Laboratory Operating Procedure

Sewages viral concentration using PEG method

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Prepared by: Julien Schaeffer

Contact: *Soizick.le.guyader@ifremer.fr*

Institution: *IFREMER, France*

LOP-Version: 1

Attachments:

Introduction

The here described method is used to elute and concentrate enteric viruses from sewage/water samples. This protocol combines, PEG (polyethylene glycol) precipitation under acidic controlled conditions and filtrations.

Sample Material

Sewage or water samples or stool suspension (after dilution).

Equipment and Reagents

Equipment

- Refrigerated centrifuge (for 1.5 ml and 2 ml tubes)
- Vortexer
- Pipettes
- Disposable gloves
- 1.5 ml and/or 2 ml tubes
- Sterile, RNase-free pipet tips (with aerosol barriers to prevent cross contamination)
- Syringe and syringe filters 5 µm; 1.2 µm; 0.45 µm and 0.22 µm porosity
- Conductimetrer

Reagents

- PEG polyethylene glycol 50% (w/v) in molecular grade water
- Chloroform/butanol (50/50 v/v)
- HCl
- NaOH
- NaCl 5M
- Glycine buffer, for 1L dissolve in molecular grade water : 3.75 g of glycine and 9 g of NaCl. Adjust pH at 9,5 with NaOH
- Omnicleave Endonuclease (Epicentre; cat. No. OC7810K)
- $MgCl_2$ 100 mM

General remarks

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Literature

- *http://www.lucigen.com/docs/manuals/omnicleave-endonuclease.pdf*
- Sima L.C., Schaeffer J., Le Seax J-C., Parnaudeau S.,Elimelech M., Le Guyader F.S. Calicivirus Removal in a Membrane Bioreactor Wastewater Treatment Plant. *Appl. Environ. Microbiol.* August 2011 vol. 77 no. 15 5170-5177.

Before getting started

- Pre-cool the refrigerated centrifuge at 4°C
- Equilibrate samples to room temperature (RT)

Procedure

1.
- Collect 40 mL of sample into 50 mL centrifuge tube
- Adjust pH to 3 with HCl (or NaOH)
- Measure conductivity using conductimeter and adjust conductivity over 2000µS with NaCl 5M.
- Add 10 mL of PEG 50%

2.
- Incubate overnight at 4°C under gentle agitation
- Centrifuge at 13 500 *x g* for 1h30
- Discard the supernatant

3.
- Dissolve PEG pellet with 2 mL of warm glycine buffer (56°C)
- Add 2mL of Chloroform/butanol
- Mix 1 min using a vortexer
- Centrifuge at 11 000 *x g* for 5 min
- Collect the upper phase

4.
- Using a syringe, filtrate the collected upper phase through syringe filter porosity 5 µm; 1.2 µm and 0.45 µm

5.
- Add 20 µL of Omnicleave enzyme mix and 200 µL of $MgCl_2$.
- Incubate 1h at 37°C
- Proceed to nucleic acid extraction protocol