Duplex assay for reliable detection of norovirus RNA and internal control

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Reliable detection of norovirus RNA by real-time RT-PCR in the presence of an internal control can be achieved without any optimization steps using the QuantiTect® Virus +ROX Vial Kit and the Rotor-Gene® Q cycler. Purification of norovirus RNA for real-time RT-PCR detection can be successfully automated on the QIAsymphony® SP using the QIAsymphony Virus/Bacteria Mini Kit. This combination of QIAGEN instrumentation and chemistries streamlines the entire workflow for analyzing norovirus RNA, from purification to detection.

Introduction

Duplex, real-time one-step RT-PCR provides a reliable method for detecting norovirus RNA. The norovirus RNA sample is spiked with internal control (IC) RNA, and both RNA targets are then reverse transcribed and amplified in the same tube. Detection of IC, but not of norovirus RNA, would indicate that real-time RT-PCR was successful and that norovirus RNA is either absent or below the limit of detection.

Success in duplex, real-time one-step RT-PCR often requires careful optimization of reaction and cycling conditions. Here we describe a method for duplex analysis that enables precise detection of norovirus RNA over a wide linear range without the need for PCR optimization. In addition, we demonstrate that this method works successfully in combination with automated purification of viral nucleic acids.

Materials and methods

Detection of in vitro transcribed norovirus RNA in a duplex assay

Serial tenfold dilutions of in vitro transcribed norovirus RNA were spiked with in vitro transcribed IC. Duplex, real-time one-step RT-PCR was then performed using the QuantiTect Virus +ROX Vial Kit in combination with 2 primer–probe sets: one specific for norovirus RNA from genogroup II (1), and the other specific for the IC. Reactions were run in triplicate on the Rotor-Gene Q real-time PCR cycler according to the appropriate protocol in the *QuantiTect Virus Handbook*, without any optimization of PCR parameters.

Detection of norovirus RNA purified from medium using an automated platform

RNA was purified in triplicate from 5 viral transport medium samples (100 µl sample diluted with 100 µl phosphate-buffered saline) from a commercially available norovirus test panel using the QIAsymphony SP in combination with the QIAsymphony Virus/Bacteria Mini Kit. Samples 1–4 contained norovirus from genogroup II, genotype 4 (GII.4), originally isolated from stool; sample 5 was the negative control and contained no norovirus. All samples were spiked with in vitro transcribed IC, and duplex, real-time one-step RT-PCR was carried out as described above.

Results

Detection of in vitro transcribed norovirus RNA in a duplex assay

The QuantiTect Virus +ROX Vial Kit and Rotor-Gene Q enabled reliable detection of the in vitro transcribed norovirus RNA, and required no optimization of reaction and cycling conditions (Figure 1). Norovirus RNA was detected over a 6-log range with high linearity (R^2 = 0.995) and precision (the C_T values for each set of triplicates showed an overall relative standard deviation of 1.30 ± 0.59%). The IC was also reliably detected, as demonstrated by the comparable C_T values for all samples (relative standard deviation of 0.59 ± 0.49%).

