Technical guide for wastewater surveillance

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Umwelt Bundesamt

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Contents

1 Background

In the AMELAG project (German: Abwassermonitoring für die epidemiologische Lagebewertung, English: Wastewater Monitoring for Epidemiological Situation Assessment), the Federal Environment Agency (UBA) and the Robert Koch Institute (RKI) collaborate with health and environmental authorities of the federal states, laboratories, and wastewater treatment plant (WWTP) operators to conduct nationwide wastewater surveillance. The project is funded by the Federal Ministry of Health. In AMELAG, the *Severe Acute Respiratory Syndrome Coronavirus 2* (SARS-CoV-2), influenza viruses, and Respiratory Syncytial Virus (RSV) are monitored in wastewater.

This guideline provides the fundamentals for conducting wastewater surveillance in AMELAG. It outlines the various steps required to process wastewater samples from the initial collection to central data analysis. The steps include sampling at the WWTP, molecular biological analysis for SARS-CoV-2, influenza viruses, and RSV, data transmission, and data processing.

The goal of this guide is to establish a largely harmonized approach across Germany. This will enhance the comparability of the generated data and its usefulness for surveillance. The described procedure reflects the experience of the AMELAG project, which is jointly coordinated by RKI and UBA.

Table 1: Target audience of the different chapters

Audience	**Important chapters**
Wastewater treatment plants	2. Sampling, 4. Data Transmission
Laboratories	3. Molecular Biological Analysis, 4. Data Transmission
States	4. Data Transmission, 5. Data Processing
Data Users	4. Data Transmission, 5. Data Processing

2 Wastewater Sampling

This section outlines the requirements for raw wastewater sampling at the inlet of the WWTP. To ensure a uniformly representative sample with respect to the genetic fragments to be analyzed, several factors at the WWTP must be considered. These factors specifically relate to the sampling location and techniques, handling of the composite sample, and sample transport. Furthermore, to ensure the quality of the raw data, certain accompanying parameters must be measured during sampling. The process of data transmission is described in detail in Section 4.

2.1 General Information

Wastewater is typically contaminated with pathogenic microorganisms and viruses. The relevant health and safety regulations and guidelines apply (e.g., TRBA 220: Wastewater Treatment Plants: Protective Measures from the Federal Institute for Occupational Safety and Health, or the DGUV Rule 103-602, German Social Accident Insurance). All relevant hygiene measures that are necessary during the sampling of raw wastewater at the respective WWTP must be observed. In general, the various steps must be performed by trained and qualified personnel.

The samples collected for wastewater surveillance must generally follow the applicable regulations of the Wastewater Ordinance. This includes, in particular, the consideration of the following standards:

- Wastewater sampling DIN 38402-11 (A11) (Edition February 2009)
- Pretreatment, homogenization, and splitting of heterogeneous water samples DIN 38402-30 (A30) (Edition July 1998)
- Preservation and handling of water samples DIN EN ISO 5667-3 (A21) (Edition July 2019)

In addition, the principles of DIN EN 16479 for performance requirements and conformity testing of automatic samplers (Edition September 2012) as well as DIN EN ISO 5667-16 for sampling in biological test methods (Edition March 2016) apply.

2.2 Sampling Location

The collection of raw wastewater samples should be carried out at an appropriate location at the inlet of the WWTP, preferably after the sand trap. If sampling is only possible at another location, such as before the sand trap, the sampling location must be provided when transmitting the WWTP's master data (see Section 4, Data Transmission).

The sampling location must not be changed during the participation in wastewater surveillance. If a change in the sampling location is absolutely necessary, the new sampling location must be updated in the master data of the WWTP, and a notification must be sent via email (abwassersurveillance@uba.de) (see Section 4, Data Transmission).

2.3 Sampling Techniques

Raw wastewater sampling at the inlet of WWTPs must be conducted twice a week as a 24-hour composite sample using an automatic sampler. Grab samples are not permitted. Requirements for the device are outlined in the "Sampler" annex (Appendix 1).

