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# Processing wastewater samples for bacterial & viral targets enrichment

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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

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 500 mL conical bottle to a clean and marked 1,000 mL bottle without disturbing the settled solids.

5m

6 Add the remaining 500 mL of the original wastewater sample to the conical bottle with settled solids and repeat step 4.

20m

7 Carefully transfer the supernatants to 1,000 mL bottles in step 5, without disturbing the pellet. Keep 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, aiming for approximately 250 mg per tube.

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