Fast-Track Communications

Rapid Method To Support Diagnosis of Swine Origin Influenza Virus Infection by Sequencing of Real-Time PCR Amplicons from Diagnostic Assays

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Since the reported emergence of swine origin influenza virus (S-OIV) A/H1N1 in April 2009, 39 countries have reported 8,480 confirmed cases (18 May 2009), and the virus appears to have potential to cause the next influenza pandemic (2). Despite the pandemic preparedness plans in place for public health laboratories around the world and the wide-spread diagnostic capability for influenza A/H5N1 (4), there was an interim period between the emergence of the virus and the development and distribution of a rapid diagnostic test specific for S-OIV. During this interim period, a “probable” diagnosis of S-OIV infection in humans was achieved by using the CDC (Centers for Disease Control and Prevention, Atlanta, GA) 2007 real-time PCR assay for human seasonal influenza (5). Nonspecific detection of S-OIV was made with a primer/probe set that targets a highly conserved region of the matrix gene of seasonal influenza A strains. An additional primer/probe set designed to subtype H1 and H3 seasonal influenza strains was unable to detect S-OIV. Therefore, a probable diagnosis of S-OIV was based upon the detection of an influenza A virus that could not be subtyped as H1 or H3. To add confidence to this test result, we have determined that sequencing of the real-time PCR matrix gene amplicon can distinguish between human seasonal A/H1N1 and S-OIV.

The CDC 2007 real-time PCR protocol for human seasonal influenza viruses is in common usage throughout the WHO Global Influenza Surveillance Network and other public health laboratories. Testing within our laboratory confirmed the detection of S-OIV from clinical samples using the seasonal matrix gene primers and probe and also confirmed that the H1 and H3 human seasonal influenza primer/probe sets do not detect S-OIV. Matrix gene real-time RT-PCR products from S-OIV and human seasonal H1N1 clinical samples were purified using a QIAquick PCR purification kit (Qiagen), including the addition of 10 μL 3 M NaCH3COO to ensure a correct pH for binding to the filter column. The purified PCR product was subjected to direct sequencing by the Sanger method on an ABI3130 sequencer, using the same forward and reverse primers described in the CDC real-time RT-PCR protocol. Informative sequence reads were consistently obtained despite the short length of the template (106 bp). An alignment of this sequenced region with all recent GenBank entries (accessed 18 May 2009) for the influenza A matrix gene (2004 to 2009) was constructed and showed a high degree of nucleotide sequence conservation within this small region both for S-OIV and for human seasonal A/H1N1 viruses. One nucleotide in the matrix gene PCR product was identified as being variable and largely discriminatory for S-OIV in comparison to human seasonal influenza A/H1N1 viruses, that being G at nucleotide (nt) 174 for seasonal H1N1 but A for S-OIV (174 G>A; Table 1). A total of 57 S-OIV sequences were available, all having an adenine at position 174. From the remainder of the available nonredundant matrix gene sequences for influenza A viruses (all subtypes, n = 2,957), only an additional 88 deposited sequences had nt 174 G>A, and only 3 of these were A/H1N1 viruses isolated from humans (Table 1). Two, A/Thailand/271/2005 and A/Aragon/RR32182008, were known zoonotic transmissions of a swine virus to humans (1, 3), and only one human seasonal A/H1N1 virus showed exact sequence identity to S-OIV across the entire sequenced region of 60 bp, that being A/Tennessee/UR06-0236/2007 (Table 1) (GenBank accession no. CY031141). The A/Tennessee/UR06-0236/2007 virus would provide a positive H1 test result for the CDC 2007 real-time RT-PCR and would thus have been excluded from identification as S-OIV. It can therefore be concluded that, for influenza A/H1N1 viruses isolated from humans, the nt 174 G>A sequence variant in the matrix gene is informative for S-OIV diagnosis.

<table>
<thead>
<tr>
<th>Virus subtype/group</th>
<th>No. of viruses</th>
<th>Segment of discriminatory region in matrix gene (nt 170–199)</th>
<th>Accession no.</th>
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<tbody>
<tr>
<td>Human A/H1N1 virus consensus</td>
<td>377</td>
<td>AGGGGATTTT AGGATTTGTG TTCACGCTCA</td>
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<tr>
<td>S-OIV consensus</td>
<td>57</td>
<td>A; Table 1)</td>
<td></td>
</tr>
<tr>
<td>A/Thailand/271/2005</td>
<td>1</td>
<td>. . . C</td>
<td>EF101750</td>
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<tr>
<td>A/Tennessee/UR06-0236/2007</td>
<td>1</td>
<td>. . . C</td>
<td>CY031141</td>
</tr>
<tr>
<td>A/Aragon/RR3218/2008</td>
<td>1</td>
<td>. . . C</td>
<td>FJ713784</td>
</tr>
</tbody>
</table>

* Nucleotide position 174 is shown in bold.
* Consensus of all human A/H1N1 viruses from 2004 to 2008.
* Consensus of all available S-OIV sequences (19 May 2009), which show 100% identity across the discriminatory region.
* Three viruses with nt 174 G>A were isolated from humans (before 2009 S-OIV emergence), as discussed in text.

* TABLE 1. Comparison of the discriminatory region in the real-time RT-PCR product of the matrix gene, including all (nonredundant) influenza A/H1N1 viruses isolated from humans between 2004 and 2009
We do not recommend that this method replace the current confirmatory testing prescribed by WHO, which requires either specific S-OIV real-time PCR, viral culture, full-length influenza gene sequencing, or a fourfold rise in S-OIV antigen-neutralizing antibodies (5). We propose that this approach could be used as a provisional diagnostic test by laboratories without access to specific S-OIV testing. We also note that this method provides a rapid validation of real-time PCR assay results, especially in the instance of unsubtypable influenza A viruses isolated from humans. If new influenza A subtypes or sequence variants were to arise in the future, sequencing of real-time PCR matrix gene products could provide an interim diagnostic test before a specific real-time PCR assay is developed.

REFERENCES