During the collection of the 24-hour composite sample, the samples must be cooled or tempered (5 ± 3 °C). Sampling can be time-proportional or flow-proportional [1], with flow-proportional sampling being recommended. The sampling frequency should be as high as possible. This should be noted when transmitting the monitoring data (see Section 4, Data Transmission). The sample volume of the 24-hour composite sample should be at least 3 liters.

2.4 Sampling Times

Two samples are taken per week. These should start on Mondays and Wednesdays, with the samples being collected 24 hours later, on Tuesdays and Thursdays, from the sampler. The sampling times should not be changed throughout the entire monitoring period. The start and end times of the sampling are recorded in the monitoring data (see Section 4, Data Transmission).

2.5 Sample Filling

If the composite sample has been collected in individual containers, the individual samples are transferred into a suitable vessel at the end of the sampling period. Before filling the transport container, the composite sample is thoroughly homogenized (special attention should be given to homogenizing the solids throughout the entire sample). Then, 1 liter of the composite sample is transferred into the prepared 1-liter sample bottle. It is important to ensure that no external contamination occurs during this process. If external contamination does occur, the sample bottle should be cleaned and disinfected after sealing.

2.6 Sample Logistics

The sample bottles (polyethylene with screw caps) are labeled with a sticker containing the following information and sent to the respective PCR analysis laboratory:

- Name of the WWTP
- Sample identification number of the WWTP
- Date and time of sample filling

The sample must be transported in a cooled/tempered state, ensuring a temperature stability of 5°C ± 3°C, especially on very cold or hot days.

The sample must reach the laboratory no later than 24 hours after being provided by the WWTP. The goal is to reduce this transport time, ideally so that an analysis result for the sample can be entered into the UBA database within 48 to 60 hours.

The sample containers (sample bottle, insulation, cooling packs) must be provided by the laboratory or federal analytics and sent to the WWTPs. They can be cleaned and reused. It must then be ensured that the containers still seal tightly.

[1] With the enactment of the revised EU Municipal Wastewater Directive 2024/3019, a 24-hour composite sample must be taken flow-proportionally. This is expected to be implemented by July 31, 2027. The required switch from time-proportional to flow-proportional sampling will take place ideally in the summer of 2026 and should be noted in the WWTP's master data sheet (see Section 4, Data Transmission).

2.7 Parameters to be Recorded

For quality assurance of the raw data, it is necessary to record WWTP-specific measurements of various parameters at each sampling. The following data should be recorded for the collected wastewater sample:

- Name of the WWTP
- Sample identification number of the WWTP
- Date and time of sample filling
- Inflow to the treatment plant (average flow rate during the sampling period, in L/s)
- Electrical conductivity (in µS/cm)
- Temperature (in °C)
- pH value
- Optional: Filterable substances (in mg/L)

These data should be transmitted along with the monitoring data (see Section 4, Data Transmission).

3 Molecular Biological Analysis

This section provides the fundamentals for the procedure of quantitatively detecting specific gene fragments of the SARS-CoV-2, Influenza A and B viruses, RSV group A and B, and viruses commonly found in feces (fecal reference viruses) using molecular biological detection methods, i.e., (reverse transcriptase) polymerase chain reaction ((RT)-PCR). It also describes the key control parameters that must be considered for quality-assured analysis.

3.1 General Information

The various steps of nucleic acid extraction and (RT)-PCR analysis must be conducted by personnel trained and qualified in molecular biology. Additionally, spatial separation of the processing phases (homogenization, sample concentration and virus enrichment, nucleic acid extraction vs. nucleic acid amplification and detection) must be adhered to. Contamination must be avoided by using sterile, nuclease-free consumables and reagents. Regular disinfection and glove changes must also be implemented. All protocols must be optimized and evaluated by the laboratories to ensure that quantification of the nucleic acids to be detected is possible, even at low virus concentrations in the wastewater samples. Furthermore, recovery rates, as well as the detection limit (Limit of Detection, LOD) and quantification limit (Limit of Quantification, LOQ) for each target gene, must be determined.

