



Wastewater Sampling and Transport from Remote Communities

This SOP provides guidance for the collection and transport of wastewater samples from remote communities for monitoring the genetic signal of SARS-CoV-2 in the community. Collected samples are forwarded to the National Microbiology Laboratory of Canada for analysis. A onetime questionnaire is provided to collect essential data about the wastewater network and community.

Safety:

Sewage sampling exposes you to sewage, which is contaminated with pathogenic microorganisms, including SARS-CoV-2. Adhere to Canadian Occupational Health and Safety regulations and guidelines, and wear personal protective equipment (PPE) including disposable gloves, safety glasses, masks or face shields. Also, follow any site-specific safety requirements (e.g. hard hat, high-visibility vest, steel-toe boots).

Sampling point:

The goal of the work is to draw a representative sample of wastewater from the community; therefore, choice of the sampling point is critical. Ideally, the sampling should be conducted at a central collection point, such as a lift station, influent wet well, or influent splitter box. The sampling site should not be stagnant in nature. Best practices for collection and analysis of wastewater from suction/sewage trucks and not well-established, and are not detailed in this document. h. Generally, trucks may be sampled provided their source can be attributed to one community and sampling can be performed safely, care must be taken to limit the carry over viral material from load-to-load. Additionally, septic tanks may be persistently positive for SARS-CoV-2 genetic material and obscure subsequent epidemiological investigation.

Sample collection:

Ideally, samples are flow-proportional or equal volume composite samples drawn over the course of 24 hours using a refrigerated autosampler. If local conditions and equipment do not allow for a composite sample, then grab sampling can be used as an alternative. If drawing grab samples from flowing wastewater, collect a series of grab samples over a period of time (e.g. every 10 minutes for 1 hour),



roughly 10 L in total, mix well and submit a sub-sample for analysis. Record all details of sample collection methods and timing.

If samples cannot be shipped shortly after collection, samples can be stored in a refrigerator. Please ensure that samples are always kept refrigerated or on ice, and arrive at the laboratory no more than 7 days after collection.

At a minimum, we are requesting that an estimate of the total daily flow be recorded on the provided testing requisition form. If total daily flow data is available to you, please submit it with the sample; or estimate the flow if possible (above average, average, or below average). If drawing samples from a collection truck, record the date, time, total volume in the truck and number of sites collected.

Materials:

- Sampling protocol (This document)
- Personal Protective Equipment (see Safety)
- Sampling and shipping materials
 - A: Telescopic rod with pitcher for grab sampling (can be extended up to 9 ft.) or rope and bucket (not pictured)
 - B: Insulated shipping box with outer cardboard box
 - C: Sampling bottle with label
 - D: Bottle seal, parafilm strip or tape for sealing cap to bottle
 - E: Funnel
 - F: Re-sealable bags
 - G: Absorbent pads
 - H: Flat frozen cold packs
 - I: Bubble wrap, optional
 - J: Brown Kraft packing paper, used for void fill as necessary
 - K: Permanent markers
 - L: Packing tape
- 20 L Bucket
- Freezer to freeze cold packs flat
- Refrigerator to store Wastewater samples post-collection



- Wastewater network questionnaire and testing requisition form



Figure 1 – Examples of sampling and shipping materials



Sampling frequency:

Please sample on a Monday and arrange for shipment immediately so that the sample arrives at the NML by Friday. If samples cannot be shipped immediately, keep in refrigerator until able to ship.

Sample collection:

1. **Freeze cold packs flat the several days before sampling.**
2. On the day of sampling, label bottles with a permanent maker indicating:
 - a. Sample location
 - b. Date/time
3. Wear Personal Protective Equipment (PPE) as per the sampler's training and/or existing community protocols and policies.
4. Remove the sampling bottle lid cover, and protect from contamination.
5. Fill sampling bottles:
 - a. Autosampler: From the 24 h composite, refrigerated autosampler (WWTP influent). Composite samplers need to purge following the collection of each discrete sample to preclude accumulation of solids in sampler tubing. Composite samples should be refrigerated during collection and storage.
 - i. If your autosampler collects individual fractions in separate tubes, then combine sample fractions into a bucket, close lid and **mix well**. It is crucial that suspended solids are thoroughly mixed and included in the sample. Fill supplied bottle to the 100 mL marking on the bottle. Do not fill bottles completely, in order to prevent issues upon freezing/storage.
 - ii. If your autosampler collects a single large fraction, then gently swirl the sampling vessel and **mix well**. It is crucial that suspended solids are thoroughly mixed and included in the sample. Fill supplied bottle to just below the shoulder of the bottle leaving a small air gap. Do not fill bottles completely, in order to prevent issues upon freezing/storage.



