RNA/DNA extraction from samples of acute gastroenteritis

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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.

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Protocol

Pretreatment of stool

Step 1.

1. Add 2.0g stool to 1.0ml phosphate buffer saline (with Mg2+ and Ca2+) in one 1.5ml Eppendorf tube with 6-8 particles of ceramic beads.
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

Step 2.

5. Throat swabs or environmental surface swabs were stored in Hank’s solution.

6. Vortex for 40 sec at 4000rpm at room temperature.

Pretreatment of water

Step 3.

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.

RNA/DNA extraction

Step 4.

11. 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).

12. RNA/DNA was suspended in 50ul of elution buffer.

13. RNA/DNA was amplified immediately or stored at −80℃.