3.2 Sample Handling (Storage, Homogenization)

After sample collection, the wastewater sample should be processed as soon as possible. Short-term storage at 4°C is acceptable for a few days. However, processing within 48 hours is strongly recommended. In the literature, it has been described that, for cooled samples, the stability of gene fragments can last up to nine days, depending on the virus (Markt et al., 2021). The wastewater sample should not be frozen, as this can lead to significant loss of viral nucleic acids.

In the laboratory, an automated homogenization of the samples in an overhead shaker for 15 minutes is recommended. Only after this should subsamples be taken for further analysis.

3.3 Concentration of the Wastewater Sample

The concentration of virus particles or viral components can be achieved through various methods. Table 1 summarizes the three most commonly used methods within the framework of AMELAG.

Solid particles from the raw wastewater are separated through an initial centrifugation step (e.g., 15-45 minutes, 4,000–5,000 x g) in many protocols (e.g., when using filter units or centrifugal ultrafiltration). After this step, the aqueous phase of the sample is processed further.

Table 1: Methods for Concentrating Virus Particles

Method	Initial Volume	Options
Precipitation with Polyethylene Glycol (PEG)	40 mL - 1 L	Polyethylene glycol 8000 (PEG 8000) (10% w/v) (or Polyethylene glycol 6000 (PEG 6000)), Additionally, NaCl (2.25% w/v) is added
Pressure or Vacuum-Based Filtration	40 mL - 1 L	Negatively or positively charged filter membrane, Pore size 0.1 µm to 0.45 µm
Centrifugal Ultrafiltration	40 mL - 200 mL	Various filter types and rotation speeds

Depending on the method used and the initial volume, the recovery rates of target gene segments and spiked control viruses may vary (see below). Key parameters for selecting the concentration method include (i) the wastewater volume that can be processed with the respective method, (ii) the efficiency of the method, (iii) the suitability of the method for the viruses under investigation, and (iv) the available laboratory equipment.

3.4 Nucleic Acid Extraction

After concentrating the viral particles or components from the wastewater sample, viral or total nucleic acids must be extracted in high quality. The most commonly used techniques for this process rely on organic extraction with phenol/chloroform, water, and alcohol, as well as binding to silica matrices. These methods are commercially available as kits from various suppliers.

The nucleic acids are usually eluted in a volume of 50–200 µL. Quantification of the extracted nucleic acids is performed photometrically at 260 nm, fluorometrically, or through fragment analysis. The purity of the sample can be checked photometrically at 230 and 280 nm or through fragment analysis.

The efficiency of the extraction method can be verified by including a known number of inactivated virus particles from another virus, such as the bacteriophage MS2 or the murine hepatitis virus (MHV), as a process control.

The extracted nucleic acids can be stored at -20°C for the medium term without significant concentration loss. For long-term storage, it is recommended to freeze aliquots at -80°C in suitable reaction vessels (e.g., low-binding tubes).

3.5 Quantification of Viral Genomic Copies Using (RT)-PCR

The quantification of viruses is performed using polymerase chain reaction (PCR). Depending on the type of virus, different methods are applied. RNA viruses require an initial reverse transcription (RT) step, during which the viral RNA is converted into complementary DNA (cDNA). This step can be carried out using a combined one-step or a two-step procedure. RNA is particularly prone to degradation and inhibition in wastewater, which makes RT a critical part of the detection process. Therefore, suitable inhibition controls (e.g., external RNA standards) are mandatory. DNA viruses, on the other hand, can be detected directly using PCR. They are generally more stable in the environment and less prone to interference in the analysis, although the inclusion of appropriate positive and negative controls is still required.

