Rapid screening system of reassortant influenza A viruses using primer dependent multiplex RT-PCR

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Abstract

A simple method for rapid screening of 6:2 cold adapted reassortnats of influenza virus, primer dependent multiplex RT-PCR screening method, is described. Primers for screening purpose were selected from highly variable regions on an attenuated donor virus in comparison to all influenza A virus from constructing multiple sequence alignments. There are two kinds of primers. One kinds of primers were selected from highly variable regions on an attenuated donor virus in comparison to other wild influenza A viruses. These showed PCR products that were amplified when the template genomes were from donor strain. The others were designed on conserved region of many kinds of influenza A viruses. These were used for the presence of each segment although there were many failures of producing PCR products from wild type virus gene were observed. As a result, the difference of primer binding affinity made it possible to show the nucleotide difference between donor strains and wild viruses and to facilitate for screening of 6:2 reassortant vaccine strain. In multiplex RT-PCR format, the process of this method is highly streamlined since time consuming post-PCR analysis is omitted. We demonstrated the utility of this method by genotyping of randomly picked reassortant viruses.

Key words : Influenza virus; Reassortant virus; Primer-dependent multiplex RT-PCR screening system.

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