# Report on the online conference
# „Monitoring of SARS-CoV-2 in the sewage system“

An event hosted by Technologieland Hessen on February 9th – 10th, 2021

## At a glance

Wastewater sampling and analytics make it possible to **monitor the spread of infections** as it happens. This way, **pandemic waves and new mutations can be recognised early on**. Thanks to the sequencing of SARS-CoV-2 fragments found in wastewater, mutations have been discovered even before they were identified in clinical diagnostics. With this **expanded early warning function**, wastewater monitoring gains an important role in the face of the rapid spread of different mutants of SARS-CoV-2.

## Table of Contents




**Contact – Hessen Trade & Invest**

Dr. Felix Kaup
felix.kaup@htai.de

Dr. Hendrik Pollmann
hendrik.pollmann@htai.de


HESSEN TECHNOLOGIELAND HESSEN

# 1 Summary

More than a year after the Covid-19 pandemic broke out, the global situation remains critical. One of the challenges of fighting SARS-CoV-2 is that case numbers determined by diagnostic tests in medical settings can only represent the spread of infections with a delay. Virus particles from stool and other excretions can be detected in wastewater, based on the PCR test of virus RNA, which offers a complementary approach to clinical testing strategies.

On the first day of the conference “Monitoring of SARS-CoV-2 in the sewage system,” scientists from Germany, the Netherlands, Switzerland, Great Britain and Sweden presented their projects in the field of monitoring wastewater for coronaviruses. They concur that the method is sensitive enough and that the amounts of viral RNA measured in wastewater reflect the spread of infections well, and faster than testing individuals. Moreover, genome sequencing technology enabled the detection of mutant viruses in the wastewater, including some that weren’t known in the clinic yet. The specific number of people infected, however, cannot be derived from wastewater data (yet). To achieve this, one would have to establish the viral load that people shed to the sewers in specific phases of the disease.

The wastewater samples analysed were typically taken from the input of water treatment plants. A study in England also investigated sewage from schools and found the virus RNA in 50 to 75% of the samples. Wastewater monitoring for coronaviruses is most advanced in the Netherlands where more than 300 locations nationwide are sampled regularly. The Dutch government publishes the results on its freely accessible corona dashboard. In Switzerland, efforts are underway to use the wastewater results in the calculation of the reproduction number.

The second day of the conference focused on the various tools that could support wastewater monitoring along the process chain from sampling to data use. Digital models of sewer networks, bio-analytical instruments, the Nextstrain software for visualising the spread of mutations, and the data management system SORMAS, originally developed for the fight against Ebola, were discussed.

The speakers from science and industry called for an international standardisation of analytical procedures. The joint effort is worthwhile, as the insights gained can also be transferred to other pathogens. The big idea is to recognise new viruses and other germs in the wastewater in time to be able to limit their spread with regional measures and avoid pandemics in the future. To achieve this, co-operation between scientific disciplines as well as between science, industry and authorities is imperative. Financial support for the relevant research and co-operation projects is equally essential.

## 2 Wastewater as an early warning system: Monitoring of SARS-CoV-2 in the sewer network

On the on 9th and 10th of February, 2021, Technologieland Hessen welcomed representatives from science, industry and administration to the conference “Monitoring of SARS-CoV-2 in the sewage system.” The meeting was held online, as the situation related to the Covid-19 pandemic in Germany and many other countries was still in a critical state, one year after the epidemic had started to spread. In Germany, around 65,000 people died from or after infection with SARS-CoV-2 by mid-February. The global death toll was 2.4 million at that time. Vaccines are offering a glimpse of hope, but virus mutations that are more infectious are becoming more prominent. There is growing concern that certain mutants may be able to evade the protection of the existing vaccines.

One of the challenges in fighting the pandemic is the fact that official case numbers represent the course of infections with an inbuilt time delay. Typically, several days pass between the infection, the onset of symptoms, and then the availability of test results. People who are infected but have no or only mild symptoms and don’t get tested will be missing from the statistics. Many countries are now considering the detection of SARS-CoV-2 in sewage as a measure complementing the clinical testing strategy. As coronaviruses and their components are not only found in saliva of the infected person but also in their excrements, although less frequently in the urine, virus materials are known to end up in the sewer system. As far as we know so far, this route does not add to the risk of spreading the infection.

