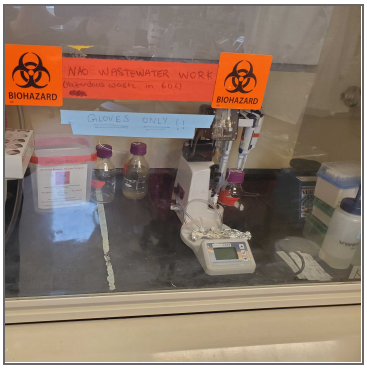

VERSION 1

JAN 08, 2024

OPEN ACCESS

External link:
https://naobservatory.org

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MANUSCRIPT CITATION:

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

Concentration and nucleic acid extraction of viruses from wastewater influent V.1

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ABSTRACT

In this protocol, 200 mL of raw influent wastewater is concentrated to a final volume of 400 uL using the Innovaprep Concentrating Pipette (CP Select). Prior to concentration, the wastewater sample is treated with Tween 20 and sonicated to dissociate viral particles from solids in the wastewater matrix. The sample is then centrifuged to remove larger solids. The pellet is discarded, and the supernatant is filtered with a 0.45 um PES 75 mm filtration unit to remove remaining suspended solids and bacteria. This filtrate is then concentrated with the CP Select, using the Ultrafiltration CPT and the recommended device settings for the Innovaprep modified wastewater processing protocol. Nucleic acids are then extracted from the concentrated product using the Zymo quick-DNA/RNA Viral kit using the manufacturer protocol with a few modifications that we have found to be helpful.

This is an in-progress draft of a complete working protocol that we are currently transferring into protocols.io.

GUIDELINES

- Status: mostly-final SOP, under review, awaiting results from experiments during the week prior to 2023-10-23
- One aliquot will be prepared for concentration and nucleic acid extraction for each sample type (North influent and South influent) unless otherwise discussed.
- **RNA processing and handling**: Please review Protocol Note: Working with RNA Samples before handling RNA samples.

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Keywords: wastewater-based epidemiology, WBE, viral concentration, Innovaprep, metagenomics, viromics, wastewater

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MATERIALS

- Centrifuge
- Rotor with 50 mL tube slots
- Bransonic M 1800 Sonicator (filled ¾ with tap water)
- Vortex with 50 mL tube holder attachment
- Vacuum Line
- Pipettes (1000 uL, 200 uL, 10 uL, 2 uL, 1 uL) and holder
- Multimeter
- Innovaprep Concentrating Pipette
- Timer
- Ice bucket
- 10% Tween 20 stock solution
- 4 x 0.45um PES 75 mm vacuum filtration units
- 4 x pyrex bottles (at least 250 mL)
- Parafilm (Millipore Sigma No. HS234526C)
- 50mL (VWR No. 21008-178) and 5 mL Falcon tubes
- Filtered pipette tips
- 50 mL serological pipettes (VWR No. 170358N)
- RNaseZap
- Kimwipes (VWR No. 34120)
- PREempt wipes (VWR No. 10822-456)
- 1.5 mL microcentrifuge tubes (VWR No. 1420-2600)
- Innovaprep ultrafiltration CP Tips
- CP Select Elution buffer (canister is reused ~5 times for sampling runs)
- Paper towels
- 125 mL collection bottles (VWR No. 414004-124)
- Ice

SAFETY WARNINGS

Biosafety precautions: All raw wastewater and settled solids samples will be received and stored with primary and secondary containment. The primary container (the bottle or falcon tube) should remain in the secondary container (a Ziploc bag containing paper towels to absorb spills) until processed. All raw samples must be handled within a dedicated fume hood or biosafety cabinet. All laboratory personnel handling these samples must use safety glasses, gloves, and lab coats. Samples will be transported between processing stations within a secondary container that has been cleaned with PreEmpt. All surfaces (outside of the fume hood/sash, centrifuge lid and rotor, etc.) will also be wiped down with PreEmpt. Please review Protocol Note: Cleaning Autoclavable Bottles for steps on how to clean the autoclavable bottles after use.

BEFORE START INSTRUCTIONS

Prepare the fume hood for wastewater handling, gather materials and reagents (7 centrifuge tubes, serological pipettes, pipette tips, micropipettes, a marker, strips of parafilm, sterile-filtered 10% Tween 20, PBS). Label 7 fresh centrifuge tubes (five tubes for a replicate of influent, and two for the negative control). Ensure proper PPE.

Part 1: Influent Handling, Dissociation, Centrifugation, Filtrat...

1 In the fume hood, add 400 uL of 10% Tween 20 stock solution each to the seven centrifuge tubes.

2 Prepare the negative control. Add 40 mL of PBS to two of the centrifuge tubes. For each step in this protocol, handle the negative control tubes in the same manner as the influent sample tubes.

3 Adhering to safety standards for handling wastewater, bring influent (refrigerated, and in primary and secondary containment) into the fume hood. Prepare to aliquot the influent by removing the secondary containment and any parafilm affixed to the primary containment. If working with multiple samples, only bring one into the fume hood at once, to minimize risks of contamination.

4 Aliquot influent into centrifuge tubes. Invert the bottle of influent several times to resuspend and homogenize contents, then carefully open it. Using a fresh 50 mL serological pipette, aspirating from the bottom of the bottle, carefully dispense 40 mL into each of five centrifuge tubes.

5 Cap and parafilm all bottles and centrifuge tubes. Return the influent bottle to secondary containment, wipe exposed surfaces with pre-empt wipes, and return the influent to refrigeration.

6 Place all centrifuge tubes on a vortexer using a 50 mL tube adapter. Shake at ~1000 rpm for 1 min.

7 Sequentially transfer all centrifuge tubes from the vortexer to a sonication bath, and sonicate for 1 min at 40 kHz. Use a paper towel to dry the tubes when finished.

8 Adhering to safety standards for handling wastewater, transfer all centrifuge tubes from the fume hood to a centrifuge. Centrifuge the samples for 5 min at 10,000 x g at 4C. After centrifugation is complete, wait 10 minutes to allow aerosols to settle before opening the centrifuge. Remove samples from the centrifuge and return them to the fume hood.

9 In the fume hood, affix two vacuum filtration tops to two pyrex bottles. One will be for the five influent centrifuge tubes, and the other for the two negative control centrifuge tubes.

10 Decant the influent supernatant directly into a vacuum filtration top. Take care not to dislodge the pellet. Pellets can be caught using the centrifuge tube cap.

10.1 Repeat for all five influent centrifuge tubes in the same vacuum filtration top.

11 Begin vacuum filtration by capping the vacuum filtration top and opening the vacuum line. When complete, cap the pyrex bottle and set aside.

12 For the negative control centrifuge tubes, decant both directly into a vacuum filtration top. There should not be a pellet. Perform vacuum filtration as was done for the influent, cap the pyrex bottle, and set aside.

Part 2: Concentration via Innovaprep Concentrating Pipette S...

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Part 3: Nucleic Acid Extraction - Zymo quick-DNA/RNA Viral Kit

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Part 4 (Optional): Qubit measurements (DNA + RNA)

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