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Version 1

Processing wastewater samples for bacterial & viral targets enrichment V.1

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Protocol status: Working

We use this protocol and it's working

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Abstract

This wastewater sample processing protocol by the Wastewater Genomics Syndicate at the National Institute of Communicable Diseases (NICD) is designed to enrich bacteria and viruses from the settled solids and supernatant components of wastewater, as different pathogens partition differently within this matrix. The end products are waterless solids and clarified wastewater supernatants, ready for downstream applications such as ultrafiltration, bead enrichment and nucleic acid extraction.

Materials

Equipment

–80°C freezer to store settled solids samples

4°C refrigerator storage of samples

Biological safety cabinet (Class II)

Refrigerated centrifuge (4°C) with adapters to accommodate 500 mL centrifuge bottles

Consumables

2 mL cryo-tubes

500 mL Conical centrifuge bottles

Labels

Waste container

Plain wooden sticks

Solutions/Reagents

70% ethanol

1% Virkon



Sample Collection & Handling

23h

- 1 Using correct personal protective equipment (PPE), collect a 1,000 ml grab wastewater sample from each location in a sterile bottle and transfer them to the laboratory in a cold chain (keep in a lab fridge at 4°C). It is important to proceed to the next step within the next 23 hours after sample collection to prevent major degradation of microorganisms.

23h



Preparing for centrifugation

10m

- 2 All work should be performed in a Class II biological safety cabinet while wearing appropriate PPE. Begin by cleaning your workspace with a 1% Virkon solution, followed by a 70% ethanol solution. Double-line your waste bucket with biohazard waste plastic, and ensure all your filter tips are refilled. Finally, label 500 mL conical centrifuge bottles, one for each wastewater sample.

10m

Centrifugation

50m

- 3 Transfer 500 mL of the wastewater samples to the corresponding conical tubes
- 4 Centrifuge at 4 650 × g (deceleration speed set at 5), at 4°C for 10 minutes.
- 5 Carefully transfer the supernatant from the 500 mL conical bottle to a clean and marked 1,000 mL bottle without disturbing the settled solids.
- 6 Add the remaining 500 mL of the original wastewater sample to the conical bottle with settled solids and repeat step 4.
- 7 Carefully transfer the supernatants to 1,000 mL bottles in step 5, without disturbing the pellet. Keep at 4°C for different downstream applications (check our page for different nucleic acid extraction protocols from clarified wastewater)

5m

15m

5m

20m

5m

Transfer settled solids

20m

- 8 Use clean, plain wooden sticks to pick out settled solids and pack them into marked 2 mL cryo-tubes, aiming for approximately 250 mg per tube.
- 9 Store the settled solids in the -80°C freezer until needed for downstream applications. (check our page for different nucleic acid extraction protocols from settled solids)

15m

5m

