentiate influenza A subtypes. Because of its good performance, ability to detect influenza A, B, and RSV simultaneously, relatively ease of use, and its status of being the only IVD product in the class, this assay is widely used by laboratories for influenza diagnosis. Many laboratories perform a subtyping assay to determine the virus type only when the ProFlu test is positive for influenza A. Therefore, a negative result by this assay would likely miss the diagnosis of 2009 H1N1 influenza unless other assays, such as virus culture are performed.

We believe that this is the first report of a sample that may indicate a mutation in the influenza A virus matrix gene. At this time, although it seems to be very rare, the true prevalence of this variant among all 2009 H1N1 viruses is unknown until more data are available. Because of the implication of misidentification with a single assay, this case underscores the need for cautious interpretation and additional testing when a negative RT-PCR result does not seem to fit clinical presentation.
References


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<td>NP aspirate</td>
<td>Rapid antigen assay</td>
<td>BinaxNOW® Influenza A&amp;B (Inverness)</td>
<td>IVD</td>
<td>Influenza virus nucleoprotein antigens</td>
<td>Influenza A: (+)</td>
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<td>NP aspirate</td>
<td>Virus culture</td>
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<td>(Standard shell vial culture)</td>
<td>Unspecified influenza A antigen</td>
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<td>NP aspirate</td>
<td>Real time RT-PCR</td>
<td>ProFlu+ (for influenza A, B, and RSV) (Gen-Probe Prodesse)</td>
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<td>Matrix gene for Influenza A</td>
<td>Influenza A, B and RSV: (-)</td>
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<td>Real time RT-PCR</td>
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