For quantification, (RT)-qPCR (relative quantification using standard curves) or digital (RT)-PCR methods (dPCR, ddPCR) are commonly used. The latter enables absolute quantification without the need for standard curves, which can be especially advantageous for complex environmental matrices.

Regardless of the virus type, the following principles must be adhered to for reliable data:

- Appropriate positive and negative controls should be included to detect false-positive or false-negative results.
- For (RT)-qPCR, the analysis of samples should be performed at least in technical duplicates.
- LOD and LOQ must be considered and documented.
- Results should be reported in a standardized form, typically as "genomic copies per liter of wastewater".

The selection of suitable target regions in the genome and the validation of the assays used are the responsibility of the laboratories. However, to ensure a standardized approach, the use of the oligonucleotides listed in Annex 2 is recommended.

3.6 Quality Control of PCR Results

Quality assurance in molecular biology analysis requires the use of appropriate controls to avoid false-positive or false-negative results and ensure comparability between laboratories. Essential elements include:

1) Process controls, i.e., surrogate viruses or nucleic acids used to verify the efficiency of concentration and extraction.
2) Internal amplification controls, i.e., markers that are ubiquitously present in wastewater (e.g., fecal reference viruses like the gut-associated bacteriophage CrAssphage or the plant virus Pepper-Mild-Mottle Virus (PMMoV)). These serve to assess (RT)-PCR inhibition and data quality.
3) Positive and negative controls, which are mandatory in every (RT)-PCR run series to detect non-specific signals or contamination.
4) Replicates – at least technical duplicates for (RT)-(qPCR) or sufficient partitions or droplets for (RT)-dPCR/ (RT)-ddPCR – to ensure statistical robustness.

!	As part of a measurement series for a WWTP site, both the sampling methods, concentration techniques, and subsequent detection methods must not be significantly altered in order to compare the individual measurements of the WWTP over time. If a change in methods is absolutely necessary, it must be communicated through the master data sheet for the laboratory, as well as via email to (abwassersurveillance@uba.de) (see Section 4 Data Transmission).

4 Data Transmission

This section explains the electronic transmission of the generated data via the PiA Monitor (from German *Pathogene-im-Abwasser*-Monitor). For wastewater surveillance, standardized and quality-assured data are essential. The data collected from WWTPs, laboratories, and monitoring are transmitted in predefined data sheets (see overview in Figure 1). Furthermore, this section outlines the responsibilities for data transmission.

4.1 General Information

The PiA-Monitor database, which compiles all necessary data for virus detection in wastewater samples, is accessible via a web application (https://app.pia-monitor.de/). The import of monitoring data can be done either directly through the web application or via an Application Programming Interface (API). Specific templates (Excel, CSV) must be used for data transmission via the web application. These templates can be requested from the UBA or downloaded from the help wiki of the web application (https://wiki.pia-monitor.de/de/Datenimport). The data formats to be used are defined within the data template. The structure of the templates must not be altered, as a preliminary plausibility check of the data is already conducted during data transmission. To use the import API, an import authentication token is required. This token can be requested from the UBA or directly from technical support. A description of the import API is available in the help wiki for the web application.

