



Wastewater Sampling and Transport from 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.

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 community health surveillance 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 are not well-established, and are not detailed in this document. 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. Ideally, grab sampling is to be conducted between 11am and 1pm. Record all details of sample collection methods and timing.



Alternatively, passive sampling may be used in situations where it is not feasible to use an autosampler. Passive samplers use absorbent material to collect viral particles from wastewater. Passive sampling is a low resource method to offer targeted monitoring of sub-sewersheds such as neighbourhoods or congregate living centres e.g. long term care facilities or dormitories.

If samples cannot be shipped shortly after collection or are being kept for batch shipping, samples may 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, or the flow through the sampling point over the collection period 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
 - o A: Telescopic rod with pitcher for grab sampling (can be extended up to 9 ft.) or rope and bucket (not pictured)
 - o B: Insulated shipping box with outer cardboard box
 - o C: Sampling bottle with label
 - o D: Bottle seal, parafilm strip or tape for sealing cap to bottle
 - o E: Funnel
 - o F: Re-sealable bags
 - o G: Absorbent pads
 - o H: Flat frozen cold packs
 - o I: Bubble wrap, optional
 - o J: Brown Kraft packing paper, used for void fill as necessary
 - o K: Permanent markers
 - o L: Packing tape
- 20 L Bucket



- Freezer to freeze cold packs flat
- Refrigerator to store Wastewater samples post-collection

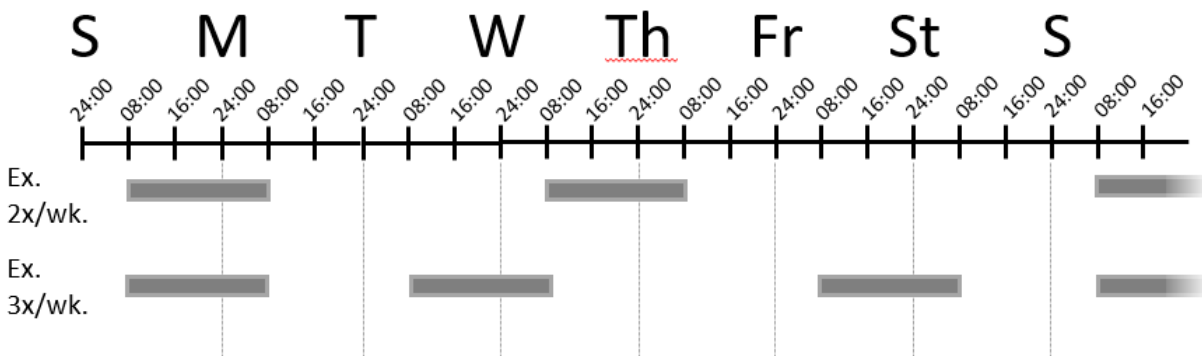


Figure 1 – Examples of sampling and shipping materials



Sampling frequency:

A sampling schedule should accommodate local site typical operations, including human resources. Importantly, sampling events should be evenly spaced, at a minimum we require 2 samples per week and three if resources allow. This affords the most optimal trending analysis. Samples must be kept refrigerated and not frozen, samples drawn from across the week can be batch shipped together. Please send samples early in the week, which guarantees timely testing of samples and that most couriers do not place samples into cold storage over weekends, this ensure high sample quality.



Adjust timings as per available resources. Importantly samples collection should be well spread through the week.

Samples are referred to by the day the sampling concludes or the day of the grab sampling event. For instance, collection of the “Monday” composite sample begins on the prior Sunday and concludes on Monday morning. In both the above examples, sampling would begin midweek and conclude on Monday. The samples would then be shipped either immediately on Monday or the following day per courier availability. Feel free to adjust the sampling schedule according to your local resources.

Sample collection:

Samples should be well mixed – consider sample collection for SARS-CoV-2 testing similar to sample collection for a TSS assay and the solids content should be representative of the influent or sewage.

