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Virus Concentration from Wastewater Using PEG Precipitation and Ultracentrifugation

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GenomeTrakr

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This method was developed at the FDA's Center for Food Safety and Applied Nutrition for GenomeTrakr's pandemic response project, monitoring SARS-CoV-2 variants in wastewater. Protocols developed for this project cover wastewater collection, concentration, RNA extraction, RT-qPCR detection, library prep, genome sequencing, quality control checks, and data submission to NCBI. This method describes the rapid concentration of intact viruses from wastewater using a combination of PEG precipitation and ultracentrifugation.

DOI

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GenomeTrakr, wastewater, SARS-CoV-2, virus, concentration, PEG precipitation, ultracentrifugation

_____ protocol,

Sep 15, 2021

Dec 02, 2021

Sep 15, 2021 Ruth Timme US Food and Drug Administration

Sep 23, 2021 Jessica Jones US Food and Drug Administration



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EQUIPMENT AND SUPPLIES:

- 1. Biological Safety Cabinet (BSC Type A2 or higher air exchange rate)
- 2. Nalgene PPCO 1L centrifuge bottles (Fisher Scientific, 05-562-25, or equivalent)
- 3. Adjustable Calibrated Micropipettor (1000 µl)
- 4. Hype-Wipe Disinfecting Towelettes (Fisher Scientific 14-412-56 or equivalent)
- 5. DNase/RNase-free microcentrifuge tubes 2.0 mL, non-stick, low retention, siliconized (Life Technologies AM12475)
- 6. Filter barrier aerosol resistant micropipettor tips DNase/RNase free (0.2 1000 µl)
- 7. Centrifuge capable of speeds of ≥12,000 x g (e.g., ThermoFisher RC6), and with rotors capable of holding 50ml conical tubes (ThermoFisher Scientific, 36-101-0816, or equivalent)
- 8. 50ml conical tubes (Fisher Scientific, 14-959-49A).
- 9. 100mL disposable graduated pipettes (Fisher Scientific, 03-395-177, or equivalent)
- 10. 50ml disposable graduated pipettes (Fisher Scientific, 13-678-11F, or equivalent)
- 11. FY14 50 carbon fiber rotor (ThermoFisher Scientific, 46922, or equivalent)
- 12. Ultracentrifuge capable of speeds of \geq 170,000 x g (e.g., Sorvall WX90) and with rotors capable of holding 70ml ultracentrifuge tubes (ThermoFisher Scientific, 75000100, or equivalent)
- 13. Refrigerated incubator shaker (Eppendorf New Brunswick™ I24R shaker M1344-0014 or equivalent)
- 14. Refrigerated incubator shaker platform (Eppendorf, M1250-9902, or equivalent)
- 15. Refrigerated incubator shaker clamps with springs (Eppendorf, ACSB-500S, or equivalent)
- 16. Carbon Fiber Rotor F40L, 8 x 100 ml:(Thermo Scientific, 096-087057, or equivalent)
- 17. Carbon Fiber ROTOR SS F10-4X1000Y LEX (Thermo Scientific, 09-604-1053, or equivalent)
- 18. 70 mL Polycarbonate ultracentrifuge tubes w/aluminum cap tubes for F40L rotor (Fisher Scientific, 010-1333, or equivalent)
- 19. Vortex mixer (Labsource, S16-200, or equivalent)
- 20. Mettler Toledo™ NewClassic ME Precision Balance, 2200g (Fisher Scientific, 01-912-408, or equivalent)
- 21. Disposable transfer pipettes (Fisher Scientific, 13-711-22, or equivalent)

MEDIA AND REAGENTS:

⊠ Glycine **Sigma** 1. Aldrich Catalog #G7126 , or equivalent **⊠** Guanidine Isothiocyanate **Thermo** 2. Fisher Catalog #15535016 , or equivalent ⋈ Hydrochloric Acid (HCI) Fisher 3. Scientific Catalog #6000710 , or equivalent 4. Technologies Catalog #AM9937 , or equivalent Sodium Chloride (NaCl) Sigma 5. Aldrich Catalog #S3014 , or equivalent **⊠** Potassium Chloride **Sigma** 6. Aldrich Catalog #P9541 , or equivalent



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7. Aldrich Catalog #P9791
                                                              , or equivalent
  Sodium Phosphate dibasic anhydrous (Na2HPO4) Sigma
8. Aldrich Catalog #S5011
                                                                     , or
equivalent
  9. Scientific Catalog #AM9856
                                                                , or equivalent
   ⊠ EDTA (0.5 M) pH 8.0 RNase-free Thermo Fisher
10. Scientific Catalog #AM9261
                                                                   , or
equivalent
   ⊠ 10X Phosphate Buffered Saline Sigma
11. Aldrich Catalog #P5493
                                                        , or equivalent

    □ PEG 20000 Sigma

12. Aldrich Catalog #81275-1KG
                                             , or equivalent
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Appropriate BSL-2 controls should be used when handling and processing wastewater samples. All steps for the concentration of viruses from wastewater should be completed in a BSC Type A2 or higher.