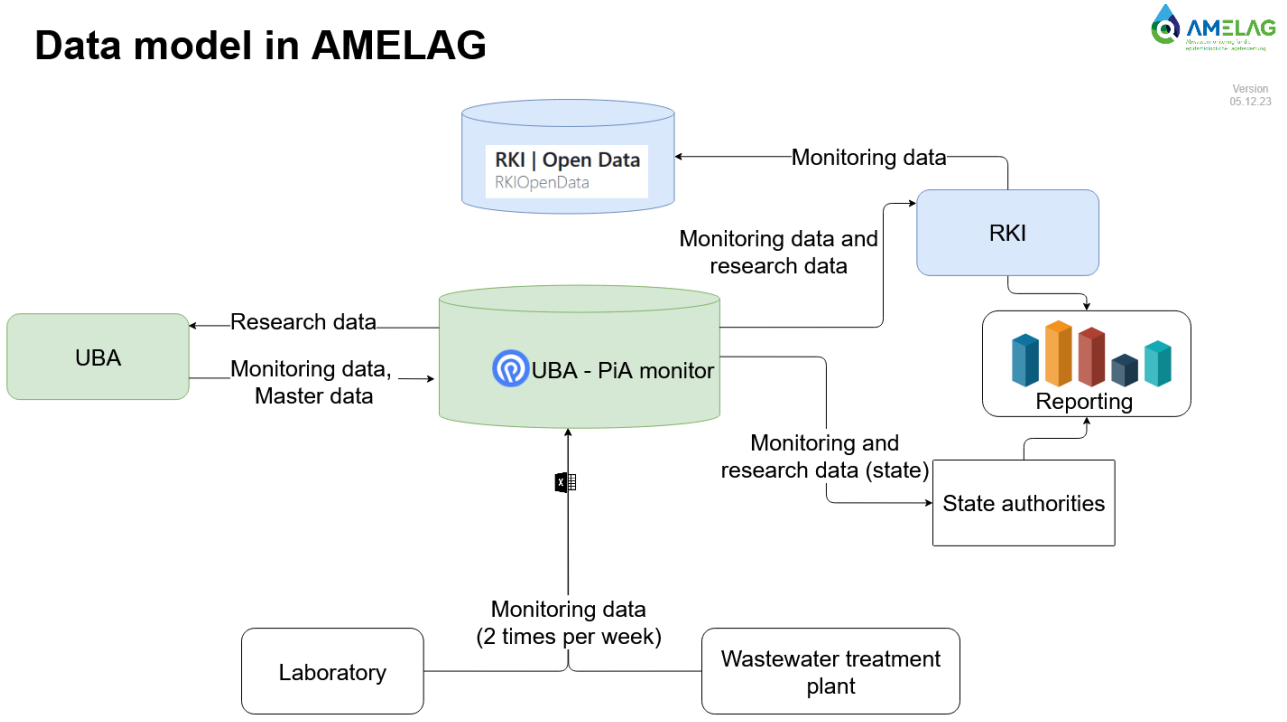

Figure 1: Data Flow in AMELAG

4.2 Data Sheets

In the data sheets, some of the data to be transmitted are designated as mandatory fields, while others are optional. An explanation of this, along with the parameters for the plausibility check, is provided directly in the Excel version of the monitoring template and in the help wiki (https://wiki.pia-monitor.de/de/Datenimport).

In addition to the monitoring data, master data for the WWTPs and laboratories are collected. The provision of this master data by the laboratories and operators, as well as the entry of this information into the database (carried out by the UBA), are prerequisites for the import of monitoring data:

Master Data Sheet for WWTP: This sheet collects the unchangeable parameters of the WWTP. These include, among others:

- EU identification code of the WWTP (DEPT key according to UWWTD reporting, can be prefilled by UBA, if unknown)
- Contact details of the WWTP
- Treatment capacity, number of connected inhabitants
- Composition of wastewater volume (total, indirect contributors, proportion of extraneous water)
- Information on the sampling site and sampler
- Volume of individual sample

Further explanations of the parameters can be found in the Excel version of the master data template under the “Description” tab. The master data sheet is submitted to the UBA once at the start of wastewater surveillance, or updated again if technical data change significantly.

Master Data Sheet for Laboratory: This sheet collects the unchangeable parameters of the laboratory. These include, among others:

- Identification code of the laboratory (assigned by the UBA)
- Contact details of the laboratory
- PCR method used, analyzed gene sequences
- Number of replicates and controls, standards
- Concentration method used
- Wastewater volume used for concentration
- Volume of RNA/nucleic acid extract used in PCR
- PCR kit used (including manufacturer)

Further explanations of the parameters can be found in the Excel version of the master data template under the “Description” tab. The master data sheet is submitted to the UBA once at the start of wastewater surveillance. If the laboratory’s analytical process changes significantly, or if the laboratory conducting the analysis changes, the laboratory master data must be updated.

