NOTES

Serotype-Specific Detection of Coxsackievirus A16 in Clinical Specimens by Reverse Transcription-Nested PCR

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We describe the development of a coxsackievirus A16 (CVA16) serotype-specific PCR which correctly differentiated between CVA16 and other enterovirus serotypes of both laboratory isolates and clinical specimens. The assay will be useful for monitoring CVA16 outbreaks and studying the disease association, epidemiology, and evolution of this common enterovirus serotype.

Enteroviruses comprise a large genus within the Picornaviridae which includes the polioviruses, group A and B coxsackieviruses, and echoviruses. Common human pathogens, they are traditionally diagnosed by virus isolation in cell cultures and, in the case of coxsackieviruses, by suckling mouse inoculation (SMI). Serotypic identification of enterovirus isolates is not usually required for patient management but is useful in studying enterovirus outbreaks; in addition, it is required to differentiate between wild or vaccine-derived polioviruses and nonpolioviruses in cases of acute flaccid paralysis (4, 5) or, more commonly, to characterize enterovirus isolates from cases of aseptic meningitis as a means of achieving certification of poliovirus eradication (14). Serotyping is achieved by neutralization of viral infectivity in cell cultures or suckling mice using individual or pooled serotype-specific neutralizing antisera or by indirect immunofluorescence using serotype-specific monoclonal antibodies. However, these methods lack sensitivity. SMI is most sensitive for coxsackievirus isolation, but it is not widely available and is slow, labor-intensive, and ethnically undesirable.

In recent years, molecular diagnostic methods have been increasingly used for enterovirus diagnosis. Although sensitive, these methods do not generally allow serotype identification. There is therefore a need for additional molecular tools for serotype or genotype identification of enteroviruses which complement existing PCR methods for generic enterovirus detection (reviewed in reference 10). The development of such assays has been limited thus far (1, 3, 6, 9, 12, 16) and, as most have been used to study enterovirus isolates rather than clinical specimens, they cannot yet completely replace traditional isolation methods.

Coxsackievirus A16 (CVA16) is the most commonly detected of the group A coxsackieviruses (CVA) and is best known for causing hand, foot, and mouth disease (HFMD). Although normally benign, fatal central nervous system complications have been observed in recent epidemics of HFMD.

TABLE 1. Primers used in the CVA16-specific PCR and generic enterovirus-reactive PCR

<table>
<thead>
<tr>
<th>PCR</th>
<th>Primer</th>
<th>Use</th>
<th>Nucleotide position on CVA16 genome</th>
<th>Region of genome</th>
<th>Sequence (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVA16 specific</td>
<td>Outer upstream</td>
<td>First-round PCR</td>
<td>3127–3148</td>
<td>VP1</td>
<td>ACC ATG ATG GCC ACT TTG AGC A</td>
</tr>
<tr>
<td></td>
<td>Outer downstream</td>
<td>Reverse transcription and first-round PCR</td>
<td>3306–3286</td>
<td></td>
<td>GGT GCA CTT GAT ATT TCC</td>
</tr>
<tr>
<td></td>
<td>Inner upstream</td>
<td>Nested PCR</td>
<td>3162–3182</td>
<td></td>
<td>GAC TGA AAA GTC ACC ACA ITC</td>
</tr>
<tr>
<td></td>
<td>Inner downstream</td>
<td>Nested PCR</td>
<td>3270–3251</td>
<td></td>
<td>GGT CTT GAA CAA ATA GGG TTG</td>
</tr>
<tr>
<td>Generic enterovirus</td>
<td>Outer upstream</td>
<td>First-round PCR</td>
<td>457–473</td>
<td>5’ Untranslated region</td>
<td>CCG CCC CTG AAT GCG GC</td>
</tr>
<tr>
<td></td>
<td>Outer downstream</td>
<td>Reverse transcription and first-round PCR</td>
<td>649–632</td>
<td></td>
<td>CAC CGG ATG GCC AAC AAT C</td>
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<tr>
<td></td>
<td>Inner upstream</td>
<td>Nested PCR</td>
<td>460–477</td>
<td></td>
<td>CCC CTG AAT GCG GCT AAT</td>
</tr>
<tr>
<td></td>
<td>Inner downstream</td>
<td>Nested PCR</td>
<td>606–587</td>
<td></td>
<td>ATT GTC ACC ATA AGC AGC CA</td>
</tr>
</tbody>
</table>

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caused by enterovirus 71 (EV71), a serotype closely related to CVA16, in the Far East (7, 15). Identifying the cause of illness was made by enterovirus 71 (EV71), a serotype closely related to CVA16. 

Next, we describe the development of a CVA16-specific PCR assay and its use for typing and serotyping of CVA16 isolates. The assay was developed based on the high degree of polymorphism of the CVA16 capsid protein VP1, which was used to design a set of primers specific for CVA16. The primers were designed to be complementary to a conserved region of the VP1 gene of CVA16, which is highly conserved across different serotypes.

The assay was tested on a variety of specimens, including stools, saliva, and cerebrospinal fluid (CSF), collected from patients with suspected CVA16 infections. The assay was able to detect CVA16 RNA in all specimens tested, with a sensitivity of 10^{-6} TCID50 per 100 µl. The assay was also able to differentiate between CVA16 and other enterovirus serotypes, with a specificity of 100%.

The assay was also found to be useful for typing and serotyping of CVA16 isolates. The assay was able to differentiate between CVA16 and other enterovirus serotypes, with a specificity of 100%.

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lates. When combined with nucleotide sequencing of PCR products, the CVA16-specific PCR may also be useful for studying the molecular epidemiology and evolution of CVA16.

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REFERENCES