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Simultaneous detection of Human Parainfluenza Viruses 1,2,3, and 4 in patients with iluenza-like illness Using a Multiplex Real-Time PCR

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Introduction

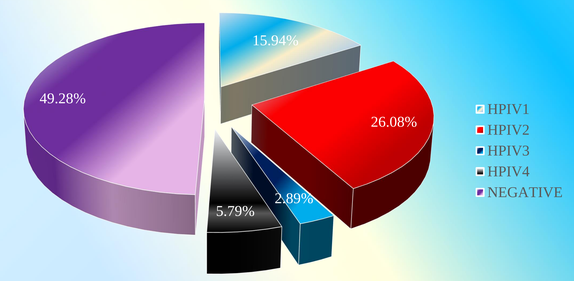
The human ParainfluenzaViruses (PIVs) are enveloped non-segmented, negative, single-stranded RNA viruses which belong to ***Paramyxoviridae Family***. They are an important cause of upper and lower respiratory tract infections with pneumonia, croup, and bronchiolitis in infants, children, and immunocompromised individuals. Four types of HPIV are circulating world-wide. The availability of HPIV-specific detection assays is important because other respiratory pathogens cause similar illnesses. Objective of the present study was to develop a one-step Multiplex quantitative RT-PCR to detect clinical speciments from 69 hospitalized patients with Influenza Like-illness in a single test.

Materials and Methods

Nasopharyngeal swabs were collected from pediatric and adult patients with Influenza-like illness. Nucleic acid extraction was performed using High Pure RNA Kit (Roche, Germany). The gene sequences of Parainfluenza viruses were obtained from NCBI and alignment was carried out using AlleleID software to identify conserved sequences for designing primers and probes. The Parainfluenza viruses-specific hydrolysis probes were labeled at the 5′ end with four different reporter dyes FAM (530 nm), TET (555 nm), ROX (610 nm) and Cy5 (660 nm), respectively. The assay was first optimized in a monoplex qRT-PCR for each type; subsequently, a one-tube multiplex real-time RT-PCR was performed to discriminate all known Human Parainfluenza viruses using a Rotor-Gene 6000 apparatus (Corbett Life Science).

Results

Of the 69 specimens, 35(50.72%) were positive by Multiplex qRT-PCR. HPIV2 (26.08%) was the most frequent detected virus followed by HPIV1(15. 94%), HPIV3(2.89%), And HPIV4(5.79%).



The chart shows the percentage of positive HPIV samples (50.72%) as compared to negative samples (49.27%).

Conclusions

This assay is mostly recognized as a sensitive and specific method for detection of respiratory RNA viruses that result can be achieve whitin 3 h, which raise clinical relevance.Thus, use of this qRT –PCR assay would develop patient management and infection control.

References

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