Literature screening report

Wastewater-based surveillance of SARS-CoV-2 to help public health decision-making: unravelling the extend of the network and outcomes

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Abstract

This literature screening reports that the implementation of national wastewater surveillance system for COVID-19 have been performed in many countries around the world. We thus focus our research on countries from Europe, America and Africa

The main reported goals of the studies and the national reports are:

- Implementation of an early warning system to anticipate the peaks of hospitalization
- Tracking trends in infection within a community
- Detection of new variants (before they are detected in the community) and spatio-temporal change in variants abundance
- Detection capacity where active cases have not been reported or where endemicity is low

Some important considerations concerning the sampling strategy, the methodology and the factors to be taken into account to estimate the concentration of virus in wastewater are also presented.

Globally, the analysed studies highlight the importance of such surveillance in complement to the epidemiological data to help public health policy. Wastewater monitoring seems to be a promising approach to detect unexpected health threats before they evolve into epidemics (Keshaviah et al 2021).

Content

Preamble

A large number of scientific publications become available on a daily basis, reflecting the rapid development of knowledge and progress of science on COVID-19 related issues. Leading authorities should base decisions or policies on this knowledge; hence they need to master the actual state of this knowledge. Due to the large number of publications shared daily, decision makers heavily depend on *accurate summaries of these publications, in the different public health domains. Therefore, the authors of this report were mandated by the Swiss School of Public Health plus (SSPH+), on request of the Federal Office of Public Health (FOPH), to inform the FOPH on recent findings from the literature.*

Background

Since the beginning of the pandemic in mid-2020, the use of wastewater-based epidemiology as a tool to monitor SARS-CoV-2 circulation arose among researchers. Precursors research groups rapidly began pilot studies for viral monitoring and highlighted the link between environmental and clinical datasets. Few months later, in August 2020, WHO published a first scientific brief[1] describing the potential of environmental testing for the surveillance of SARS-CoV-2 and the potential considerations for its implementation. In September 2020, CDC launched the National Wastewater Surveillance System (NWSS) to coordinate wastewater surveillance at the national level[2]. In November 2020, an expert consultation related to surveillance of SARS-CoV-2 was organized by WHO Europe[3] which led to the adoption of a recommendation on monitoring COVID-19 and its variants in wastewater in the EU[4] by the European Commission, in March 2021. Now, the question to which extent countries monitor the SARS-CoV-2 takes a particular dimension as the early detection of the emergence of new variants is critical.

The number and proportion of persons infected with SARS-CoV-2 are mainly determined based on individual testing and laboratory-based bio-molecular diagnostics. It is therefore expected that the actual infection extent in a specific region can only be very roughly estimated because non-detected cases contributes significantly to the spread of the infection. Recently, various studies detected SARS-CoV-2 RNA in wastewater worldwide (see details in this report). Although the viral RNA was detected in wastewater, it did not imply that SARS-CoV-2 coronavirus was current active. In fact, viruses are often killed or inactivated during water treatment or purification processes (Giordana Rimoldi et al. 2020). **Virus concentrations are affected by a number of environmental processes categorized into two major processes of dilution and decay. Dilution is driven by seasonal dynamics of water flows, the decay of viruses in receiving waters it is governed by environmental factors such as heat, light, dryness, pH, salinity, ammonia, organic matter, microbial activity, and biofilms** (Kumar et al., 2022). Wastewater-based epidemiology (WBE) is a promising approach that has been used to analyze a large number of markers in wastewater treatment plant (WWTP) to characterize emerging chemicals, drug use patterns, or disease spread within communities (Choi et al. 2018). This surveillance allows understanding the status of disease outbreaks by monitoring: the presence of viruses (e.g. SARS-

[1] https://www.who.int/news-room/commentaries/detail/status-of-environmental-surveillance-for-sars-cov-2-virus Accessed 19/01/2022
[2] https://www.cdc.gov/mmwr/volumes/70/wr/mm7036a2.htm Accessed 19/01/2022
[3] https://apps.who.int/iris/bitstream/handle/10665/339487/WHO-EURO-2021-1965-41716-57097-eng.pdf Accessed 19/01/2022
[4] https://ec.europa.eu/jrc/en/news/coronavirus-commission-adopts-common-approach-track-covid-19-through-wastewater-monitoring Accessed 19/01/2022

CoV-2, other respiratory virus such as influenza A and enteric viruses) (Gonzalez et al. 2020; Heijen and Medema et al. 2011). WBE, when combined with clinical datasets, can be used as an early warning method to inform about the efficacy of the current public health interventions (Kumar et al. 2020).

