Influence of different one-step reverse transcription qPCR reagents for no-doubt detection of norovirus genogroup II

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Introduction

Human noroviruses, members of Caliciviridae family are one of the main cause of acute non-bacterial gastroenteritis in humans of all ages. They appear in both, epidemic and sporadic cases. Noroviruses can not be propagated in cell cultures. Thus real-time PCR molecular methods for caliciviral detection, which represent the basis of molecular epidemiology, have been introduced [1, 2, and 3].

Material and Methods

The total RNA was extracted from 15 collected clinical stool samples using iPrep PureLink Virus kit (Invitrogen by Life Technologies) followed manufacture’s instruction.

For targeting a 95 base-pair fragment in ORF1–ORF2 (open reading frame) junction of the genome, genogroup II specific primers (COG2F/COG2R) were used [1, 2, and 3]. For the purpose of our study the detection probe RING2-TP from the same author was slightly modified: a 20 base-long dual fluorogenic probe (FAM-BHQ) (Metabolion) was used.

A home-brew assay for detection of luciferase (Luc) RNA (Promega) was used as internal amplification control (IAC) to assess the inhibition of RT-qPCR chemistry. Luc was spiked to each RT-qPCR reaction.

RT-qPCR reactions were prepared as shown in Figure 1. RT-qPCR reactions were run on StepOne instrument (Applied Biosystems by Life Technologies) using specific cycling conditions ("fast" or "standard" protocol) (Figure 1).

Data was acquired and analyzed using the Sequence Detection System Software (version 2.01). To determine the dynamic range of detection and efficiency of amplification for both RT-qPCR standard curves were generated using 10-fold serial dilutions of positive stool sample from a patient with confirmed norovirus infection.

Aim of the study

The aim of the study was to compare two different one-step reverse transcription real-time PCR (RT-qPCR) reagents for detection of norovirus strains belonging to genogroup II. The linear range of detection, sensitivity, efficiency of amplification were established and comparison of detected fluorescence using two different RT-qPCR reagents was made.

The efficiency was determined by plotting the Ct values as a function of log, relative concentration of positive sample. RT-qPCR efficiency was calculated by the following equation: PCR efficiency [E] = 10^{(1/S)} (slope (S)) [4]. Comparison of detected fluorescence from RT-qPCR reaction was compared from linear view of amplification curve.

Discussion and Conclusion

Conclusions:
- same time frame of RT-qPCR amplification for both RT-qPCR reagents ("fast" protocol)
- broader linear range of TaqMan Fast Virus 1-Step Master Mix (6-log linear range for TaqMan Fast Virus 1-Step Master Mix ("standard" or "fast" protocol) and 5-log for RT-qPCR reagent Producer A ("standard" or "fast" protocol)
- comparable efficiency and R² for both kits
- in the view of detected ΔRn for same samples more reliable detection with TaqMan Fast Virus 1-Step Master Mix

The results show that the use of a TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems by Life Technologies) offers fast, sensitive and reliable (robust) detection of norovirus genogroup II in comparison to the reagent from Producer A.

References