

**SAMRC COVID-19 PREVENTION RESEARCH PROGRAMME:
wastewater surveillance for SARS-CoV-2**

WASTEWATER SAMPLING GUIDE



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SARS-CoV-2 Wastewater Sampling Guide

Pre-fieldwork vaccinations for wastewater samplers:

The below table provides the list of vaccinations required for individuals working with wastewater or sludge. These include:

Item	Doses	*Total Price
Hepatitis A and B (<i>Twinrix</i>)	3	R1425
Tetanus, Diphtheria, Pertussis, Polio (Tdap-IPV) vaccination	1	R420
Typhoid vaccination	1	R350
TOTAL	5	R2 195

*Prices as of 2021. Average consultation cost is R350. For vaccine-specific contraindications, please consult a healthcare worker.

The Hepatitis A and B must be done in three doses - First dose, then second dose 1 month after the first dose, and third dose 6 months after the first dose. The cost is ±R475 per dose (R1425 in total).

Preparation for fieldwork:

- Notify wastewater treatment plant (WWTP) managers/operators of arrival and samples required.
- Daily itinerary (with maps / GIS points).
- List of samples required at each WWTP.
- Check local conditions for travel (weather, planned protests).

Personal Protective Equipment (PPE)

This includes:

- Disposable nitrile/latex gloves.
- Surgical face mask.
- Clear face screen.
- Steel-capped boots.
- Weatherproof clothing.

PPE should be worn at all times during collection and handling of the wastewater on site. A first aid kit should accompany each field trip.

Wastewater Collection:

Wastewater should be collected using 24-hr time-proportional composite sampling (10min interval, 09:00 to 09:00). If composite sampling is not possible, grab sampling (raw wastewater after grit

screening or at the Primary Settling Tank sludge outlet) can be done. The collection of basic metadata includes: (1) time of sample collection, (2) volume of sample, (3) site location and (4) date of collection. Wastewater should be collected in clean sampling bottles (prepared as described below) and kept on ice while transported to the laboratory. For routing / surveillance sampling, sample collection date and time should be standardized.

General equipment required for the successful collection of wastewater includes:

- Grab sampling device that can reach up to 2m.
- \pm 20 L container for pooling composite samples.
- Cooler box.
- Ice packs (no ice).
- Paper towels.
- Waterproof markers.
- Field notebook and pen.
- Ample labels.
- 70% v/v ethanol spray bottle.
- Sealed container for contaminated gloves, paper towels and all BioSafety Level 2 (BSL-2) waste.

All disposable items should be discarded as BSL-2 waste and re-usable items should be cleaned daily after each use with water and soap followed by 70% v/v ethanol.

Sample bottle preparation:

Sample bottles need to be prepared before going into the field. Plastic or glass bottles with watertight lids should be used for wastewater collection and prepared as follows:

- Scrubbed with a brush to remove any particles and rinsed well with deionized water (dH₂O).
- Soak in a 10% bleach solution (5min), including the lid.
- Rinse well with dH₂O.
- Rinse with methanol, including the lid (methanol can be re-used between bottles).
- Rinse twice with dH₂O, including the lid.
- Keep sampling bottles closed until used for sampling.

Sample bottle labelling

Labels should be waterproof and covered by clear tape to prevent water and ethanol damage and prepared before going out into the field.

Collection bottles should be labelled according to specific predetermined labelling codes as discussed with the receiving laboratory. An example is provided below:

- WWTP name, abbreviation or number.
- Week of sampling.
- Abbreviated letter for the type of sample (Grab = G, Composite = C, Sludge = S).

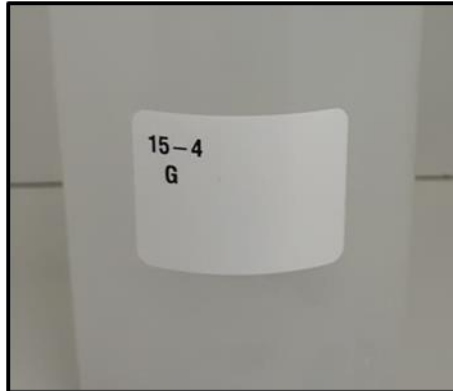


Figure 1: Labelling example. Site 15, week 4 of sampling, grab sample.

