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RNA/DNA extraction from samples of acute gastroenteritis V.1

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Protocol status: Working
We use this protocol in our group and it is working.

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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.

Pretreatment of stool

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°C.
4. Collect the supernatant.

Preparing swabs

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

3

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

RNA/DNA extraction

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°C.