

ULTRAFILTRATION SOP FOR RAW SEW AGE CORONAVIRUS PROJECT

Description

The aim of the protocol is to concentrate viruses present in 70 mL of sewage into a final volume of 150-300 μ L. 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

- Sterile 50 mL plastic tube (e.g. BD Falcon)
- Millipore-Sigma Centricon Plus-70 Ultrafilter (UFC701008)
- High speed swinging-bucket centrifuge (~4200 x g)
- Sterile 5 mL plastic tube
- Sterile serological pipettes (50 or 25 mL)
- Micropipettes and filter tips
- Ultrapure water
- 70% Ethanol
- Murine Hepatitis Virus (MHV) viral stock (approximately 10^6 gc/mL)

Method

A. Preparation

1. Pre-condition the Centricon Plus-70 Ultrafilter by adding 50 mL of ultrapure water. Centrifuge the ultrafilter for 15 min at 3000 x g.
2. Aliquot 2 x 40 mL of each wastewater sample to 2 x 50 mL tubes, for a total volume of 80 mL across two tubes.

B. Spiking with control process virus

1. Add 800 μ L of MHV viral stock (10^6 gc/mL) to all 40 mL tubes designated as MHV control samples (1600 μ L per sample).
2. Shake the designated MHV control samples at room temperature at 220 rpm on an orbital shaker for 20 minutes.

C. Isolation of viral particles by ultrafiltration

1. The mixed sample of 2 x 40 mL raw sewage is centrifuged for 30 min (~4200 x g) to remove large particles.
2. Using a serological pipette, remove 34 mL from each pair of tubes, carefully so as not to disturb the pellet, and dispense into a pre-conditioned Centricon Plus-70 Ultrafilter, such that each ultrafilter contains 68 mL of wastewater.
3. Centrifuge the ultrafilter at 3000 x g for 30 min. Discard the filtrate and proceed with step 4.
4. To elute the viral concentrate, invert the concentrate cup from the ultrafilter and centrifuge at 1000 x g for 3 min.
5. Approximately 150 to 280 µL of viral concentrate should be recovered. This is carefully pipetted into a 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_	Locarno
10_	Zürich
11.1_	Kloten+Flughafen (KF)
11.2_	Kloten (K)
12_	Lausanne
13_	Lenzburg
14_	Bern
15_	Basel
16_	Genf
17_	Chur
18_	Luzern
19_	Altenrhein
20_	Schaffhausen
21_	Freienbach
22_	Fribourg
23_	Ergolz 1
24_	Verbier
25_	Laupen

e.g.: A sample from Lausanne collected the 4th 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
2.1	Anina Kull	2021-02-11	Removed filtration by using SteriCup
3.0b	A.J. Devaux	2021-03-12	Beta protocol removing glass bottles + stirrers
3.0	A.J. Devaux	2021-03-15	Incorporated beta protocol changes. Increase volume of concentrated wastewater to 70 mL.
3.1	A.J. Devaux	2021-03-19	Decreased Centricon loading volume from 70 to 68 mL