Sample collection:

1. Raw influent wastewater collection (composite and grab):

1. Composite sample collection:

As far as possible, wastewater should be collected as a 24-hr time-proportional composite sample (10min interval, 09:00 to 09:00). The autosampler should be set up to collect the wastewater at the inlet works, after coarse screens and grit screens.

To collect the **composite** sample (500 mL):

- Pool the composite sample in the clean, 20 L container.
- Rinse the clean sampling device in the pooled sample.
- Mix the sample well and take the sample using the sampling device.
- Pour carefully into the clean, labelled sample bottle.
- Repeat until the sample bottle is filled to the brim and close the lid tightly.
- Avoid contaminating the outside of the bottle.
- Spray down the sample bottle with 70% v/v ethanol and wipe down with a paper towel.
- Place the sample bottle on ice, in the cooler box.
- Discard all of the remaining composite sample in the raw feed of the WWTW.
- Rinse the sampling device and container with water and spray down with 70% v/v ethanol and let air dry.

2. Grab sample collection

Grab sampling can be done at the inlet works, after the coarse- and grit screening.

To collect the **grab** sample (500 mL):

- Rinse the clean sampling device in the wastewater.
- Take the sample using the sampling device, scooping in the direction of the flow.
- Pour carefully into the clean, labelled sample bottle.
- Repeat until the sample bottle is filled to the brim and close the lid tightly.
- Avoid contaminating the outside of the bottle.
- Spray down the sample bottle with 70% v/v ethanol and wipe down with a paper towel.
- Place the sample bottle on ice, in the cooler box.
- Rinse the sampling device with water and spray down with 70% v/v ethanol and let air dry.



Figure 2: Wastewater grab sampling. A) Collect wastewater with the grab sampling device, after rinsing in the wastewater. B) Pour into the labelled sample bottle, avoid contaminating the outside of the bottle. C) Rinse the inside of the sample bottle with the wastewater and discard of the contents. D) Repeat steps A&B and fill the sample bottle to the brim and close the lid tightly. E) Spray down the sample bottle with 70% ethanol and transport on ice.

3. Waste activated sludge collection (250 mL):

Waste Activated Sludge (WAS) should also be collected at the effluent of the Primary Settling Tank (PST).

- Rinse the clean sampling device in the wastewater.
- Take the sample using the sampling device, scooping in the direction of the flow.
- Pour carefully into the clean, labelled sample bottle.
- Repeat until the sample bottle is filled to the brim and close the lid tightly.
- Avoid contaminating the outside of the bottle.
- Spray down the sample bottle with 70% v/v ethanol and wipe down with a paper towel.
- Place the sample bottle on ice, in the cooler box.
- Rinse the sampling device with water and spray down with 70% v/v ethanol and let air dry.

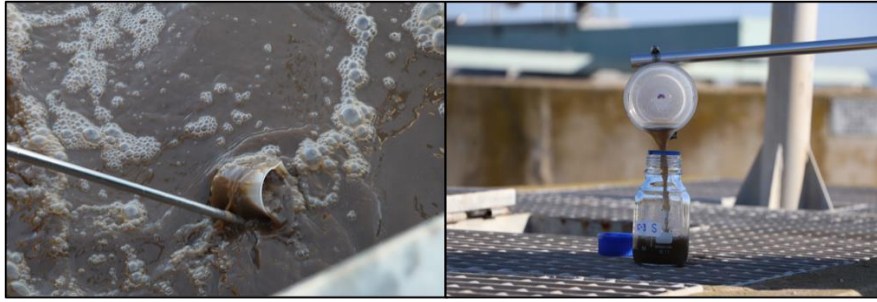


Figure 3: Collection of Waste Activated Sludge (WAS).

