Letters to the Editor

Improved Sensitivity of a Commercial Reverse Transcription-PCR Test for Subtyping of the 2009 H1N1 Influenza A Virus

Rapid detection of influenza A virus and determination of its subtype are important globally for public health surveillance and locally for the selection of antiviral treatment (1). We have used the ProFlu+ assay to test respiratory samples from children for influenza A virus, influenza B virus, and respiratory syncytial virus (RSV) and the ProFlu ST test to subtype influenza A virus (Gen-Probe Prodesse, Inc., Waukesha, WI). ProFlu ST is a multiplex assay that detects the 2009 H1N1 virus by targeting the nucleoprotein gene and also detects the seasonal H1N1 and seasonal H3N2 viruses by targeting the hemagglutinin gene. Here we report that the new subtyping test from Prodesse, ProFAST, is more sensitive than ProFlu ST for the 2009 H1N1 virus.

Between 2 October 2009 and 10 May 2010, we tested respiratory specimens using ProFlu+, and if influenza A virus was detected, subtyping was performed using the ProFlu ST test. RNA was extracted using the Qiagen QIAamp MinElute virus spin kit with the QiaCube system (Qiagen, Valencia, CA). The Prodesse ProFlu+ assay was performed according to the manufacturer’s instructions using the Cepheid SmartCycler system (Cepheid, Sunnyvale, VA). Of the 2,249 specimens tested with ProFlu+, 269 (12.0%) were positive for influenza A virus. Of these, 210 (78.1%) were subtyped as the 2009 H1N1 virus and the remaining 60 samples (21.9%) were negative with the ProFlu ST test. Nearly all influenza A virus circulating in the United States during the 2009-2010 influenza season was 2009 H1N1 (FluView; www.cdc.gov/flu), so it was likely that the negative specimens from the ProFlu ST test were of this subtype. As such, the ProFlu+ results compared to the ProFlu ST data suggested an inadequate sensitivity in the subtyping assay.

Recently, Prodesse introduced a new influenza A virus subtyping kit, ProFAST, to replace the ProFlu ST test. ProFAST targets the hemagglutinin gene for all three subtypes of influenza A virus. We used nucleic acid samples from selected specimens collected in previous influenza seasons and stored at −80°C to determine the performance of the ProFAST test. These samples had previously been tested by both the ProFlu+ and ProFlu ST tests. All of the specimens that were positive for 2009 H1N1, seasonal H1N1, or H3N2 viruses in the ProFlu ST assay were determined to be the same subtype in the ProFAST assay (Table). We also used the ProFAST assay to test 24 specimens from the 2009-2010 influenza season which were positive for influenza A virus in the ProFlu+ test but which did not give a detectable subtype in the ProFlu ST test. These same specimens were tested by the state public health laboratory using PCR reagents and protocols provided by the CDC (2). All 24 of these samples were positive for 2009 H1N1 using the CDC panels. More than half (14/24) were positive for 2009 H1N1 in the ProFAST assay (Table 1). There was no clear difference between the specimen types of the 27 samples that were subtyped with the ProFlu ST test (16 nasopharyngeal swabs, 6 nasopharyngeal aspirates, 4 nasopharyngeal washes, and 1 sputum sample) and the 24 that were not (16 nasopharyngeal swabs, 4 nasopharyngeal aspirates, 2 sputa, 1 nasopharyngeal wash, and 1 endotracheal tube aspirate sample). Our data support implementation of the new ProFAST subtyping test in our algorithm to significantly reduce the number of influenza virus specimens that are refractory to subtyping.

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<tr>
<th>TABLE 1. Results of subtyping of selected specimens that were positive for influenza A virus in the ProFlu+ test</th>
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<tbody>
<tr>
<td>No. of samples</td>
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<tr>
<td>27</td>
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<td>24</td>
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* ND. Not determined, as these specimens were not tested using the CDC subtyping test.

REFERENCES


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*Published ahead of print on 5 January 2011.