Questions addressed

- 1. Which countries monitor SARS-CoV-2 through wastewater testing?
- 2. What general experience do these countries have with wastewater investigations?
- 3. Are wastewater surveillance systems integral part of national surveillance and public health systems?
- 4. What were the most relevant outcomes for public health
- 5. In particular, what conclusions relevant to public health could be drown?

Methodology

A literature search was carried out to collect evidence from peer-reviewed studied, government agencies and national sanitary agencies reports. The research focussed on national reports of SARS-CoV-2 surveillance in wastewater on the period from 2020 to nowadays, using the query TI=(SARS-CoV-2 or COVID-19 or coronavirus) AND TI=(wastewater or sewage) AND CU=(“country”) in PubMed and Web of Sciences, in Europe, North America and Africa.

The structure of the review is organized by geographical areas (Europe, America, Africa). Each geographical area is presented in tables (Synoptic tables) reporting the main results of the scientific papers and a summary of the national reports (if available in English or translated via google translation).

Results and Findings

1. Which countries monitor SARS-CoV-2 through wastewater testing?

Summary:

This report focusses only on countries from America (USA, Canada and South America), Europe and Africa. In total, our literature search allowed to identify 21 European countries which have monitored SARS-CoV-2 in their Waste Water Treatment Plants (WWTPs) and 4 African countries. Concerning the USA, we identified different studies from 12 states. Three studies were carried out in South America and 3 in Canada.

Results:

The details on the studies having performed WBE are in the synoptic tables 1 to 6

2. What general experience do these countries have with wastewater investigations?

Summary:

The concept of using wastewater-based epidemiology (WBE) quickly emerged at the start of the pandemic in early 2020 to monitor SARS-CoV-2 in communities. In fact, WBE is a relatively new approach as the methodology was outlined for the first time in 2001 as a tool to evaluate licit and illicit drug use within a community (Daughton, 2001). However, wastewater has been used decades ago to monitor the progress of poliomyelitis vaccination, allowing the identification of “silent” circulation or introduction of wild and vaccine-derived polioviruses into the population in the absence of clinical cases (Blomqvist et al., 2012). Since 2005, the WBE discipline rapidly develop with the goal of monitoring real-time data on geographical and temporal trends by chemical or biological markers detection (i.e. pathogens, antibiotic resistant genes, pharmaceuticals and other markers characterising human lifestyle and health)

Results:

General experience with water investigation can be divided in 4 categories

1. **Countries with research lab having a strong expertise in water testing**

Since the development of wastewater surveillance, various project such as acute gastro-enteritis prevalence and genotyping or Hepatitis E surveys in wastewater (Miura et al., 2016; Ozawa, Yoshida, Usuku, & Schaffner, 2019; Prevost et al., 2015) shown the validity of quantitative surveillance of viral epidemics. Before pandemics, several sewage surveillance network such as European network called Sewage Analysis CORe group Europe (SCORE), Global Sewage Surveillance Project, Global polio monitoring network and NORMAN network existed across the world.

As soon as viral excretion in faeces and SARS-CoV-2 genome were described, these laboratories rapidly set up pilot studies to develop consistent methodologies to detect and quantify SARS-CoV-2 viral particles in sewage. Driven by the preliminary results obtained in Italy, Spain, Australia, Netherlands, USA, France and Pakistan; WHO published a first communication “Status of environmental surveillance for SARS-CoV-2 virus” in August highlighting the potential of WBE for early warning of viral circulation and surveillance in limited clinical surveillance. WBE became a complementary tool for epidemic monitoring and collaboration between national laboratory, universities and private laboratory arose to provide strong surveillance networks.

2. Countries having a surveillance network under the umbrella of international organisations

As mentioned previously, environmental testing has a long history of use in public health, particularly for polioviruses and other enteroviruses. Through the Global Polio Eradication Initiative (GPEI), coordinated by WHO, 146 WHO accredited polio laboratory through 92 countries have been implemented across the world. Additionally to clinical testing, sewage samples or other environmental samples are tested to provide insights into the international spread of poliovirus, particularly in areas with suboptimal polio surveillance, inadequate immunization activities or areas with a high-risk of wild-poliovirus importations (Zaidi, Asghar, Sharif, & Alam, 2016).