- b. Grab sample from flow. Into a bucket, draw samples of approximately the same volume from the flowing wastewater every 10 minutes for an hour, seal lid and mix by swirling the bucket. The total volume should be approximately 10 L. **Mix the sample well**, ensuring suspended solids are thoroughly mixed, and draw a sub-sample by filling the supplied bottle to just below the shoulder of the bottle leaving a small air gap. Do not fill bottles completely, in order to prevent damage of the bottles upon freezing/storage.
- c. Grab sample from lift stations. Using the bucket attached to the telescopic rod, draw samples of approximately the same volume every 10 minutes for an hour, acquiring a total of approximately 10 L and **mix well**. Ensure that suspended solids are thoroughly mixed and included in the sample. With the aid of a funnel (narrow mouth bottles), fill supplied bottle to just below the shoulder of the bottle leaving a small air gap (see Figure 2).



Figure 2 - Bottle filled with the aid of a funnel

6. Place sampling bottle cap back onto bottle and firmly hand tighten cap closed.



Sample packing for shipping:

Ship samples as per site-specific logistics plan developed for you. Always ensure that the containers remain in an upright position to prevent leakage of sample.

1. A pre-affixed seal is provided with each bottle. Remove the seal and reaffix at the neck of the bottle in a clockwise direction, i.e. same direction as tightening the cap. If Parafilm is used to seal, place the parafilm so that it cover part of the lid and bottle, then stretch and wrap the lid in a clockwise direction (see Figure 4).

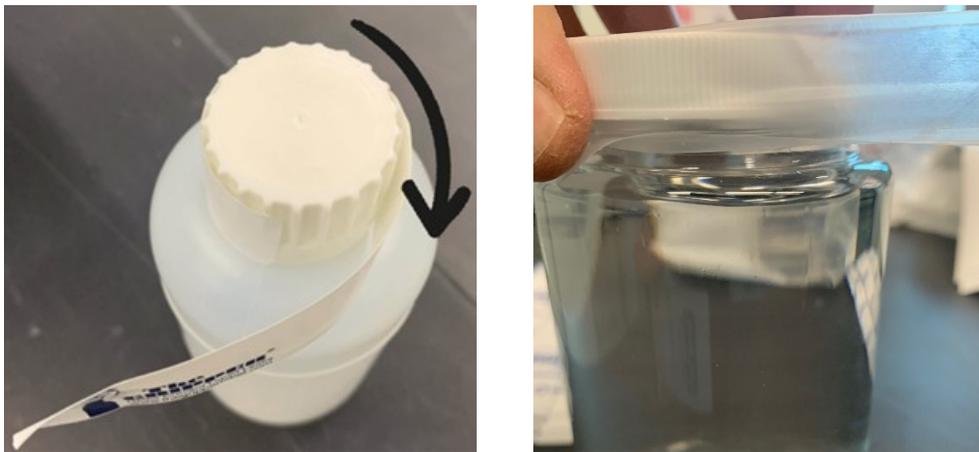


Figure 4 – Example of samples being sealed using bottle cap seal and parafilm

2. Place the filled bottle into the clear re-sealable leak-proof bag containing the absorbent material. Seal the bag while expressing as much air as possible (see Figure 5).



Figure 5 – Examples of samples ready for packing



3. Bubble wrap may be used to help keep the sample upright during shipment, preventing possible leakage (see Figure 6).



Figure 6 - Bubble wrapping of sample pack

4. Place the flat frozen cold packs inside the lining of the insulated shipping box with one ice pack on each side. Then place the bubble wrapped bottle, upright, in the middle of the box (as shown in Figure 7).



Figure 7 - Sample in cooler



5. Kraft paper may also be used to help stabilize and minimize movement during shipment.
(see Figure 8).



Figure 8 – Examples of brown Kraft paper used for stabilization of sample during transport



6. Apply the lid of the insulated shipping box to the top of the box. Place the completed testing requisition form on top of the closed shipping cooler (see Figure 9).



Figure 9- Proper placement of form

7. Once all contents are packaged in the shipping box, the outer cardboard box can be closed by folding down the side flaps first, followed by the other two flaps.
8. Seal the cardboard box tightly with packing tape by placing the tape along the edge where the flaps meet, sealing halfway down both sides of the box (see Figure 10).



Figure 10- Sealing of sample package



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Clean-up of sampling material:

Using water, rinse all sampling material used including buckets, grab samplers, and funnels before disinfecting. Materials may be disinfected using a dilute bleach solution. Follow the directions on the bleach bottle for preparing a diluted bleach solution. If your bottle does not have directions, you can make a bleach solution by mixing: 100ml bleach and 900ml water. Contaminated items should remain wet with the bleach solution for at least 1 min before rinsing again with water. Hands should be washed with soap and water after sampling and after material clean up.

Shipping:

Arrange for shipping to the address below using the fastest shipping method available (e.g. priority overnight, express, etc.).

ATTN: Dr. Chand S. Mangat
Public Health Agency of Canada
JCWIDRC rm J1103
1015 Arlington St
Winnipeg MB, R3E 3R2

Tele: 204-789-6508

Should you have any questions regarding the sampling protocol, please contact: chand.mangat@phac-aspc.gc.ca

Thank you for your participation in this study.