ULTRAFILTRATION SOP FOR RAW SEW AGE CORONAVIRUS PROJECT

Description

The aim of the protocol is to concentrate viruses present in 50 mL of sewage into a final volume of 150-300 µL. The sample should be a 24 h 1 L composite sample to be representative, though this method will also work for grab samples.

Required Instruments & Consumables

- 250 mL glass bottle (i.e., Schott)
- Magnetic stir bar, magnetic stir bar retriever, magnetic stirrer
- Centricon Plus-70 Ultrafilter (UFC701008)
- High speed centrifuge (3'000 x g)
- 2 µm glass fiber pre-filter (AP2007500)
- SteriCup filter 0.22 µm (SCGVU02RE)
- Vacuum pump
- Tweezers (flame sterilized)
- Sterile 1.5 mL plastic tube
- Sterile graduate disposable pipettes
- Micropipettes and filter tips
- ultrapure water
- Ethanol
- RNase AWAY
- MHV or Sendai virus viral stock (approximately 10^6 gc/mL)

Method

A. Preparation

1. Add a with Ethanol and RNase AWAY cleaned magnetic stir bar into a 250 mL glass bottle. Shake the wastewater sample to mix it. Add 50 mL of the sample to the glass bottle.
2. Pre-condition the Centricon Plus-70 Ultrafilter by adding 50 mL of ultrapure water. Centrifuge the ultrafilter for 15 min at 3000 x g.

B. Spiking with control process virus

1. Add 250 µL of MHV and 500 µL of Sendai virus viral stocks to the 50 mL sample.
2. Let the sample stir for 20 min at room temperature (ca. 200 rpm).

C. Isolation of viral particles by ultrafiltration

1. The mixed sample of 50 mL raw sewage is either:
    a. Pre-filtered by using a glass fibre 2 µm filter that was placed over the SteriCup filter 0.22 µm with the help of sterile tweezers. When pouring the sample, a magnetic stir bar retriever should be held against the glass bottle to make sure the magnetic stir bar remains in the bottle. Rinse the empty glass bottle (including the magnetic stir bar) and then the 2 µm glass fibre pre-filter with 10 mL ultrapure water and filter it. Keep the filtrate.
    b. Centrifuged for 20 min (10'000 x g) to remove large particles. Decant supernatant into pre-conditioned Centricon-Plus-70 Ultrafilter
2. Place the filtrate (1a) or supernatant (1b) into the pre-conditioned Centricon Plus-70 Ultrafilter.
3. Centrifuge the ultrafilter at 3'000 x g for 30 min. Discard the filtrate and proceed with step 5.
4. To elute the viruses, invert the concentrate cup from the ultrafilter and centrifuge at 1'000 x g for 3 min.
5. Approximately 150 to 300 µL of viral concentrate should be recovered. This is carefully pipetted into a 1.5 mL plastic tube.
6. Keep the viral concentrate on ice at 4° C for subsequent extraction or freeze at -80°C for later use.

Sample codification and labelling

Samples should be labelled following the format:
(WWTP code) _ year (XXXX) _ month (XX) _ day (xx)

Internal code for WWTP are provided in Table 1:

01_	Vacallo/Chiasso
02_	Rancate
03_	Barbengo/Lugano
04_	Croglio/Purasca
05_	Bioggio
06_	Foce Ticino/Gordola
07_	Giubiasco
08_	Biasca
09_	Foce Maggia
10_	Zürich
11.1_	Kloten+Flughafen (KF)
11.2_	Kloten (K)
12_	Lausanne

i.e.: A sample from Lausanne collected the 4rd of March 2020 would be 12_2020_03_04

Version History

Version	Author	Date	Changes
1.0	Anina Kull	2020-10-05	
2.0	All	2020-10-09	Added centrifugation as pre-conditioning step