In African regions, polio teams are supporting COVID-19 response efforts (World Health Organization, 2020). Polio surveillance field and laboratory staff were repurposed to the COVID-19 response, as recommended by the GPEI, and surveillance staff and logistics supported COVID-19 surveillance, contact tracing and data management. But the lack of qualified personnel and equipment has prevented many countries from carrying out environmental monitoring either for COVID-19 nor polio. However, the use of existing environmental surveillance network has shown its effectiveness in Pakistan. Using routine polio environment surveillance samples, this team has monitored efficiently viral circulation of SARS-CoV-2 in settings where person to person testing capacity is limited (Sharif et al., 2021).

3. Countries involved in international projects.

Capacity building projects were written to perform SARS-CoV-2 monitoring in Low and Middle Income Countries (LMICs). As example, the University of Bath obtained a grant for the project entitled "Building an Early Warning System for community-wide infectious disease spread: SARS-Cov2 tracking in Africa via environment fingerprinting". This project able South Africa and Nigeria to implement a surveillance strategy in their respective countries.

4. Countries with no general experience

Many countries do not have a strong experience in wastewater investigation. Following international recommendations such as the European Commission recommendation of 17.3.21 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU, they implemented a wastewater network surveillance more or less deployed in their countries.

3. Are wastewater surveillance systems integral part of national surveillance and public health systems?

Summary:

At the time of writing the manuscript, wastewater surveillance systems are more and more becoming part of surveillance and public health systems. Most of the time, this monitoring consists to measure viral concentration weekly or twice a week in samples collected in WWTP over the country. The results are often available to the public via a dashboard on a public website. Sometimes the website is dedicated to the COVID-19 with a lot of other information including the follow-up of the clinical cases and sometimes the website is entirely dedicated to the wastewater surveillance.

Results:

Concerning USA, in response to the COVID-19 pandemic, The Center for Disease Control and Prevention (CDC) and the US Department of Health and Human Services (HHS), initiated the National Wastewater Surveillance System (NWSS, September 2020). Many US states (43 as of August 2021) have joined this surveillance system, sharing the wastewater testing data into a common database (Kirby et al., 2021). Harmonized sampling and testing methods ensure data comparability across jurisdictions. The data generated by NWSS will help public health officials to better understand the extent of SARS-CoV-2 (the virus that causes COVID-19) infections in communities. (https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html).

In Europe, wastewater monitoring is usually performed with the support of public health systems. Network of national or university research lab and even private companies, aggregate their results under the governance of reference laboratories. Following the recommendation of European Commission for wastewater surveillance in march 21, numerous countries began a surveillance from summer 2021. Actually, each country published its own dashboard for SARS-CoV-2 detection, using their respective methods of viral concentration, quantification and calculation methods (Figure 1). **The details concerning the goals and the methodology of national surveillance of these European countries are in synoptic tables part 1. Table 1 and a summary of each country with the Website link are also available in Synoptic tables part 2.**

Figure 1 Available Dashboard in Europe. Retrieved from COVIDPoops19 accessed 30.01.21

In absence of recommendation for monitoring implementation such as CDC or European Union recommendation, COVID-19 surveillance is generally not implemented on national surveillance systems. Brazil (Prado et al., 2021) (Fongaro et al., 2021) and Argentina (Iglesias et al., 2021), in the south part of America, were involved in SARS-CoV-2 surveillance at local level, in collaboration with health departments. National University of Mexico (Universidad Nacional Autonoma de Mexico) monitored two wastewater treatment plants, demonstrating a trend between SARS-CoV-2 RNA quantification and cumulative COVID-19 confirmed cases (Carrillo-Reyes, Barragán-Trinidad, & Buitrón, 2021). Canada performed few studies on SARS-Cov-2 monitoring in wastewater but they were not integrated on a national surveillance system. Results shown a correlation between SARS-CoV-2 found in sewage and clinical cases but were not strong enough to generate a relevant outcome (D'Aoust et al., 2021); (Hinz et al., 2021), however they have been used to generate wastewater-based epidemic models (Nourbakhsh et al., 2021). Interesting approach was the real-time monitoring of hospitals and build environment wastewaters to see the correlation between results and hospital-acquired cases and outbreaks (Hinz et al. 2021; Acosta et al. 2021).

