

Technical Guideline Part 1 for SARS-CoV-2 Wastewater Surveillance - Sampling Wastewater

This Technical Guideline is part of four working documents. The working documents are closely related from a technical point of view and should be considered together:

- Technical Guideline Part 1 for SARS-CoV-2 Wastewater Surveillance
 - Sampling Wastewater
- Technical Guideline Part 2 for SARS-CoV-2 Wastewater Surveillance
 - Molecular Biological Analysis
- Technical Guideline Part 3 for SARS-CoV-2 Wastewater Surveillance
 - Sample Logistics and Data Submission
- Technical Guideline Part 4 for SARS-CoV-2 Wastewater Surveillance
 - Data Processing

This guideline provides the basic principles for the sampling of raw wastewater at wastewater treatment plants for analysis of SARS-CoV-2 gene sequences. The procedure described is intended to provide uniformly representative sampling for the determination of SARS-CoV-2 gene fragments. It also lists the information required for the normalization of the SARS-CoV-2 raw data to be submitted continuously with each respective sample of the associated wastewater treatment plants. The data submission process is described in detail in the Technical Guideline Part 3 - Sample logistics and data submission.

The guideline describes the following areas:

- 1. General information**
- 2. Sampling point**
- 3. Sampling methods**
- 4. Homogenization of the composite samples, filling and sample volume**
- 5. Sample shipment/transport**
- 6. Parameters to be recorded**

In general, the various operations must be carried out by trained and qualified personnel.

1. General information

Wastewater is usually contaminated with pathogenic micro-organisms and viruses. The relevant health and safety regulations and guidelines apply. All relevant hygiene measures necessary for the sampling of raw wastewater at the respective wastewater treatment plant must be followed.

Sampling in the course of SARS-CoV-2 monitoring must always be carried out in accordance with the relevant provisions of the Wastewater Ordinance. In particular, the following standards must be implemented:

- German standard methods for the examination of water, waste water and sludge - General information (group A) - Part 11: Sampling of wastewater (A 11)
- German standard methods for the examination of water, wastewater and sludge - General information (group A) - Part 30: Pretreatment, homogenization and aliquotation of non-homogeneous water samples (A 30) (July 1998 edition)
- Water quality - Sampling - Part 3: Preservation and handling of water samples DIN EN ISO 5667-3 (A21) (July 2019 edition)

In addition, the water quality principles of DIN EN 16479 on performance requirements and conformity test procedures for water monitoring equipment - Automated sampling devices (samplers) for water and waste water (September 2012 edition) - and DIN EN ISO 5667-16 Sampling - Guidance on biotesting of samples (March 2016 edition) - apply.

2. Sampling point

The raw wastewater samples shall be taken at a suitable location, if possible after the grit chamber of the wastewater treatment plant. If this is not possible, the sampling point must be reported in the wastewater treatment plants general master data (see Technical Guideline Part 3 - Sample Logistics and Data Submission). Samples should also be collected at locations that precede addition of chemicals, like precipitants for phosphorus removal, or mixing of additional waste streams at the wastewater treatment plant.

For the duration of participation in SARS-CoV-2 monitoring, it is important not to change the sampling point and the sampling period.

3. Sampling methods

Sampling of raw wastewater at the inlet of wastewater treatment plants must be carried out twice a week as a 24-hour composite sample using an automated sampler. The requirements for the instrument are explained in the appendix "Sampler specifications".

Sampling with composite sampler

Composite samples are taken with automated samplers over a period of 24 hours. The sampler should be capable of cooling/heating (5 ± 3 °C). Sampling can be proportional to time or volume. This must be considered when submitting the monitoring data (see Technical Guideline Part 3 - Sample Logistics and Data Submission). The sample volume of the 24 h composite sample should be at least 3 - 5 litres. At the end of the sampling period, the field sample is filled from the individual samples of the sampler into a suitable container/ bottle (collection in individual bottles or containers) or directly from the sampler (collection in one container/bottle).

Sampling times

At least two samples should be taken each week. These should start on Monday and Wednesday and be collected by the sampler on Tuesday and Thursday. The sampling times should not be changed over the entire investigation period and should be reported in the monitoring data (see Technical Guideline Part 3 - Sample Logistics and Data Submission).

4. Homogenization of the composite samples, filling and sample volume

The volume of the sample to be sent for SARS-CoV2 analysis is 1 litre of raw wastewater. Prior to filling into the transport container, the composite sample shall be homogenized and transferred to the sample bottle (1 litre) in such a way that no external contamination of the container occurs. If external contamination nevertheless occurs, the sample bottle must be cleaned and disinfected after closure.

5. Sample shipment/transport

The sample bottles are labelled with the following information for the respective PCR analysis laboratory:

- Name of the wastewater treatment plant
- Sample identification number of the wastewater treatment plant
- Date and time of sample collection
(most laboratories prefer the end of the sampling period)

The data are submitted digitally in parallel as monitoring data (see Technical Guideline Part 3 - Sample Logistics and Data Submission).

6. Parameters to be recorded

For normalization and quality assurance of the SARS-CoV-2 raw data, it is necessary to record wastewater treatment plant-specific measured values of several parameters for each sampling. The following data should be recorded for the wastewater samples taken:

- Discharge rate at the time of sampling (mean wastewater flow, volumetric flow rate)
- Conductivity
- Temperature
- pH value
- Optional: Suspended solids

These are submitted with the monitoring data (see Technical Guideline Part 3 - Sample Logistics and Data Submission).

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Further literature	<ul style="list-style-type: none"> • Handout sampling guidelines (German: "Handreichung zur Probenahme"), ESI-CorA document • Annex - Specification of the sampler - to the Technical Guideline Part 1 for SARS-CoV-2 Wastewater Surveillance • Standard ISO/WD 7014:2022 "SARS-CoV-2 in wastewater" 	