Laboratory and Epidemiology Communications

Neuraminidase Subtyping of Human Influenza A Viruses by RT-PCR and Its Application to Clinical Isolates

Shinichi Takao*, Yukie Shimazu, Shinji Fukuda, Masaru Kuwayama and Kazuo Miyazaki

Division of Microbiology II, Hiroshima Prefectural Institute of Health and Environment, Minami-machi 1-6-29, Minami-ku, Hiroshima 734-0007

Communicated by Hiroo Inouye

(Accepted December 13, 2002)

In Japan, prefectural and municipal public health institutes are primarily responsible for isolating influenza viruses from clinical specimens. They determine the viruses’ hemagglutinin (HA) subtypes by means of hemagglutinin inhibition (HI) test, but usually not neuraminidase (NA) subtypes because NA subtype-specific antisera are commercially unavailable and the procedure of the neuraminidase inhibition (NI) test is too cumbersome to be conducted for many samples.

It has been considered that the influenza A virus epidemics that have occurred since 1977 were caused by subtypes A(H1N1) and A(H3N2). However, if the NA subtype is not actually determined, the NA subtypes of these isolates remain open to assumption. Reports on A(H1N2) outbreaks from Asian and other countries during the 2001/02- influenza season (1-3) and a report of A(H1N2) isolation in Yokohama (4) appeared to necessitate the determination of NA subtypes in addition to HA subtypes. We communicate here human influenza A virus NA subtyping using RT-PCR and its application to clinical isolates.

Viral RNAs were extracted from the supernatants of cultured MDCK cells infected with the influenza A virus by using a commercial kit (Isogen-LS, Nippon Gene, Tokyo) and dissolved in 40 µl of distilled water. The RT-PCR was performed using a one-tube RT-PCR method (Access RT-PCR system, Promega, Madison, Wis., USA) by employing the NA subtype-specific primer pairs (5) as shown in Table 1. For RT-PCR, the first strand cDNA synthesis was conducted for 45 min at 48°C. After denaturation for 2 min at 94°C, the samples were submitted to 35 cycles of PCR amplification consisting of denaturation for 10 s at 94°C, primer annealing for 10 s at 55°C, and extension for 1 min at 65°C except the final extension which continued for 5 min.

First, we examined the specificity of the RT-PCR method. A total of 11 standard strains of influenza viruses, four A(H1N1), six A(H3N2), and one A(H2N2), were tested. As shown in Fig. 1, NA subtype-specific products were amplified from all the standard strains. The specificity of the RT-PCR method was confirmed by direct sequencing of the RT-PCR products.

We then determined the NA subtypes of 45 A(H1) and 51 A(H3), which had been isolated from the patients with influenza-like illnesses from January 1996 to March 2002.

Table 1. Oligonucleotide primers designed for NA subtyping of influenza A viruses

<table>
<thead>
<tr>
<th>Primer</th>
<th>Gene target</th>
<th>Sequence</th>
<th>Base location</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN1B</td>
<td>A(N1)</td>
<td>5’-TTGCTTGGTGCAGCAAGTGA-3’</td>
<td>505-524</td>
<td>708</td>
</tr>
<tr>
<td>AN1DII</td>
<td>A(N1)</td>
<td>5’-TTAGCTCAGGATGTTGAAG-3’</td>
<td>1193-1212</td>
<td></td>
</tr>
<tr>
<td>AN2B</td>
<td>A(N2)</td>
<td>5’-GGTGACGACGACACCTTATG-3’</td>
<td>345-364</td>
<td></td>
</tr>
<tr>
<td>AN2CII</td>
<td>A(N2)</td>
<td>5’-CCTGAGCACACATAACTGGA-3’</td>
<td>940-959</td>
<td>615</td>
</tr>
</tbody>
</table>

*Corresponding author: Fax: +81-82-252-8642, E-mail: takao@urban.ne.jp
Of a total of 96 isolates, 45 were classified as A(H1N1) subtype and 51 as A(H3N2) subtype. These results suggested the usefulness of RT-PCR for NA subtyping of human influenza A virus. NA subtyping of isolates in the coming influenza season in 2002-2003 will reveal whether or not the A(H1N2) epidemic expands.

REFERENCES