4. What were the most relevant outcomes for public health

Summary:

The most relevant outcomes for public health could be illustrated with a figure published in a review of the literature on the same topic as this one (Bonanno-Ferrara et al. 2021) (Fig 2).

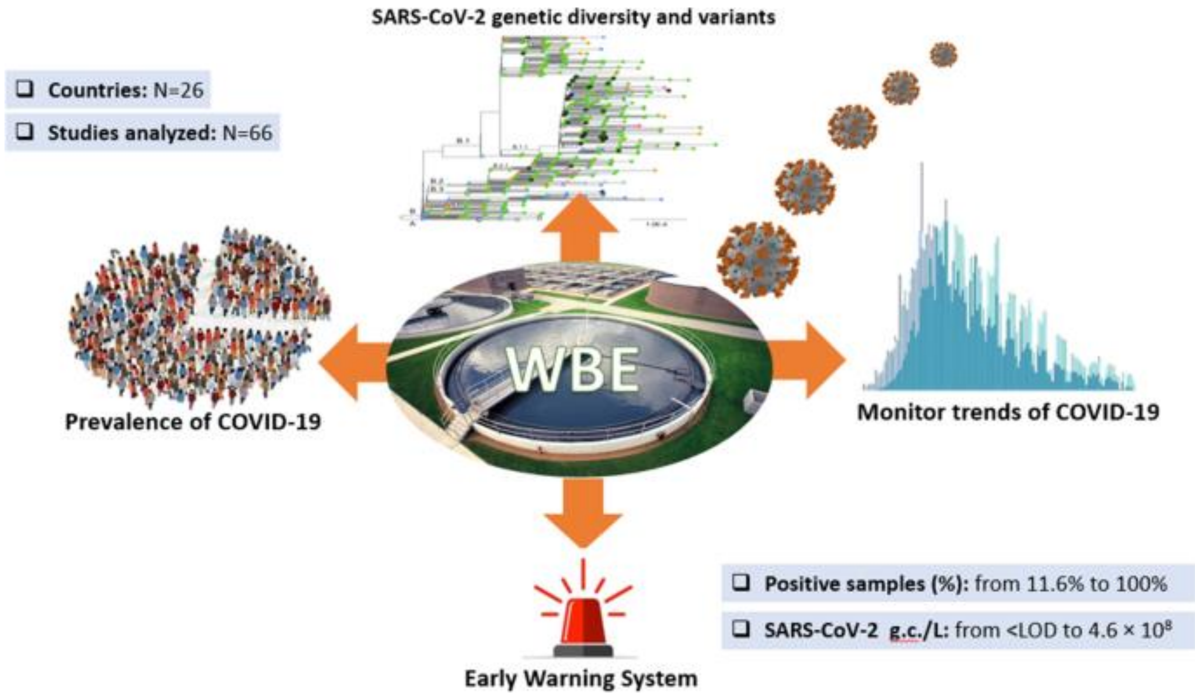


Figure 2. Waste water based epidemiology (WBE) as a tool for monitoring spread of SARS-Cov-2 within a community

Concerning the sampling strategy, it could change depending on the pandemic phase and the objective as summarized in the figure published by Keshaviah et al 2021.(Fig 3)

	Pandemic phase			
	Initiation	Acceleration	Deceleration	Preparation for future pandemic
Hypothetical number of cases				
Measurement objective	Detection	Quantification	Quantification	Detection
Sample frequency	Weekly	At least 2 to 3 times per week	At least 2 to 3 times per week	Weekly
Sampling prioritization	Sentinel sites	Vulnerable populations	Transmission nodes	Sentinel sites
Sampling method	Composite sample or grab sample	Flow-based composite sample	Flow-based composite sample	Composite sample or grab sample

Figure 3: Design considerations for wastewater surveillance in communities at different stages of a pandemic. The figure provides recommendations for sampling frequency, location, and type during different phases of disease threat prevalence, defined using the Pandemic Intervals Framework from the Centers for Disease Control and Prevention.

Results:

Results from USA: Only results arised from consolidated national surveillance and public health systems are presented.

