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Wastewater sample collection, transportation and storage V.2

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Wastewater Surveillanc...



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We use this protocol and it's working

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Abstract

This SOP describes the procedures for the safe collection, transportation, handling, and storage of wastewater samples from wastewater collections sites such as sewer systems, and wastewater treatment plants to the laboratory. The aim is to ensure that samples are managed in a way that prevents significant changes in their composition between collection, storage, and analysis. This is critical to maintaining sample integrity and ensuring valid, reliable test results.

This SOP applies to wastewater sample collection by trained lab personnel and other authorised individuals.

Wastewater sample locations and frequencies of sample collection are specified according to each institute's permit.

Guidelines

All procedures can be performed by suitably trained members of staff.



Materials

- Sample containers – Must be sterile prior to collection and leak proof
- Cooler with ice packs or cubed wet ice in double-sealed plastic bags (e.g Ziploc bags)
- Absorbent shipping pad or paper towels
- Reusable insulated shipping cooler
- Packaging/boxes
- Alcohol or bleach wipes
- Alcohol resistant marker for labelling
- Temperature monitoring device (e.g Tinytag temperature data loggers)
- Sampling PPE (Waterproof gloves, Lab coat, Safety goggles / face shield, face mask, rubber boots)
- Disinfectant (e.g 70% ethanol or 10% bleach solution)
- Liquid hand soap
- Chain of custody form

Safety warnings

! Hazardous Material – Sample bottles may contain hazardous material. Personnel collecting the samples must handle and fill these bottles with care to avoid exposure to harmful fumes or direct contact with hazardous substances. Appropriate personal protective equipment (PPE) must be worn during sample collection.

Infectious agents – Wastewater contains pathogens, including viruses, bacteria and fungi. Appropriate safety precautions must be exercised when handling wastewater samples.

Leakage / Cracking - Containers showing any signs of leakage or cracking should be transferred to fresh tubes inside the MSCII or MSC1 cabinet in BSL2 or simply autoclaved and discarded.

Before start

All procedures can be performed by suitably trained members of staff



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Safety information

Hazardous Material – Sample bottles may contain hazardous material. Personnel collecting the samples must handle and fill these bottles with care to avoid exposure to harmful fumes or direct contact with hazardous substances. Appropriate personal protective equipment (PPE) must be worn during sample collection.

Infectious agents – Wastewater contains pathogens, including viruses, bacteria and fungi. Appropriate safety precautions must be exercised when handling wastewater samples.

Wastewater sample collection

- 2 Wastewater samples should be collected by trained operators or approved contractors, following the agreed-upon sampling procedures.
- 3 On the day of sample collection, the sample collector(s) must take a chain of custody form to document field activities from the time of collection through to arrival in the lab. This form must include:
 - a. Name of sample collector(s)
 - b. Unique sample identifier or log number
 - c. Date and time of sample collection
 - d. Sample information including:
 - Sample point name/location
 - Wastewater plant/system name
 - Sample source
 - Sample types (e.g., Grab, composite)
 - e. Date and time of sample transfer at each sample handoff with name and initials of the person receiving the sample
 - f. Comments (If any)

Note

Make sure to fill in the form 1 and 3 information on Redcap

- 4 Sample collectors must wear appropriate PPE (gloves, lab coat, goggles, face mask, rubber boots) when collecting samples.

**Note**

A clean pair of new, non-powdered disposable gloves must be worn each time a different location is sampled.
Avoid allowing the gloves to touch the sample. Ensure to replace gloves at any point during sampling if they become contaminated or otherwise compromised.

- 5 Collect a minimum sample volume of 250 mL / 0.25 L.

5.1 Example of how to collect the wastewater**Note**

Ensure to collect the sample from a well-mixed section of the wastewater. This is typically near the center of the flow channel at approximately 40–60% of the water depth, where turbulence is at a maximum and the likelihood of solids settling is minimised.
Avoid skimming the water surface or disturbing the channel bottom. However, allow for fluctuations in water depth due to flow variations

- a. Attach the sample bottle to the sampling stick
 - b. Immerse the bottle in the wastewater source
 - c. Discard the water into the sample bottle
 - d. Repeat this process until at least 250 mL of wastewater has been collected in the sample bottle
 - e. Close the sample bottle tightly.
- 6 Check that the sample bottle caps are tightly secured and will not leak.
- 7 After filling the sample bottles and ensuring that the bottle caps are tightly secured, wipe the outside of the bottles with 70% ethanol or 10% bleach solution
- 8 Clearly label the sample bottles with alcohol resistant marker using a unique sample identifier followed by year, month and day (YYYY-MM-DD)

Note

Apply the label to the sample bottle, not to the sample bottle cap.



