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Wastewater grab sample processing with PEG-8000 precipitation

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ES_multipathogen

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Protocol status: Working
We use this protocol and it's working

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Abstract

PEG (Polyethylene Glycol) is a chemically inert, nontoxic, water-soluble synthetic polymer and has been used in aqueous polymer two-phase systems that help concentrating and isolating viruses from a variety of environmental samples. PEG is known as a good inductor of attractive interactions that crystallize viruses in the interpolymer spaces between PEG molecules. So, the combination of PEG concentration and RNA extraction steps enable 900– 1500X concentration of wastewater samples and sufficiently eliminate most of the organic matter, which could inhibit the subsequent qPCR assay.

Materials

1. PEG 8000 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**2139-1KG
2. Sodium Chloride Fisher BioReagents™ **Fisher Scientific Catalog #**BP358-1
3. 1X PBS (Phosphate-buffered saline)
4. Ethanol Absolute Honeywell Catalog #02875
5. Whirl-Pak® Sample Bag Merck Catalog #WPB01027WA-500EA
6. Nalgene® centrifuge bottles, Style 3122 Merck Catalog #B0408-4EA
7. SPINIX™ – Vortex Shaker Tarsons Catalog #3020
8. Eppendorf® Centrifuge 5910 R Merck Catalog #EP5943000246
9. Nalgene Centrifuge Bottle Sealing Cap Assembly Catalog number: DS3131-0038.

Sample

1 Wastewater grab samples

Procedural steps - PEG 8000 concentration method: 2h 15m

2 Collect 350 mL of sewage samples in a sterile Whirl-Pak bag and disinfect the sampling bag with ethanol to avoid contamination.

3 Transfer the bag to the laboratory in a cold chain 4 °C .

4 The sewage sample is transferred to a sterile 250 mL Nalgene centrifuge bottle.

5 Add 25 g of PEG 8000 and 5.6 g of NaCl in 250 mL of the sample to get the final concentrations of 10 % (w/v) and [M] 0.3 Molarity (M) respectively.

6 Vortex the mixture at 500 rpm for 00:15:00 until the reagents are completely dissolved in the suspension. 15m

7 Thereafter, seal the Nalgene bottles using the cap assembly, and centrifuge the mixture at 12000 x g, 02:00:00 until the pellet is visibly seen. 2h

8 Resuspend the pellet in 3 mL of PBS (pH 7.4). Then, aliquot 1 mL of the suspension into each of 3 screw cap tubes.

9 The resulting pellet is stored at -20 °C until further process.

Freeze-thawing of primary concentrates is limited to once.

Protocol references

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2. Kumar M, Patel AK, Shah AV, Raval J, Rajpara N, Joshi M, et al. First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2. Science of The Total Environment. 2020 Dec 1;746:141326