Monitoring Data: The monitoring data for the pathogens are transmitted with the assignment to the location (WWTP) and the analyzing laboratory, along with data on the quality control of the workflows. This includes information on sampling at the respective location, such as:

- WWTP location (DEPT key, name)
- Date/time of sampling, sample volume
- Average flow rate during the sampling period
- pH, conductivity, temperature, filtrable substances

And laboratory analysis data, including:

- Laboratory ID, sample ID
- Temperature upon arrival at the laboratory, time of sample preparation start
- Detected gene copies of various viruses, detection limits
- Detected gene copies of surrogate viruses (PMMoV, CrAssphage)

The data import can be performed simultaneously for any number of samples and WWTPs. Data corrections after the import are possible within a limited scope:

- Deleting a current import process/import file if the data has not yet been reported to the RKI,
- Correcting accompanying parameters, and
- Reimporting older data upon request (at UBA and RKI).

A description of the correction and reimport options can be found in the help wiki for the web application (https://wiki.pia-monitor.de/).

4.3 Responsibilities

Depending on the procedure in a federal state, the responsibility for data transmission may lie directly with the central state office or with the individual WWTPs/laboratories (decentralized solution).

In the case of centralized responsibility with a state office, it is up to the state office to decide in what format they receive the data from the WWTP(s) and laboratory(ies) to then import it either directly via the data sheet through the application or through the import API into the UBA database.

In the case of a decentralized solution, the respective data flow and data provision must be clarified in advance by the respective WWTP/laboratory. If the WWTP is involved in federal analytics, it transmits its data via the data sheet to the federal analytics laboratory. The laboratory is responsible for transmitting the monitoring data into the UBA database.

At any time, and without restrictions, the transmitting partners can view their data and use it for their own purposes.

Access data for PiA-Monitor, along with the specific data sheets, will be provided upon request to the UBA (abwassersurveillance@uba.de). This also includes access to the help wiki and the description of the web application, import data, and import options.

Data must be transmitted regularly and in a timely manner.

5 Data Processing

This section describes the procedures for data quality assessment, data calculation, and statistical analysis within the framework of AMELAG. These steps are carried out by UBA and RKI.

5.1 Introduction

The raw wastewater inflow to WWTPs is subject to diurnal, weekday-related, and seasonal fluctuations. Additionally, changes in inflow occur due to continuous or discontinuous indirect dischargers, extraneous water, and rainwater. These changes in flow volume lead to the dilution of the respective parameters being analyzed, which in turn influences the concentration of gene fragments from various viruses.

Normalization may help to compensate for fluctuations in raw data. However, an analysis of long-term time series in AMELAG showed that normalization did not result in a general improvement in data quality for the SARS-CoV-2 viral load (Saravia et al. 2024). As of 01.08.2025, normalization for SARS-CoV-2 using the average flow rate at the inflow of the WWTP will be discontinued. Therefore, only non-normalized data are currently being analyzed. However, normalized data will still be provided (see Section 5.3). Data for influenza viruses and RSV are also not normalized, as no improved data quality through normalization was observed.

Other normalization methods (e.g., using fecal reference viruses or chemical human markers) are being investigated in AMELAG, but are not yet applied in the presentation of results.

Measured virus loads in wastewater generally show high variation over time, so trend calculations are not based on the measured (possibly normalized) values but on smoothed values. Several statistical methods exist for smoothing the measurements.

5.2 PCR Data Calculation

For SARS-CoV-2, only samples that have been analyzed for at least two different gene fragments are considered in the data processing to exclude false-positive results. If fewer than two measurements (i.e., values for at least two gene fragments) are available, the sample is excluded from further analysis and marked as "not determined".

