May 07, 2025

Clarification of wastewater samples

DOI
https://dx.doi.org/10.17504/protocols.io.5jyl8qo99l2w/v1

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Wastewater Genomics S...

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Protocol Citation: Victor Mabasa, Natasha Singh, Emmanuel Phalane, Mokgaetji Macheke, Sipho Gwala, Thabo Mangena, Lethabo Monamets, Lebohang Rabotapi, Nkosenhle Ndlovu, Fiona Els, Sibonginkosi Maposa, Said Rachida, Kerrigan McCarthy, Mukhlid Yousif 2025. Clarification of wastewater samples. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5jyl8qo99l2w/v1

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

Created: May 07, 2025

Last Modified: May 07, 2025

Protocol Integer ID: 217828

Keywords: Wastewater solids, wastewater, NICD, South Africa, WBE, WBS, wastewater genomics syndicate, wastewater sample processing protocol, wastewater sample, clarification of wastewater sample, supernatant components of wastewater, wastewater supernatant, nucleic acid extraction, different pathogen, such as ultrafiltration, ultrafiltration, bacteria, extraction, virus, national institute of communicable disease, sample

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 or 500 mL Conical centrifuge bottles
Waterproof Labels
Waste container
Plain wooden sticks

Solutions/Reagents

70% ethanol
1% Virkon

Sample Collection & Handling

1 Wearing appropriate personal protective equipment (PPE), collect a 1 000 mL grab wastewater sample from each location using a sterile bottle. Transport the samples to the laboratory under cold chain conditions and store them at 4 °C. To minimise microbial degradation, proceed to the next step within 23 hours of sample collection.

Preparing for centrifugation

2 Conduct all work in a Class II biological safety cabinet while wearing appropriate PPE. Clean the workspace using 1% Virkon followed by 70% ethanol. Double-line the waste container with biohazard waste bags, ensure all filter tip racks are refilled, and label a 250 mL conical centrifuge bottle for each wastewater sample.

Centrifugation

3 Transfer each wastewater sample to its corresponding conical centrifuge bottle, taking care not to overfill to prevent spillage during centrifugation.

4 Centrifuge the samples at 4 700 × g for 10 minutes at 4 °C.

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

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

Storage

7 Aliquot 6 × 5 mL cryotubes of the clarified supernatant and store them at −80 °C for long-term preservation. Keep the remaining clarified wastewater at 4 °C for other downstream applications.

8 Using clean, plain wooden sticks, collect settled solids and transfer them into labelled 2 mL cryotubes. Briefly centrifuge to remove excess liquid, then store the tubes at −80 °C until needed for downstream applications.