Several researchers around the world, including Prof. Dr. Susanne Lackner from the Technical University Darmstadt, Germany, have been able to show that the quantities of viral RNA detected in sewage reflects the course of the virus spread, and does so faster than the tests conducted on individuals affected. Like the clinical diagnostics, the wastewater analyses rely on PCR tests. With additional sequencing methods, the presence of virus mutants can also be detected in the sewage.

The idea of monitoring wastewater isn’t a new one. Ten years ago, the World Health Organisation (WHO) included sewage tests for poliovirus in its programme aiming to eradicate polio. Among experts, the term “wastewater-based epidemiology”, or WBE for short, has become widely accepted. The state of Hesse has recognised the potential of this approach and supported a project at the TU Darmstadt between June and December 2020 with funds of the state and from the European Fonds for Regional Development totalling 270,000 euros.

With the online conference, Technologieland Hessen aimed to achieve a stronger connectedness between the different European research initiatives in this field, as well as a

more profound cooperation between science and industry. The event began with a digital science session covering five European research projects. The second day saw presentations of different problem-solving approaches along the process chain. At the end, an expert panel discussed the challenges and opportunities of wastewater monitoring.

## 3 European research projects introduce themselves

Science session on February 9th, 2021

***On the first day of the online conference, researchers from Germany, the Netherlands, Switzerland, Great Britain and Sweden discussed the current status of their research into SARS-CoV-2 in sewage.***

### 3.1 SARS-CoV-2 in the sewage of Frankfurt (Main), Germany

*Prof. Dr. Susanne Lackner and Dr. Shelesh Agrawal (Darmstadt, Germany)*
*Technical University of Darmstadt, Department of Civil and Environmental Engineering, Wastewater Engineering at Institute IWAR*

The project of Professor Susanne Lackner at TU Darmstadt deals with SARS-CoV-2 particles in sewage from the metropolitan region of Frankfurt. The researchers analysed 24h-composite samples taken twice a week to represent the input from treatment plants serving 1.8 million residents of the districts Sindlingen and Niederrad, as well as a partial sewage feed of the district Griesheim. In addition, the researchers also analysed wastewater produced by Frankfurt Airport, in order to find out more about the origins and pathways of viral spread in the area. However, Lackner didn’t cover this last part of the project in her talk, as it is currently limited by the severely reduced volume of air traffic.

After concentrating the samples by filtration with electronegative membranes or by ultracentrifugation, the researchers isolated viral RNA and detected it using quantitative PCR (qPCR). Even during the period of low case rates in the summer of 2020 they could detect the viral genome in most samples. Thus, the method was proven to be sufficiently sensitive. The amount of viral RNA detected in wastewater reflects the case numbers reported by the Robert Koch Institute (RKI). A key advantage is that trends – such as a rise or fall in infection rates – become apparent from the wastewater up to ten days earlier than in the clinical test results.

Wastewater also carries information about the SARS-CoV-2 mutants in circulation. Genome sequences obtained from Frankfurt wastewater between October and December showed a shift towards the mutant first detected in the UK, which is associated with higher infection

rates. The researchers also detected other mutants that have been described in clinical cases in other countries but had not been detected in patients in Germany before.

Asked if the amount of viral RNA in the wastewater makes it possible to calculate the number of infected individuals, Lackner replied that this is not yet possible and requires further investigation. For instance, it is not yet known how many virus particles an infected person releases into the wastewater stream in a given stage of the disease.

