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Processing wastewater samples for bacterial & viral targets enrichment V.2

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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
250 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. Clean your workspace with a 1% Virkon solution and a 70% ethanol solution. Double-line your waste bucket with biohazard waste plastic, and ensure all your filter tips are refilled. Finally, label 250 mL conical centrifuge bottles, one for each wastewater sample.

10m

Centrifugation

50m

3 Transfer 250 mL of the wastewater samples to the corresponding conical tubes

5m

4 Centrifuge at 4 650 × g (deceleration speed set at 5), at 4^{O}C for 10 minutes.

15m

5 Carefully transfer the supernatant from the 250 mL conical bottle to a clean, marked 1 000 mL bottle without disturbing the settled solids.

5m

6 Repeat steps 3 to 5 until the original sample is finished.

20m

7 Keep the clarified wastewater sample at 4^{O}C for different downstream applications (check our page for different nucleic acid extraction protocols from clarified wastewater).

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. Centrifuge briefly to remove water.

15m

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).

5m