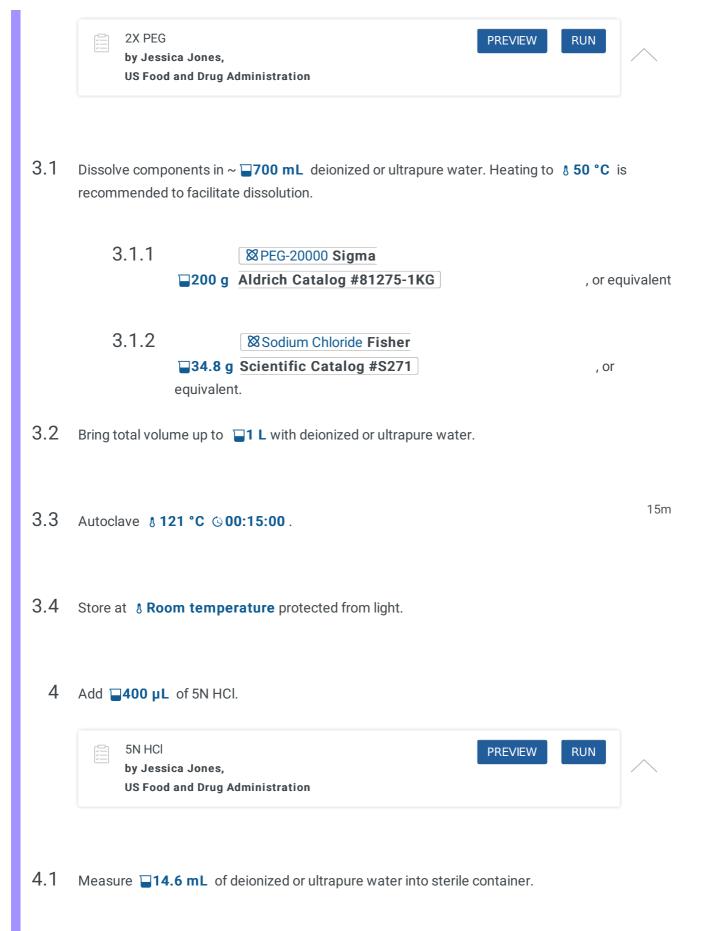
If sample is frozen, thaw under refrigeration (§ 2-8 °C). For an 800 mL sample, thawing may take up to five (5) days.

To preserve RNA viruses, samples should be kept on ice whenever possible during the process. To avoid any RNA degradation, sterile glassware and DNase/RNase-free microcentrifuge tubes should be used.

Virus concentration 30m

- 1 Place **■800 mL** of wastewater into an 1L centrifuge bottle.
- 2 Add **200** μL of extraction control (concentration of 10² per mL), as described here: <u>Preparation of Murine Norovirus for Use as an Extraction Control for Concentration of Viruses from Wastewater (protocols.io)</u>.
- 3 Add \square 40 mL of 2X PEG.





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4.2

⊠ Hydrochloric Acid (HCl) Fisher

Slowly add 10.4 mL Scientific Catalog #6000710 equivalent to the water.

, or



This solution should be prepared under a chemical fume hood.

- 4.3 Store at & Room temperature.
 - 5 Shake briefly and place bottles in refrigerated shaker at \$\triangle\$100 rpm, 6-8°C for \$\igottime\$02:00:00.
 - 6 Centrifuge **8000 x g, 2-6°C, 00:30:00**.

30m

For ThermoFisher RC6, set acceleration at 7 and deceleration at 3, or equivalent speeds for other models.