### 3.2 SARS-CoV-2 in the wastewater in the Netherlands

*Dr. David Weissbrodt and David Calderón (Delft, Netherlands)*
*Delft University of Technology, Faculty of Applied Sciences, Environmental Biotechnology*

In the Netherlands, there are several initiatives dealing with the detection of SARS-CoV-2 in wastewater. Scientists at the water research institute KWR at Nieuwegein are among the pioneers in this field. They started as early February 2020 to analyse wastewater in search of the new virus, although the first confirmed case of COVID-19 in the Netherlands was only reported at the end of that month. Accordingly, the use of the datasets from wastewater has already reached an advanced stage. The Dutch government already uses this data to complement clinical tests in its national COVID-19 surveillance strategy. The official coronavirus dashboard of the Netherlands, which is freely accessible online, includes a map updated weekly with details of SARS-CoV-2 in the wastewater of different regions. The survey includes data from 318 water treatment plants run by 21 wastewater authorities.

Using Amsterdam as an example, the researchers from Delft showed that measures like the lockdown of spring 2020 and the partial lockdown in the autumn, but also the re-opening of bars in the summer leave their traces in the wastewater. In these studies, too, the changing trends appeared in the wastewater much earlier than in the clinical data.

Since September 2020, the Dutch researchers have normalised the detected amounts of viral RNA with respect to the resident population and have been publishing their results in terms of viral particles per 100,000 residents. They were also able to detect mutants in the wastewater, including some that had not yet been identified in patient samples.

David Calderón described the analytical methodology in detail. Among other things he recommended the sample preparation using electronegative membranes. He advised against the use RNA purification methods in several steps, as these tend to lose too much of the RNA. Before PCR, the RNA is not necessarily to be transcribed to cDNA. The PCR test used in Delft is based on three different genes (the spike gene S, the nucleocapsid gene N, and the ORF1ab gene, which plays an important role for the replication of the viral RNA. In wastewater analyses (like in clinical tests) one has to consider the possibility that

coronaviruses might come up with mutations in the genes targeted by PCR, making them invisible to one specific type of test.

### 3.3 SARS-CoV-2 in the wastewater of Zurich and Lausanne

*Dr. Tim Julian (Dübendorf, Swiss)*
*Eawag (Swiss Federal Institute of Aquatic Science and Technology), Department Environmental Microbiology*

Swiss researchers at Eawag and EPFL (Ecole Polytechnique Fédérale de Lausanne) were among the first teams in Europe to investigate the detection of the novel coronavirus in wastewater samples. Since the end of February 2020 they have been analysing 24h-composite samples from the feeds of the water treatment plants at Zürich Werdhölzli und Lausanne-Vidy. By now, they are receiving these samples on a daily basis.

The first infection with SARS-CoV-2 in Switzerland was confirmed on February 25th. Since then, the course of the pandemic there has resembled the one in Germany. Confirmed case numbers were low in the summer, rose significantly in October, and then started to fall again. The amounts of viral RNA detected in Swiss wastewater samples reflect this trend.

Dr. Tim Julian emphasised that the data from wastewater – like the numbers of clinically detected cases – come with a degree of uncertainty, because, among other things, the virus material shed by infected people varies and is distributed unevenly across the time span of the disease and possibly beyond. In order to calculate case numbers from wastewater data, one has to take this so called shedding load distribution into account. Julian presented models that incorporate this aspect.

The Swiss wastewater-based datasets are now due to be included in the calculation of the reproduction number (R value). So far, the team of mathematician Prof. Dr. Tanja Stadler at the ETH Zurich has been modelling the R value based on the case numbers published by the Swiss Federal Health Office. Due to the delay between infection and a positive test result, the R value has been lagging behind the current course of the disease spread. The wastewater researchers at Eawag and EPFL are now cooperating with Stadler in order to calculate a near real-time R value from the wastewater results.

The Swiss researchers are also looking into the detection of mutant coronaviruses. In wastewater samples collected in a Swiss ski resort in December, they were able to detect the UK variant of the virus before the Federal Health Office had confirmed any such cases.