- 9 Place the bottles inside a bag and seal / tie the bag.
- 10 Place the sample in a cooler with ice packs / or polystyrene shipping box, with ice packs. This ensures sample preservation during transportation to the lab.

Note

Recommend using synthetic ice packs (e.g freezer gel packs) or using ice that is kept in watertight containers to prevent the melted ice from destroying sample labels.

- 11 Place a temperature monitoring device (e.g., Tingtag temperature data logger) inside the cooler or polystyrene shipping box. This is to verify that the samples remain within the required temperature range (at or below 8°C, but above freezing) throughout shipment / transportation to the lab.
- 12 Fill the remaining space with absorbent material (paper towels, Styrofoam, or newspapers) to prevent samples from shifting or moving during transportation.

Note

Secure samples in a manner to prevent breakage and/or leakage during transport / shipping.

- 13 Fill out / complete the chain of custody from your participating lab and place the form in a watertight container (zip or press-lock poly bags) to prevent it from damage during shipping.

Note

All documentation for a sampling event (e.g bottle labels, chain of custody form) must be consistent in recording the metadata for a sampling site, (e.g location, date, time, sample identifier, and parameters sampled).

- 14 Label the outside of the shipping container with a current return address and contact person, and a current laboratory address and contact person.

Note

Make sure contact information is current



Recommendation for transportation of raw wastewater

- 15 The samples must be shipped to the lab immediately after collection. Analysis should start as soon as possible to ensure that the results accurately reflect the original sample conditions.
- 16 Ship / transfer the samples in insulated containers with ice packs or ice in a cooler (4°C) from the field to the lab.

Receiving wastewater samples in the lab

- 17 Upon arrival at the lab, make sure to unpack the box containing the samples in the Biosafety level 2 (BSL2) or equivalent within a BSC type II/A2.
- 18 Once samples arrived at the laboratory, check the temperature recorded by the temperature monitoring device (e.g., Tingtag temperature data logger) to confirm that the samples were maintained within the required temperature range during transport.
- 18.1 **How to perform temperature check of a Tingtag temperature data logger**
 - a. Retrieve the temperature logger from the shipping container
 - b. Connect the logger to a computer or reader
 - c. Download and review the recorded temperature data
 - d. Verify that the temperatures stayed within the acceptable range (at or below 8°C, but above freezing)
 - e. Document the results in the sample receipt
- 19 Wipe down the containers with 70% ethanol or 10% bleach

Safety information

Leakage / Cracking - Containers showing any signs of leakage or cracking should be transferred to fresh tubes inside a biological safety cabinet (BSC type II/A2) in BSL2 or simply autoclaved and discarded.

- 20 If samples are to be processed at a later stage, make aliquots of the samples and store the samples immediately (see section: Wastewater sample storage).

Wastewater sample storage

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**Note**

Aliquot samples before freezing at -80°C to avoid repeated thawing which affects sample integrity. Aliquoting of the samples must be done in the BSL2 or equivalent within a BSC type II/A2.

- 22 Mix the wastewater sample thoroughly by inverting the container several times.
- 23 Prepare at least six aliquots by transferring the sample into 50 mL falcon tubes, filling each tube to 40 mL
- 24 Seal the cap of each tube tightly with parafilm to prevent leaks or contamination.
- 25 Immediately transfer the sealed tubes containing the wastewater in ULT freezers at -70°C or -80°C freezer for storage until ready for processing. Keep two of the six 40 mL aliquots for archiving purposes. Store the tubes in upright position

Note

Ensure to keep the samples undisturbed to preserve the integrity of the sample until ready for processing.

Protocol references

1. Gordon Webster, Shrinivas Nivrutti Dighe, William B. Perry, Ewan H. Stenhouse, Davey L. Jones, Peter Kille, Andrew J. Weightman. Wastewater sample storage for physicochemical and microbiological analysis, Journal of Virological Methods, Volume 332, 2025,115063, ISSN 0166-0934, <https://doi.org/10.1016/j.jviromet.2024.115063>. (<https://www.sciencedirect.com/science/article/pii/S0166093424001885>.)