RT-PCR for NoV

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ABSTRACT

This assay amplified the partial VP1 gene (Region C) of the norovirus genome. The yield products could be sequenced.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Liu L, Guan H, Zhang Y, Wang C, Yang G, Ruan S, Zhao H, Han X (2018) The prevalence of non-GII.4 norovirus genotypes in acute gastroenteritis outbreaks in Jinan, China. PLoS ONE 13(12): e0209245. doi: 10.1371/journal.pone.0209245

PROTOCOL STATUS

Working

Reagents

1 SuperScript™ III One-Step RT-PCR with Platinum™ Taq
By Life Technologies
Catalog #:12574026

RNA extraction

2 Extract total RNA of specimens with the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions.

Reaction system

3 The primers (G1-SKF/G1-SKR for GI, COG2F/G2SKR for GII) were derived from the studies of Kojima, S. and Shinohara, M. et al.

The reagent composition	Volume 25 ul
2×Reaction Mix	12.5 ul
Forward Primer (25 uM)	0.5 ul
Reverse Primer (25 uM)	0.5ul
Taq mix	1.0 ul
Rnase Inhibitor (30-40U/ μl)	0.5 ul
RNA	3 ul
DEPC H_2O	to 25ul

Amplification

4 The amplification conditions were as follows: 42 for 30 min; 95 for 15 min, 40 cycles of 95 for 30 s, 50 for 30 s, and 72 for 30 s and a final extension of 72 for 10 min.

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Products

5 The expected amplicon size of GI was 330 bp and GII was 387 bp. The yield products were sequenced to validate the the genotype of NoV.

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