

**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:

### Grab sampling

#### 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
<b>TOTAL</b>	<b>5</b>	<b>R2 195</b>

\*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.

## General equipment required for the successful collection of wastewater includes:

- Grab sampling device that can reach up to 2m.
- Sample collection bottles.
- 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:

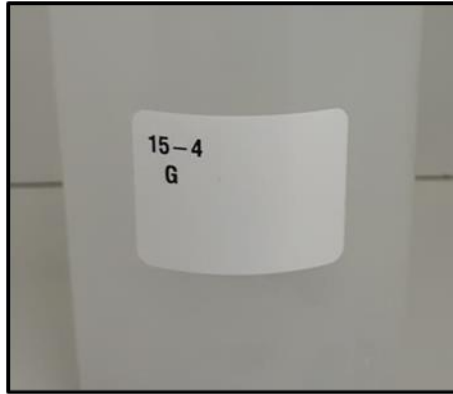
- Scrubbed with a brush to remove any particles and rinsed well with deionized water (dH<sub>2</sub>O).
- Soak in a 10% bleach solution (5min), including the lid.
- Rinse well with dH<sub>2</sub>O.
- Rinse with methanol, including the lid (methanol can be re-used between bottles).
- Rinse twice with dH<sub>2</sub>O, 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).



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

### Sample collection:

#### **Raw influent wastewater collection (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**

### 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	02/11/2021	Wildevoelviei WWTW	WV	Grab (G) Influent	07:37	Normal flows
3						
4	02/11/2021	Simon's Town WWTW	ST	Grab (G) Influent	08:14	Normal flows
5						
6	02/11/2021	Miller's Point WWTW	MT	Grab (G) Influent	08:38	Very low flow
7						
8	02/11/2021	Cape Flats WWTW	CF	Grab (G) Influent	09:34	Normal flow
9						
10	02/11/2021	Mitchell's Plain WWTW	MP	Grab (G) Influent	10:05	Normal flow
11						
12	02/11/2021	Zandvliet WWTW	ZV	Grab (G) Influent	10:35	Normal flow
13						
14	02/11/2021	Macassar WWTW	MC	Grab (G) Influent	10:55	High flows, chemical smell
15						
16	02/11/2021	Gordon's Bay WWTW	GB	Grab (G) Influent	11:27	High flows at the time of sampling
17						
18						
19						

**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.

**Routine sampling**

For routine/ surveillance sampling, sample collection date and time should be standardized.

**Checklist for washing of bottles**

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

### Checklist of field items

Checklist	Items
<b>PPE</b>	
<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
<b>General sampling equipment</b>	
<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