A) Monitoring the presence of infection within a community

Detected presence/absence, rather than quantity of SARS-CoV-2 in wastewater could be useful when community transmission was low. Considered the limitations due to sampling design, sensitivity of the test used and the amount of SARS-CoV-2 being shed by the community, found RNA of SARS-CoV-2 in wastewater meant that there was at least one individual infected by COVID-19 (Keshaviah, Hu, & Henry, 2021).

B) Tracking trends in infection within a community.

Table 7. Trend results between WW (wastewater) data and confirmed cases of SARS-CoV-2 infection in USA. Wastewater trend classification was the statistical analysis of changes in the normalized concentration of SARS-CoV-2 in wastewater. To calculate the trend, a non-parametric statistics such as regression analysis was used.

Country	SARS-CoV-2 target/s quantity	Confirmed cases	Statistics (ww data vs cases)	References
Ohio	Copies/L	5-days moving average	Non parametric Spearman rank correlation (Spearman average r = 0.85)	(Ai et al. 2021)
Wisconsin	Copies/L	7-day moving average	Non parametric Spearman rank correlation (r = 0.59 to 0.87)	(Feng et al. 2021)
California	Copies/L	7-day moving average	Non parametric Kendall's Tau-b coefficients (t 0.43; p<0.0001)	(Greenwald et al. 2021)
Minnesota	Log (copies/L)	New confirmed cases	Pearson's correlation (r = 0.39)	(Melvin et al. 2021)
Utah	Copies/mL	New Covid-19 cases/100.000 inhabitants	Non parametric Spearman rank correlation (Spearman average r = 0.54)	(Weidhaas et al. 2021)
Texas	Log (copies/day)	Positivity rate	Non parametric Spearman rank correlation (r = 0.45 to 0.89)	(Stadler et al. 2020)

C) Early warning system

Detected SARS-CoV-2 in the environment before the identification of clinical cases, or a rise in SARS-CoV-2 concentrations in the environment before these trends became visible in the numbers of cases (Bonanno-Ferraro et al. 2021).

Table 8. Predictive results between WW (wastewater) data and confirmed cases of SARS-CoV-2 infection in USA

Country	SARS-CoV-2 target/s quantity	Confirmed cases	Statistics (WW data vs cases)	Results	References
Montana	Log (copies/L)	Frequencies of reported case and symptom onset	Local polynomial regression and Pearson's correlation	SARS-CoV-2 RNA concentration in the wastewater lagged behind symptom onset between 5 and 8 days (r = 0.989 -0.92), and preceded laboratory test results for individuals by 2-4 days (r = 0.969 - 0.953).	(Nemudryi et al. 2020)
Minnesota	Log (copies/L)	New confirmed cases	Pearson's correlation, lag analyses	Wastewater predicted confirmed new cases by 17d (r = 0.68, P < 0.001).	(Melvin et al. 2021)
Massachusetts	Copies/L	Positive PCR test confirmed	Approximate Bayesian computation	Before August 2020, the wastewater signal preceded clinical cases by approximately 6.2 days; after August 15, 2020, the wastewater signal was more in phase with clinical cases mean time lag 1.0 days	(Xiao et al. 2022)

D) Provides a non-invasive, near-real-time analysis of SARS-CoV-2 dissemination

Since the method do not allow for identification of individuals, the approach protect the anonymity of sample providers and prevent stigmatisation. Furthermore, it able to detect the presence of asymptomatic carriers in the community that would not be detected by traditional testing methods.

Results from Europe

In Europe, the results of 8 studies showed that a peak of concentration of SARS-Cov-2 in WW **preceded the peak of clinical cases between 6 days to 4 weeks**, while only one study did not find such precedence. Almost all the studies (14) having compared WW and epidemiological results, have found a positive correlation between the WW concentration of SARS-CoV-2 and the number of reported clinical cases. **Three studies succeeded in the identification of hot spot** in the community and **10 studies have carried out genetic investigations to estimate the proportion of new variants.** (Table 1 in annexe)

Results from Africa

Tracking virus circulation in settings where person to person testing capacity is limited: Environmental surveillance is a useful tool to detect viral spreading in remote or confined communities (Sharif et al., 2021). Furthermore, the potential of wastewater testing is high in settings showing a low number of tests per 100'000 persons leading to potential under-sampling, which could be related to the expense of testing or test availability.