The LODs of the applied methods are requested during data submission for the respective gene fragments.

If the measurement values are above LOD and LOQ, further analysis proceeds as described below.

If a measurement value is below the LOQ, the value is replaced with 0.5 * LOQ and used for further analysis.

Currently, the geometric mean is calculated from the (at least) two available gene fragment values:

$$Geometric\ Mean = \sqrt[n]{x1 * x2 * \ldots * xn}$$

Where x_1, x_2, …, x_n represent the genomic copies per liter (GC/L) for each gene concentration.

5.3 Normalization of PCR Data

The raw data for viruses studied in AMELAG are currently not normalized for evaluations in the AMELAG weekly report, as no improvement in data quality through normalization was observed.

However, normalized data for SARS-CoV-2 are still available on GitHub (https://github.com/robert-koch-institut/Abwassersurveillance_AMELAG).

For the normalization of the data, the dry weather inflow of the WWTP was used as a reference. The dry weather inflow is the flow rate that is not influenced by precipitation events or thawing.

In the previously used normalization method, the ratio of the inflow flow rate during the sampling period ($Q_{WWTP,\ current}$) to the dry weather inflow was calculated and then multiplied by the geometric mean of the PCR measurements (*Genes averaged*). The median of all previously reported inflow values ($Q_{WWTP,\ median}$) was used to determine the dry weather inflow.

Based on the assumptions described, the normalized value was calculated as follows:

$$Gene\ normalized = \frac{Q_{WWTP,current}}{Q_{WWTP,median}} * Gene\ averaged$$

Where:

$Q_{WWTP,\ current}$*: Inflow rate of the WWTP during the sampling period;*

$Q_{WWTP,\ median}$*: Median inflow rate of the WWTP.*

5.4 Statistical Analyses

For a pathogen in wastewater surveillance, smoothed values for a calibration curve are calculated for each location using a generalized additive model (GAM) with adaptive smoothing. This calculation is based on the logarithmic viral load in wastewater. Adaptive smoothing allows the smoothness of the curve to vary over time. This means that the method can automatically assign more flexibility for periods in which the data show complex patterns and less flexibility for periods in which the data are less variable. The strengths of the smoothing are set for each location-laboratory combination using the cross-validation criterion to optimize the predictive quality of the curve. The idea behind this is to find a balance between a high fit of the GAM curve to the available data and a low variability of the GAM curve, i.e. a curve that is as smooth as possible. This results in a smooth curve for each location, and for each time point (even between measurement points), a predicted viral load. Pointwise confidence intervals are then calculated using the t-distribution.

To obtain a nationwide trajectory of the viral load of a pathogen in wastewater, the time series from different locations are aggregated. First, the mean of the weekly averaged logarithmic measurements for each location is calculated. Then, for each location each week, the difference from the weekly mean of all locations in that week is determined. For each location-laboratory combination, the mean of these differences is calculated across all weeks, and this mean is subtracted from the originally measured values. This adjustment accounts for average differences in viral loads between different location-laboratory combinations.

Finally, for every week where data is available from at least 20 locations, the mean of these adjusted values is calculated, weighted by the population size of the connected WWTP. The resulting time series is then smoothed using a GAM regression, as described above.

5.5 Software

The processing of PCR data is carried out in PiA-Monitor (https://app.pia-monitor.de). The application also includes an automated quality control process for the data to be imported. Login credentials for the application and a description of its features can be requested separately.

