Development of a sensitive and specific real-time RT-PCR System for the qualitative detection of Enterovirus and Rhinovirus RNA

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Introduction
The genus Enterovirus (family Picomaviridae) contains 12 different species (9 enterovirus and 3 rhinovirus species). They carry positive, single-stranded RNA genomes of around 7500 nucleotides. Non-polio enteroviruses often occur seasonally and are commonly associated with cardiae and respiratory symptoms, cutaneous and mucosal infections, neonatal sepsis or viral meningitis and encephalitis. Rhinovirus infections are usually restricted to the respiratory tract with symptoms of a common cold but more severe illness like pneumonia can occur. The Poliovirus group is most closely associated with poliomyelitis.

The clinical symptoms are mostly unspecific, which makes infections caused by enteroviruses difficult to distinguish from those caused by other agents. Therefore, there is need for reliable enterovirus in vitro diagnostics. The RealStar\(^\text{®} \) Enterovirus RT-PCR Kit facilitates detection of all human entero- and rhinoviruses in different types of patient specimens. It detects all currently known strains, including emerging C-group enteroviruses. The assay contains an internal control for reliable interpretation of results.

Here we present data on the analytical specificity and sensitivity of RealStar\(^\text{®} \) Enterovirus RT-PCR Kit. Furthermore, 20 pretested samples were analyzed with the RealStar\(^\text{®} \) Enterovirus RT-PCR Kit in comparison to different real-time RT-PCR assays.

Methods

The analytical sensitivity was determined by probe analysis. In vitro transcripts containing the respective target region of Enterovirus A71 and Rhinovirus B72 were diluted in half-logarithmic steps and tested in replicates with the RealStar\(^\text{®} \) assay. Hit rates were determined and the 95% limit of detection (LoD95) was calculated.

Reactivity was confirmed by testing QCMD proficiency panels and viral RNA of different entero- and rhinoviruses. Possible cross-reactivity of the RealStar\(^\text{®} \) Enterovirus RT-PCR Kit was assessed by testing nucleic acids from various viruses and bacteria that may cause similar symptoms or are present in the same sample type as entero- and rhinoviruses. Nucleic acid extraction was performed using the QiAamp\(^\text{®} \) Viral RNA Mini Kit (Qiagen, Hilden, Germany) according manufacturer’s instructions.

Results

Analytical Sensitivity

The limit of detection (LoD95) was calculated using probe analysis and was determined by testing replicates of half-logarithmic dilutions specific in vitro transcribed RNA of Enterovirus A71 and Rhinovirus B72. The X-axis shows the concentration of RNA and the Y-axis the proportion of positive results. The LoD95 is 0.3 ng/µl (95% confidence interval 0.2 ng/µl to 0.6 ng/µl) for Enterovirus A71 and 1.1 ng/µl (95% confidence interval 0.7 ng/µl to 2.0 ng/µl) for Rhinovirus B72.

Analytical Specificity

The specificity of primers and probes selected for the specific detection of all enterovirus and rhinovirus serotypes was experimentally determined by testing strains of genus Enterovirus (Table 1). All strains were successfully detected with the RealStar\(^\text{®} \) Enterovirus RT-PCR Kit.

Diagnostic validation

Here we present data on the analytical specificity and sensitivity of RealStar\(^\text{®} \) Enterovirus RT-PCR Kit. Furthermore, 20 pretested samples were analyzed with the RealStar\(^\text{®} \) Enterovirus RT-PCR Kit in comparison to different real-time RT-PCR assays.

The cross-reactivity was tested with DNA/RNA of CMV, EBV, HAV (A, B), HEP (A, B), VZV, HSV, HCV, HIV, BKV, JCV, Adenovirus, Parvovirus B19, Influenza (A/H1N1, H3N2, B), IVV (1-4), RSV (A/B), MPV, murine virus, measles virus, parvovirus, Coronavirus, Norovirus, Gastro-virus, Rotavirus, Nocardia asteroides, Bordetella pertussis, Bordetella parapertussis, Sphingomonadaceae, Neisseria meningitidis, and Enterococcus sp. No unspecific cross-reactivity was observed.

Conclusion

The results presented here show that the RealStar\(^\text{®} \) Enterovirus RT-PCR Kit is highly sensitive and allows the detection of low-level of virus RNA in different sample types. The Internal Control included in the assay enables reliable diagnostics. Therefore the RealStar\(^\text{®} \) Enterovirus RT-PCR Kit will be a valuable tool for the detection of enterovirus and rhinovirus RNA in patients samples.

References


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