### 3.4 SARS-CoV-2 in wastewater in Great Britain

*Dr. Andrew Singer (Wallingford, England)*
*UK Centre for Ecology & Hydrology*

In Great Britain, the research into SARS-CoV-2 in wastewater forms part of the National Wastewater Epidemiological Surveillance Programme, in which universities and research institutes as well as the water companies and authorities take part. England, Scotland and Wales have set up their own SARS-CoV-2 monitoring projects. England, for instance, covered 80 percent of its population with 44 sampling locations, Scotland half the population with 28 locations. In Wales, wastewater samples are taken in 21 locations.

Using England as an example, Dr Andrew Singer made clear that the amount of SARS-CoV-2 particles in wastewater reflects the spread of infections. The effects of school closures this winter, for instance, resulted in a decrease in the amounts of viral RNA in wastewater.

In addition, the researchers investigated the wastewater of 24 primary and secondary schools in the English municipalities of Pendle, Exeter, Bristol and Brent. Between October and December, none of the schools was spared cases of infection, as the wastewater samples revealed. In the beginning, every other sample contained virus particles. With the spread of the more infectious UK variant of the virus, the fraction of positive results rose to 75%.

In the course of the English project TERM, the wastewater of twelve prisons in the North of England will be monitored for the presence of SARS-CoV-2 and other pathogens. The wastewater from care homes is also due to be sampled. As most residents of British care homes for the elderly have already received at least one dose of a Covid-19 vaccination, wastewater analytics can help to monitor the efficacy of the vaccination programme in care homes.

Taking samples from sewers of schools and other institutions isn’t all that simple, says Singer, as automatic sampling devices aren’t designed for this situation. Further challenges he identified include the privatisation of the English water providers leading to a lack of transparency, as well as Brexit and the resulting delivery problems affecting, for instance, analytical kits.

### 3.5 SARS-CoV-2 in the wastewater of Stockholm

*Prof. Dr. Zeynep Cetecioglu Gurol (Stockholm, Sweden)*
*KTH Royal Institute of Technology, Division of Resource Recovery, Bioconversion Group*

Inspired by the pioneering work of Dutch researchers, the team of Prof. Dr. Zeynep Cetecioglu Gurol from the KTH Royal Institute of Technology in Stockholm started to

investigate wastewater samples for SARS-CoV-2 in April 2020. As Gurol previously worked with wastewater but not with viruses, she teamed up with the cancer researcher Prof. Dr. Cecilia Williams at KTH, who is an expert in the bio-analytical methods required.

The KTH researchers analysed wastewater samples from the three Stockholm water treatment plants Bromma, Käppala and Hendriksdal. Samples underwent centrifugation and ultrafiltration in Gurol's lab and then transferred to Williams' team for RNA isolation and amplification by PCR. As an external control, the researchers used a bovine coronavirus added to the samples, while pepper mild mottle virus served as an internal reference. This plant virus is the most abundant RNA virus found in human excrements.

The amount of viral RNA in the wastewater of Stockholm traces the spread of infection well and closer to real time than the case numbers obtained from patient diagnostics. Thus, the second wave of the pandemic already became apparent in wastewater samples at the end of August (week 35), while the officially confirmed case numbers only started to increase from week 38 onwards. Samples from weeks 40, 41 and 42 showed only small amounts of viral RNA. The reason for this glitch was not related to the pandemic itself, as Gurol explained, but to the storage of the samples. Due to delays affecting the delivery of reagents, the samples could not be analysed immediately but were stored at -20°C, which led to the loss of some genetic material.

The quantification of virus particles and the normalisation of analytical methods remains a challenge, as Gurol emphasised. Nevertheless she considers wastewater monitoring suitable for the prediction of pandemic waves and for the surveillance of infections in care homes and other institutions.

## 4 Transferring wastewater monitoring from research to practical application

Best practice talks on February 10th, 2021

***On the second day of the conference, representatives of companies and other institutions discussed the specific challenges of monitoring wastewater for SARS-CoV-2 and presented solutions along a possible process chain, which could accelerate the transfer from research to application.***

Monitoring wastewater for SARS-CoV-2 captures many people at once with very limited effort – including, as some studies indicate, both patients with disease symptoms and those who show little or no signs of disease. After retrieval of samples from the sewage system, analyses are carried out based on the PCR detection of specific genes. Detection of variants

requires additional sequencing technology. The processed data will have to be transferred to the relevant health authorities, but, at least in Germany, there is no established protocol for this.