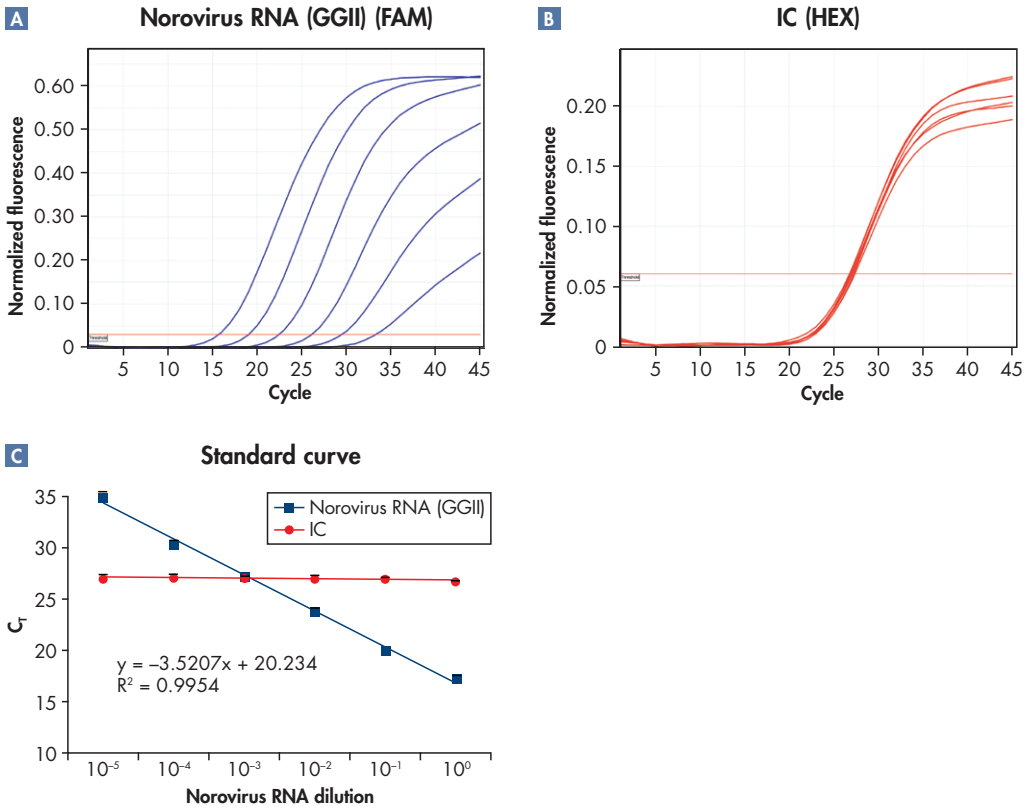

Figure 1. Precise detection of in vitro transcribed norovirus RNA over a wide linear range. Duplex, real-time one-step RT-PCR was carried out using the QuantiTect Virus +ROX Vial Kit and the Rotor-Gene Q according to the supplied protocol and without any PCR optimization. The template was serial tenfold dilutions of in vitro transcribed norovirus RNA (genogroup II; GGII) containing a fixed amount of IC. Each dilution was analyzed in triplicate. A Amplification plots for norovirus RNA (one replicate is shown for each template dilution). B Amplification plots for the IC (one replicate is shown for each norovirus RNA dilution analyzed). C Plot of log template amount versus mean C_T value, demonstrating the high linearity and precision in detection of norovirus RNA.

Detection of norovirus RNA purified from medium using an automated platform

Norovirus RNA purified using the QIAsymphony Virus/Bacteria Mini Kit on the QIAsymphony SP was reliably detected using the QuantiTect Virus +ROX Vial Kit and the Rotor-Gene Q (Figure 2). For samples 1–4, norovirus RNA was detected with high precision, with the C_T values for each set of triplicates showing an overall relative standard deviation of 1.16 ± 0.86%. As expected, norovirus RNA was not detected in sample 5, the negative control. The IC was detected with comparable C_T values in samples 1–5 (relative standard deviation of 1.12 ± 0.51%).

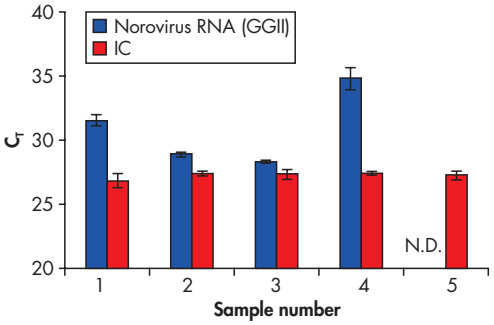

Figure 2. Precise detection of different norovirus GGII RNA isolates. Duplex, real-time one-step RT-PCR was carried out using the QuantiTect Virus +ROX Vial Kit and the Rotor-Gene Q. The template was viral RNA purified in triplicate from 5 viral transport medium samples on the QIAsymphony SP using the QIAsymphony Virus/Bacteria Mini Kit. After purification, each sample was spiked with a fixed amount of IC. The bar chart shows the C_T values obtained: samples 1–4 contain norovirus RNA; sample 5 is the negative control and contains no norovirus RNA.

Conclusions

- The QuantiTect Virus +ROX Vial Kit and Rotor-Gene Q allowed precise detection of norovirus RNA over a wide linear range.
- Reliable detection of norovirus RNA and internal control in a duplex assay was achieved without any optimization of reaction and cycling conditions.
- The QIAsymphony Virus/Bacteria Mini Kit and QIAsymphony SP provided reliable purification of norovirus RNA from viral transport medium for real-time RT-PCR analysis.
- The combination of QIAsymphony, Rotor-Gene, and QuantiTect technologies streamlined the entire workflow for purifying and detecting norovirus RNA.

References

(1). Höhne, M. and Schreier, E. (2004) Detection and characterization of norovirus outbreaks in Germany: Application of a one-tube RT-PCR using a fluorogenic real-time detection system. J. Med. Virol. **72**, 312.

Ordering Information

Product	Contents	Cat. no.
QuantiTect Virus +ROX Vial Kit (50)*	For 50 x 50 µl reactions: 5x Master Mix (without ROX dye), ROX Dye Solution, 100x RT Mix, RNase-Free Water, and Nucleic Acid Dilution Buffer	211031
Rotor-Gene Q	Real-time PCR cycler with 2, 5, or 6 channels, and with or without high-resolution melting (HRM®) analyzer	Varies†
QIAsymphony SP	QIAsymphony sample prep module, 1-year warranty on parts and labor	9001297
QIAsymphony Virus/Bacteria Mini Kit (192)	For 192 preps of 200 µl each: Includes 2 reagent cartridges and enzyme racks and accessories	931036

* For use with Rotor-Gene cyclers, the Applied Biosystems® 7500, and cyclers from Bio-Rad, Cepheid, Eppendorf, Roche, and Stratagene. Larger kit sizes available; please inquire. Kits for use with other Applied Biosystems instruments also available; please inquire.

† For ordering information, visit www.qiagen.com/Products/Rotor-GeneQ.aspx.

The QuantiTect Virus +ROX Vial Kit is intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

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