D-MRTPCRHPIV: Designing a multiplex reverse transcriptase-PCR for simultaneous detection of parainfluenza viruses types 1-4

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Abstract

Introduction: Human Parainfluenza virus (HPIV) is an enveloped RNA virus from Paramyxoviridae Family with single stranded genome. The viral envelope contains a virulence factor and contains F-protein and Hemagglutinin-Neuraminidase (HA). Molecular diagnosis methods are inexpensive and applicable for virus detection. Multiplex RT-PCR, one of these methods, is relatively inexpensive and fast. The aim of this study is to evaluate its reliability for HPIV detection.

Materials & Methods: The gene sequences of HPIV types 1 to 4 were obtained from NCBI (National Center for Biotechnology Information) and aligned. Forward and reverse primers for each type were designed based on the conserved sequences of HN gene. Specific forward and reverse primers of HPIV-1, HPIV-2, HPIV-3 and HPIV-4 amplify 451 to 599, 786 to 1122, 1006 to 1488 and 546 to 1103 regions of related HN genes, respectively. Four HPIV HN genes were synthesized and inserted into pBSK Vector (BIOMATIK, Denmark) and plasmids were extracted using Mini Extraction kit (BIONIR, South Korea) and linearized. The exact amount of extracted linear plasmids was determined with Spectrophotometer at OD 260 nm, and different concentrations were prepared utilizing serial dilution method, and were used as template in conventional RT-PCR using designed primers. Finally, the minimum detectable template concentration was determined for each pair of primers.

Results & Conclusions: Analysis of the copy number results (using www.web.uri.edu-USA) shows that this in-house RT-PCR has acceptable ability to detect 6.22×104, 2.75×104, 1.91×104 and 1.66×104 copy number of HPIV-1, 2, 3 and 4 genomes, respectively. Considering the results of our study, multiplex RT-PCR seems to be a reliable method and can be used to detect HPIVs genome in respiratory samples.

Biography

Maryam GhelichKhan is a student in microbiology at Department of Microbiology, Faculty of Biological Sciences, Shahid-Beheshti University, Tehran, Iran and she is working on her thesis on HPIV at Influenza Research Lab, Pasteur Institute of Iran, Tehran, Iran.

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