### 4.1 Digital models of the sewage system

To identify suitable sampling locations and make the best possible use of the wastewater data, one needs detailed knowledge of the sewage system. Dr. Oliver Kraft from BGS Wasserwirtschaft GmbH at Darmstadt presented computer models from residential water suppliers, which represent sewer networks. Pollution with ammonium, nitrite or traces of pharmaceuticals has already been tracked with such models. They can also help to identify the hotspots of viral load in wastewater. The models can also take into account local emitters like hospitals.

Jan Hanken, founder of the Frankfurt-based company idatase GmbH, emphasised the complexity of the sewer system. The total length of Germany's sewers adds up to 600,000 km, which is 1.5 times the distance to the Moon. Moreover, the structures have grown gradually with the settlements they serve. In Frankfurt, more than 10% of current sewers are more than 100 years old. idatase develops "digital twins" of complex networks, in order to facilitiate their maintenance and administration. Such digital twins could also be created for sewage systems, which could help to plan sampling. Relevant infrastructure information including water treatment plans, sewers, wastewater emitters, as well as topological data would be accommodated in the model.

### 4.2 Tools of bioanalytics

The foundation of a reliable system of wastewater monitoring is analytics. Wastewater analysers – like clinical diagnosticians – have to take into account the possibility that mutated viruses could evade the PCR test, as Dr. Rolf-R. Marell from BAG Diagnostics GmbH from Lich emphasised in his talk. He referred to SARS-CoV-2 as a moving target and also pointed to the problems that can arise from other pathogens present in wastewater and from PCR inhibitors.

Nicolai Wilk and Dr. Thea Ziegler from Thermo Fisher Scientific GmbH at Darmstadt explained how the analytics industry meets these challenges. They emphasised that both the PCR systems and the sequencers of their company are suitable for wastewater monitoring. As the RNA is isolated from the wastewater, the sample matrix isn't a key problem, Ziegler said. For the removal of inhibiting substances, magnetic beads and other special techniques are available, although their suitability for wastewater samples remains to be tested. Reassuring news regarding the problems of mutant viruses: Commercial Covid-19 assays

target several different viral RNA fragments and thus have an in-built redundance. The company plans to introduce a new PCR kit enabling customers to choose the target genes, so they can address specific mutations that may be spreading in their area.

But where do we expect which mutations to occur and how are they distributed geographically? Dr. Richard Neher from the Biozentrum of the University of Basel, Switzerland, presented a helpful tool that can answer such questions, namely the software Nextstrain. Based on entries in sequence databases (eg GISAID, which Neher's group uses), Nextstrain analyses the tree-like transmission networks of the mutants and displays them graphically, such that users can call up the regional spread of a given mutant.

### 4.3 Data management: from the wastewater lab to the health authorities

Professor Dr. Gérard Krause from the Helmholtz-Centre for Infection Research at Braunschweig presented another digital tool that has already proved itself in the fight against the pandemic. The „Surveillance Outbreak Response Management and Analysis System", or SORMAS for short, was originally developed for the management of contacts during outbreaks of Ebola in Africa. Based on information fed into the system by doctors, laboratories, infected patients and other relevant participants, SORMAS analyses infection chains and can automatically issue findings and quarantine orders. SORMAS was expanded with a new module specific to Covid-19, which has already been implemented in Ghana and Nigeria, before the first cases of Covid-19 in Africa had been detected. Around half of Germany's local health departments have at least installed the system, and it is also used in France, Switzerland and Fiji. Krause emphasised that it is relatively simple to include data obtained from wastewater monitoring within SORMAS. When the laboratories analysing the wastewater are defined as SORMAS users, they can feed their results directly into the system.

In short, there are already various tools available to make wastewater monitoring efficient and transfer it from research to practical application. The workshop helped to promote the urgently required exchange of information between the participants on different stages and to inspire cooperations.