Spillage:

In case of accidental spillage during transport, remove all collection bottles and ice packs from the cooler box. Discard the spilled sample in the BSL-2 waste container and disinfect with 70% v/v ethanol while wiping down the entire internal surface with paper towels. Spray down the collection bottles and ice packs with 70% v/v ethanol and wipe down with paper towels. Discard all contaminated paper towels in the BSL-2 waste container.

Field log entries:

Keep a detailed field log of the date, time of sampling and the site. Also, note any conditions that could influence the outcome of the study during wastewater collection and record in detail. These conditions include, but are not limited to:

- The wastewater level during sampling.
- The consistency of the wastewater.
- The colour of the wastewater.
- How the above-mentioned conditions compare to previous sampling events.
- Heavy rains prior to and during sampling.

If possible, other physico-chemical data such as temperature, electrical conductivity, dissolved oxygen and pH should be measured upon collection or received from the wastewater treatment plant operator.

Example of sampling log sheet:

	A	B	C	D	E	F
1	Date	Site	Code	Type of sample	Time sampled	Comments
2	09/07/2020	Gordons Bay WWTW	3-1	Grab (G)	7:40	
3				Sludge (S)	7:34	
4	09/07/2020	Macassar WWTW	14-1	Grab (G)	8:40	
5				Composite (C)	8:45	24 hour composite, ending at 09:00; error on autosampler program = ±21 hour composite
6				Sludge 1 (S1)	8:30	Reactor 1
7				Sludge 2 (S2)	8:35	Reactor 2
8	09/07/2020	Zandvliet WWTW	18-1	Grab (G)	9:16	
9				Composite (C)	9:21	24 hour composite, ending at 09:00
10				Sludge (S)	9:04	
11	09/07/2020	Mitchells Plain WWTW	22-1	Grab (G)	10:14	
12				Sludge (S)	10:18	
13	09/07/2020	Cape Flats WWTW	13-1	Grab (G)	11:01	
14				Composite (C)	11:07	24 hour composite, ending at 09:00
15				Sludge (S)		Primary settling tank out of order, no sludge sample
16	09/07/2020	Athlone WWTW	17-1	Grab (G)	11:32	
17				Composite (C)	11:36	24 hour composite, ending at 09:00
18				Sludge (S)	11:40	
19	09/07/2020	Simons Town WWTW	4-1	Grab (G)	12:38	Heavy rain 2 hours prior to sampling
20				Sludge (S)	12:42	Heavy rain 2 hours prior to sampling

Turnaround time:

Samples should be transported to the laboratory on same day as collected.

Chain of custody

Each day the receiving laboratory should sign off on the number of samples received and list of the sample codes.

Checklist for washing of bottles

- Scrub Brush.
- 10% bleach solution.
- dH₂O.
- Methanol.

Checklist	Item
<input type="checkbox"/>	Scrubbing brush
<input type="checkbox"/>	10% bleach
<input type="checkbox"/>	dH ₂ O
<input type="checkbox"/>	Methanol

Checklist of field items

Checklist	Items
PPE	
<input type="checkbox"/>	Nitrile/Latex gloves
<input type="checkbox"/>	Surgical face mask
<input type="checkbox"/>	Clear face screen
<input type="checkbox"/>	Steel-capped boots
<input type="checkbox"/>	Weatherproof clothing
<input type="checkbox"/>	Hand sanitizer / soap and water
	First aid kit
General sampling equipment	
<input type="checkbox"/>	Labelled sample bottles
<input type="checkbox"/>	Sampling rod
<input type="checkbox"/>	Cooler box
<input type="checkbox"/>	Ice pack
<input type="checkbox"/>	Paper towel
<input type="checkbox"/>	Waterproof markers
<input type="checkbox"/>	Field notebook and pen
<input type="checkbox"/>	70% v/v ethanol spray bottle
<input type="checkbox"/>	Sealed container for contaminated gloves, paper towels and all BioSafety Level 2 (BSL-2) waste