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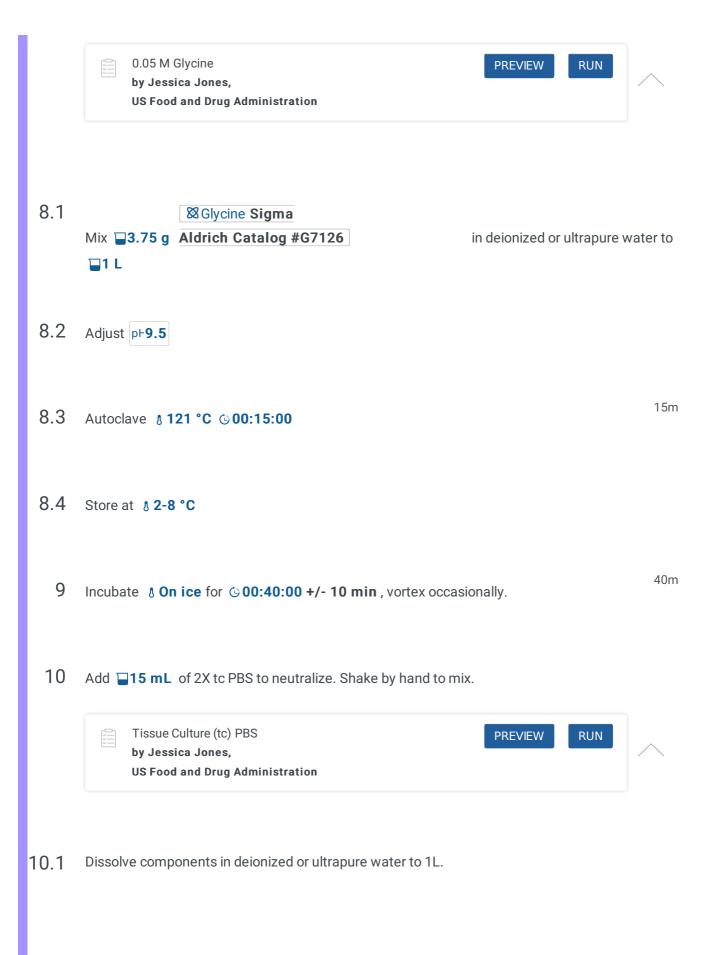


Carefully pipette supernatant and discard, care should be taken not to disrupt the pellet.

Up to **15 mL** of residual supernatant may remain, it is not necessary to remove this volume and risk disrupting the pellet.

8 Resuspend pellet in **15 mL** of 0.05M glycine and transfer entire volume to a 50 ml conical tube.

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10.1.1

    Sodium Chloride (NaCl) Sigma

                       ■8.0 g Aldrich Catalog #S3014
            10.1.2
                               ⊠ Potassium Chloride Sigma
                       ■0.2 g Aldrich Catalog #P9541
            10.1.3 □0.12 g

    ⊠ Potassium Phosphate dibasic (KH2PO4) Sigma

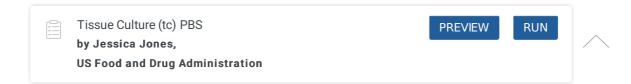
                       Aldrich Catalog #P9791
            10.1.4 □0.91 g
                       Sodium Phosphate dibasic anhydrous (Na2HPO4) Sigma
                       Aldrich Catalog #S5011
 10.2 Adjust p⊦7.5
                                                                                         15m
 10.3 Autoclave § 121 °C © 00:15:00
 10.4 Store at § 2-8 °C
           For 2X tc PBS, double components in 1.1 - 1.4
 10.5
           Alternatively, 1X and 2X PBS can be made by dilution of
            ⊠10X PBS Sigma
           Aldrich Catalog #P5493
                                                      into sterile deionized or ultrapure water.
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11 Centrifuge at **3000** x g, 2-6°C, 00:15:00.

15m

- 12 Transfer supernatant to clean ultracentrifuge tube (careful not to disturb pellet).
- Bring total volume up to **65 mL** or **125 g** total weight (includes bottle and cap) with addition of 1X tc PBS.



13.1 Dissolve components in deionized or ultrapure water to 1L.

13.1.4 **□0.91** g

Sodium Phosphate dibasic anhydrous (Na2HPO4) Sigma

Aldrich Catalog #S5011

13.2 Adjust p**⊦7.5**

13.3 Autoclave § 121 °C © 00:15:00

15m

13.4 Store at 8 2-8 °C

For 2X tc PBS, double components in 1.1 - 1.4

13.5

Alternatively, 1X and 2X PBS can be made by dilution of

⊠10X PBS **Sigma**

Aldrich Catalog #P5493

into sterile deionized or ultrapure water.

- 14 Balance tubes to within $\bigcirc 0.05$ g of each other using 1X tc PBS.
- 15 Centrifuge at **(3)170000 x g, 2-6°C, 00:45:00**.

45m

Minimum volume for ultracentrifugation using Fiberlite rotor and tubes is 50 mL.

- 16 Discard supernatant and resuspend pellet in $\blacksquare 800 \, \mu L$ of 1X tc PBS.
- 17 Evenly distribute sample into four 2.0 ml microcentrifuge tubes.
- Store concentrates from Step 17 at & -70 °C or proceed directly to RNA Extraction from Wastewater Concentrates Using RNeasy and Zymo Kits (protocols.io).

Only 1 tube is required for RNA extraction. The remaining tubes should be stored at 8 -70 °C as reserve.