# 5 Use of wastewater monitoring for the containment of SARS-CoV-2 and other pathogens

Conclusion and outlook

***Researchers around the world agree that wastewater monitoring can contribute to coping with the Covid-19 pandemic. Nevertheless, some obstacles remain to be overcome. The effort is worthwhile, especially because the methods can be transferred to other pathogens. This was the conclusion of the panel discussion at the end of the online conference.***

The potential of wastewater monitoring for SARS-CoV-2 is undisputed in the scientific community. As Prof. Dr. Susanne Lackner from the TU Darmstadt highlighted in the final panel debate, the greatest opportunity it provides is the chance to detect changes in the rise and fall of infection rates much more quickly than with clinical testing strategies. In addition, using modern methods of genome sequencing, virus mutants were discovered in wastewater even before they had been detected clinically.

Data from wastewater monitoring do not enable the calculation of the number of infected people yet. More research is needed in this area. Thus, it is not yet clear, how many virus particles an infected person excretes over what timescales. Such gaps in our knowledge will gradually close, as there are numerous initiatives that are dealing with SARS-CoV-2 in wastewater. It would be an advantage if the different monitoring projects could swiftly agree on shared protocols and procedures. Only then would datasets be comparable. The lack of standardisation is one of the reasons why Germany and many other countries have already collected large amounts of data from wastewater, but haven't used these to fight the pandemic. It would also be desirable to have as much data transparency as possible, to ensure that as much information as possible can be generated from the wastewater.

## 5.1 Global standardisation of analytic procedures as the biggest challenge

The presentations from five European research projects in the course of this event have shown that the procedures are fundamentally similar, but differ in detail, from the sampling procedure (different volumes and timings of the sampling) via the processing of samples (different methods for concentration, isolation and purification of viral RNA) through to the analytical method (RNA vs. cDNA used for PCR, use of different kinds of external and internal controls).

Thus, one of the biggest challenges is to optimise and standardise the analytical procedure including sample preparation. This is not only a task for the researchers developing the

methods, but also involves the producers of reagents and lab equipment, whose products are used in wastewater monitoring and who are developing tailored systems for this purpose, such as specific test kits. This is a topic of global relevance, and the companies involved are operating globally, emphasised panelist Dr. Peter Quick, CEO of Promega GmbH at Walldorf and chair of the division Life Science Research at Verband der Diagnostica-Industrie. The companies cannot develop separate solutions for every country. Therefore, international standards have to be introduced quickly. Until then, the requirements of large markets are a guide. If, for instance, the CDC (Centers for Disease Control and Prevention) in the US recommends a specific procedure, this deserves careful consideration, Quick said.

### 5.2 Germany has to catch up

The science session on the first day made clear that the different European projects have reached very different levels. Wastewater monitoring is most advanced in the Netherlands, where the data are already routinely used by the government. Germany isn't very far advanced in this respect, as Quick remarked.

Optimism for Germany can be drawn from the fact that all stakeholders involved are willing to cooperate. This includes the operators of water treatment plants, who play a key role in providing the samples for analyses. Thus, Dr. Susanne Schmid, head of the department for water treatment in Frankfurt, explained in the final panel discussion that her department is happy to provide samples as requested by researchers. The scientist can for instance choose if they want a sample from a specific timepoint or a composite sample covering a time span. Moreover, the sewer network is well documented, such that the department can identify suitable sampling locations together with the researchers.

### 5.3 Preventing future pandemics

Quick pointed out that data from wastewater must be communicated to politicians and the general public in an accessible way. In the panel discussion, Prof. Dr. Richard Neher from Basel even called for a maximum of transparency. Why not publish all genome data from the wastewater samples online? Data analysts might detect something unexpected in there.

At the moment, all eyes are on SARS-CoV-2, but the current insights can be transferred to other pathogens. The generalised, long-term goal is to recognise viruses and other germs in wastewater sufficiently early such that regional protection measures can be used to contain its spread. A dramatic situation like the one produced by SARS-CoV-2 just now should urgently be prevented. For this, wastewater monitoring is a suitable method.