RNA/DNA extraction from samples of acute gastroenteritis

Version 2

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ABSTRACT

Many types of sample would be collected in outbreaks of acute gastroenteritis. Therefore we have settled this protocol to extract RNA/DNA for virus detection rapidly and effectively.

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

Pretreatment of stool/anal swab

1. Add 0.2g stool to 1.0ml phosphate buffer saline (with Mg2+ and Ca2+) in one 1.5ml Eppendorf tube with 6-8 particles of ceramic beads. Put the anal swab into 1.0ml phosphate buffer saline (with Mg^{2+} and Ca^{2+}) and repeatedly squeeze it.
2. Vortex for 2×20 sec at 4000rpm at room temperature.
3. Centrifuge at 8000 rpm for 10 min at 4℃.
4. Collect the supernatant.

Preparing swabs

5. Throat swabs or environmental surface swabs were stored in 3.5ml Hank's solution （Yocon Catalog #:MT0301-1）.
6. Vortex for 40 sec at 4000rpm at room temperature.

Pretreatment of water

7. Add 15ml contaminated water to centrifugal filter (Merck Millipore Ltd., Ireland).
8. Centrifuge at 8000rpm for 5min at 4℃.
9. Repeat step 8 for three times.
10. Collect the supernatant.

Pretreatment of food:

1. Select randomly six points on the surface of food specimen, then cut them into 2mm×2mm×2mm pieces by sterilized scissors.
2. Add 0.2g food pieces to 1.0ml phosphate buffer saline (with Mg^{2+} and Ca^{2+}) in one 1.5ml Eppendorf tube with 6-8 particles of ceramic beads.
3. Vortex for 2×20 sec at 4000rpm at room temperature.
4. Centrifuge at 8000 rpm for 10 min at 4℃.

5. Collect the supernatant.

RNA/DNA extraction

5 16. Add each above 200ul supernatant in sample cartridge to extract RNA/DNA followed the manufacturer's instructions (MagNA Pure LC Total Nucleic Acid Isolation Kit, Roche, Germany).
17. RNA/DNA was suspended in 50ul of elution buffer.
18. RNA/DNA was amplified immediately or stored at −80℃.

Note:

6 The left supernatant was stored at −80℃ after the RNA/DNA extraction.