1. Freeze cold packs flat several days before sampling.
2. On the day of sampling, label bottles with a permanent marker 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 just below the shoulder of the bottle (approximately 200ml for a 250ml bottle, approximately 450ml for a 500ml bottle). 200ml is the minimum required volume to conduct testing. Do not fill bottles completely, in order to prevent issues during transport and storage/freezing.
 - 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. Further guidance for packaging wastewater for transport can be found in our companion packaging wastewater for shipment document.

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).

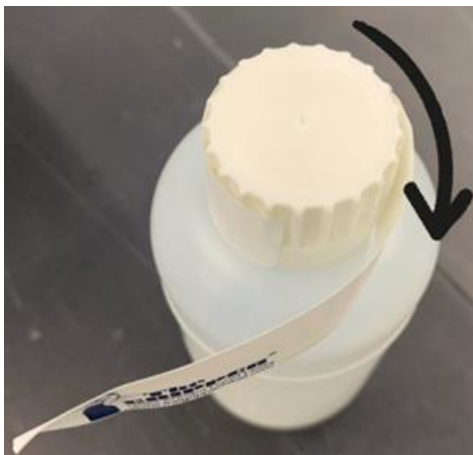


Figure 4 – Example of samples being sealed using bottle cap seal and parafilm



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).

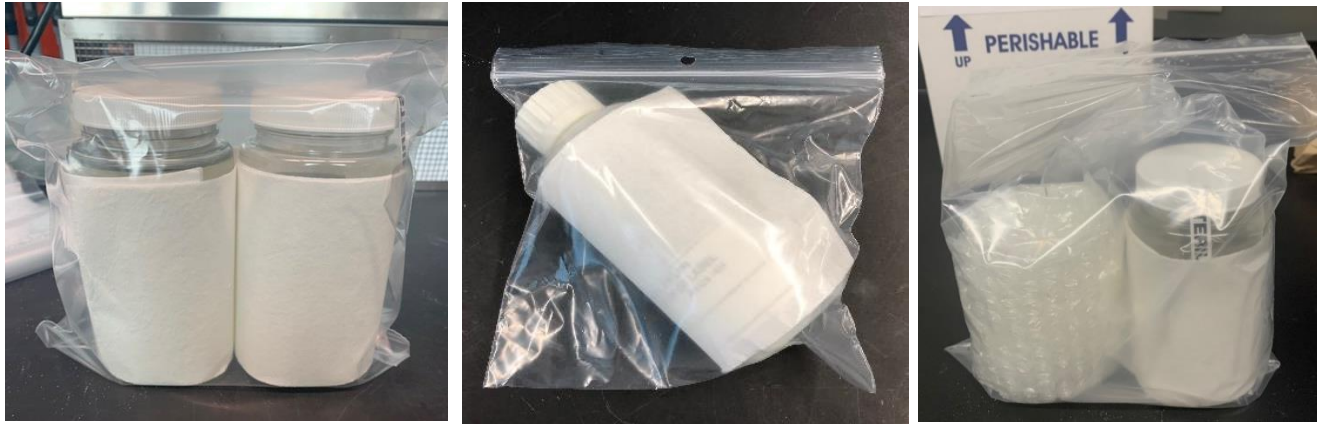


Figure 5 – Examples of samples ready for packing

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).

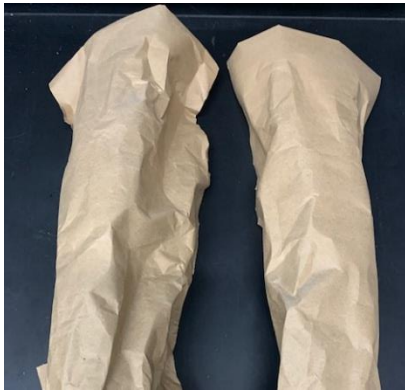


Figure 8 – Examples of brown Kraft paper used for stabilization of sample during transport



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

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.



Public Health
Agency of Canada

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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.