The GAM method used for trend calculation is implemented in statistical software packages like R. The software code used in AMELAG is available at https://github.com/robert-koch-institut/Abwassersurveillance_AMELAG/

6 Abbreviations

AICc	Correction factor of the Akaike Information Criterion
AMELAG	Abwassermonitoring zur epidemiologischen Lagebewertung, Wastewater Monitoring for Epidemiological Assessment
API	Application Programming Interface
cDNA	Complementary DNA
DGUV	Deutsche Gesetzliche Unfallversicherung, German Social Accident Insurance
dPCR	Digital Polymerase Chain Reaction
LOESS	Locally Estimated Scatterplot Smoothing
LOD	Limit of Detection
LOQ	Limit of Quantification
PEG	Polyethylene Glycol
PiA	Pathogene im Abwasser, Pathogens in Wastewater
PMMoV	Pepper Mild Mottle Virus
Q	Inflow (here: to the wastewater treatment plant)
qPCR	Quantitative Polymerase Chain Reaction
RKI	Robert Koch Institute
RSV	Respiratory Syncytial Virus
RT	Reverse Transcription
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
UBA	Umweltbundesamt, German Environment Agency
WWTP	Wastewater Treatment Plant

7 Additional Information

Contact	Umweltbundesamt, abwassersurveillance@uba.de Robert Koch-Institut, abwassersurveillance@rki.de
Websites	https://www.umweltbundesamt.de/amelag https://www.rki.de/abwassersurveillance
Acknowledgment	The contents of the Technical Guide were developed in collaboration with the participating laboratories and experts of the AMELAG project.
Funding	The Federal Ministry of Health (BMG) funds wastewater monitoring as part of the project "Abwassermonitoring für die epidemiologische Lagebewertung (AMELAG)".
Further Reading	Markt R, Mayr M, Peer E, Wagner AO, Lackner N, Insam H. Detection and Stability of SARS-CoV-2 Fragments in Wastewater: Impact of Storage Temperature. Pathogens. 2021 Sep 18;10(9):1215. doi: 10.3390/pathogens10091215. Marquar N, Pütz P, Buchholz U, Exner T, Fretschner T, Greiner T, Helmrich M, Lukas M, Marty M, Obermaier N, Saravia Arzabe C, Schattschneider A, Schneider B, Selinka H-C, Ullrich A, Walter B, Braun U., Schumacher J (2024). SARS-CoV-2-Abwassersurveillance in Deutschland im Rahmen des Projekts AMELAG. *Epidemiologisches Bulletin 34:16-26*. Saravia CJ, Pütz P, Wurzbacher C, Uchaikina A, Drewes J, Braun U, Bannick CG, Obermaier N. Wastewater-based Epidemiology: Deriving a SARS-CoV-2 Data Validation Method to Assess Data Quality and to Improve Trend Recognition. Frontiers in Public Health 2024, 12:1497100. Schattschneider A, Greiner T, Beyer S, Hans J, Correa Martinez C, Eckmanns T, Diercke M, Schumacher J (2024). Abwasser enthält Informationen für die öffentliche Gesundheit: Mögliche Anwendungen für eine Abwassersurveillance. *Epidemiologisches Bulletin 34:3-15*.

8 Appendix

8.1 Appendix 1: Description of the Sample Collector's Performance

The requirements from EN 16479 apply, particularly:

Technical requirements (mandatory)

Fully automatic, stationary sample collector for discontinuous time- and quantity-proportional sampling

Suitable for outdoor use

Sample temperature control with adjustable interior temperature (ambient temperatures from -20°C to +40°C)

Pressure-vacuum sampling system with dosing container for variable single samples of at least 50 mL - 200 mL

The minimum sampling interval must be at least 5 minutes

Space for at least 12 x 2-liter bottles or at least 20-liter single containers

Technical Requirements (optional)

Suitable for flow-proportional sampling

GSM modem, remote control, SMS, and program start via mobile phone, or full access to the sampler software via PC/notebook

X-Y distributor for direct dosing of the sample into the sample bottle with free input of any bottle positions directly at the control

Door contact linked to the control system (when the door is opened, the program should pause and the X-Y distributor should move to a park position for sample bottle removal.