# Biosurveillance in Wastewater

## Focus on SARS-CoV-2 Detection

### Objective

- Real-time monitoring of regional prevalences within large populations
- Early identification of hot spots
- Cost-effective analysis of large populations instead of individual testing

### Challenges

- Representative sampling
- Increased number of samples
- Large volumes/ low concentration
- Harsh environment

### Solution

- Automated and controlled sample collection for reproducable sample quality
- Efficient enrichment allows low limits of detection
- Automated nucleic acid extraction for minimal hands-on time
- From sampling to final result within a few hours
- Reliable results even in challenging wastewater matrix
- Detection based on highly specific real-time PCR – the gold standard for viral diagnostics
- One workflow - multiple biological parameters

Wastewater-based epidemiology (WBE) is the approach to identify both biological and chemical parameters in one gathering ground. It reflects the regional load and allows a comprehensive real-time monitoring of public health.
Screening wastewater for detecting pathogens such as SARS-CoV-2 has recently been shown in various scientific publications[a] and can be a valuable tool for targeted COVID-19 management. Similar approaches[b] provided valuable insights into epidemiologic aspects of other diseases, too.

For these issues real-time PCR - the gold standard for the direct and highly sensitive detection of SARS-CoV-2 in both research and clinical settings – is a qualified method for wastewater analysis, as well. Due to its complex composition and high dilution handling of wastewater in this context is challenging.

Here, an easy to implement and comprehensive workflow starting from representative wastewater sampling, continuing with effective enrichment and nucleic acid extraction and closing with the real-time PCR is presented.

[a] Lancet Gastroenterol Hepatol. 2020 Jun;5(6):533-534. doi: 10.1016/S2468-1253(20)30087-X. Epub 2020 Apr 1
Sci Total Environ. 2021 Mar 1;758:143578. doi: 10.1016/j.scitotenv.2020.143578. Epub 2020 Nov 10
[b] Proc Natl Acad Sci U S A. 2018 Nov 6;115(45):E10625-E10633. doi: 10.1073/pnas.1808798115. Epub 2018 Oct 18

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# Real-time PCR-based Detection Workflow

-24 h

## 1. Sample collection

Fully automated sample collection (over individual period, e.g. 24 h) by automatic water sampler Liquistation CSF48 (by Endress+Hauser)

- Stationary automatic sampler
- Complies with worldwide water regulations
- Menu-guided sample programming
- Tool-free maintenance

0 min

## 2. Enrichment

Efficient target enrichment by filtration (third party). The filter membrane is homogenized using the SpeedMill PLUS (Analytik Jena GmbH)

- Entire and reproducible homogenization
- Small foot print
- Passive cooling function for maximum sample integrity

15 min

## 3. Nucleic acid extraction

Reproducible extraction of DNA and/or RNA using the innuPREP AniPath DNA/RNA Kit-IPC16 (Innuscreen GmbH) on InnuPure C16 *touch* (Analytik Jena GmbH)

- Highly efficient extraction of viral/bacterial DNA and/or RNA from broad range of starting materials
- Automated extraction from 1-16 samples in parallel
- Easy and intuitive to use

100 min

## 4. Amplification and detection

Highly sensitive target detection using real-time cycler from the qTOWER$^3$ family (Analytik Jena GmbH) in combination with specific real-time qPCR Assay (by third party, e.g. Water SARS-CoV-2 RT-PCR Test by IDEXX Laboratories Inc.)

- Innovative sample block technology for best thermal conductivity
- Patented fiber optic system for optimal real-time polymerase chain reaction (PCR)
- Expandable filter module system offers maximum flexibility

180 min

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