5. In particular, what conclusions relevant to public health could be drown?

Summary:

Wastewater based epidemiology (WBE) for SARS-CoV-2 in conjunction with clinical data was useful for:

- Tracking changes or trends in virus concentrations.
- Provide complementary information on the extent of community infection and the effectiveness of public health interventions.
- Identify "hot spots" in areas, such as small rural regions, for which testing rates are low or when epidemics is decreasing.

- Sampling sewersheds directly from hospitals or buildings may allow for a more refined picture of infected individuals and pinpoint epidemic hot spot and local outbreaks. Although this approach had some limitations and needed further investigations.
- Detection of new variants

Results: see results above and in annexes.

Most of the studies worldwide give recommendations for the future and highlight the importance of WBE as a potential early warning tool for detection outbreak and offering cost-effective strategy for measuring prevalence. This could allow the implementation of timely response measures for the containment of outbreaks (Bonanno-Ferrero et al. 2021).

Beside these conclusions, a study from Switzerland (Huisman et al 21) propose a way to derive the **effective reproductive number (Re) from WW to track the dynamic of the COVID-19.**

It should be noted that the National Academy of medicine in France (National Academy of medicine, 2020), classify the detection on SARS-CoV-2 in wastewater as an urgent measure at a time of epidemic reflux. Indeed, in a phase of regression of the COVID-19 pandemic, wastewater analysis provides a major strategic tool which must be used to monitor the circulation of the virus and to detect early possible resurgence.

Synoptic tables

1. Studies using Wastewater testing in Europe, America and Africa

Table 1: Characteristics of studies from Europe: Sampling period (first wave ; second wave ; and other waves).Outcomes (anticipation of clinical peak cases; no anticipation of clinical peak; correlation between SARS-Cov-2 RNA in WW and clinical cases; identification of hot-spots;genetic:detection/abundances of new variants; estimation of Re). Quality of data according judgement of experts: +++ = internal control and big sample size and sophisticated data interpretation; ++ = internal control + correct sample size; + = not very original or part of a sub-project.

Country (ref)	Sampling period and duration	Goals/Outcomes	Measured parameters frequency	Number of WWTP Population	Quality of data	Derived measure/recommandations
France, Marseille (Wurtz et al., 2021)	6 months July-december 20	Quantification +correlation between SARS-CoV-2 RNA copies and + cases. No precedence observed	1 sample/day (collected 24h) Biofire kit: 3 different target sequences	2 sewer networks ≈ 1mio inhabitants	++	-
France, Paris (Wurtzer et al., 2020)	1 month, March 20	Quantification + correlation between SARS and fatal cases. Anticipation of clinical peak cases	Viral E gene	3 WWTP 3 mio	+	-
France, 10 departments (Lazuka et al., 2021)	5 months	Quantification + correlation between	1 sample every 2 weeks,	10 WWTP	++	Need to take into account average rainfall

	August-december 20	SARS and incidence in population	every week, twice a week N1 and N2 genes	2,85 mio PE		
France, Paris (Wurtzer et al., 2020)	2 months March-April 20	Quantification +correlation with asymptomatic and symptomatic patients. Virus could be detected before the the epidemic peak.	Daily samples E gene	3 WWTP 3 mio inhabitants	+	-
France, Montpellier (Trottier et al., 2020)	3 months post lockdown, May-July 20	Precedence 2-3 weeks before the peak of new positive patients	Weekly N1 N3 genes	1 WWTP 470'000 inhabitans	++	-
Germany, Frankfurt, (Shelesh Agrawal, Laura Orschler, & Susanne Lackner, 2021; S. Agrawal, L. Orschler, & S. Lackner, 2021)	1 day Dec 20	Mutation detection	Nearly full genome sequencing	3 WWTP Not mentionned	++	In future: insights into emerging variants.

Germany, Frankfurt, (Shelesh Agrawal et al., 2021)	5 months April- August 20	Quantification + correlation between SARS and registrered cases. Precedence observed. Spatio-temporal differences observed between different sampling points.	Twice a week N, S and ORF1ab gene	2 WWTP 1,82 mio PE	++	In future: Use as early warning of peak
EU : 20 european countries, (Agrawal et al., 2022)	1 day in March 21	Insight into the abundance and the profile of the mutation associated with the VOCs	NGS: Near-full genome sequencing	54 WWTP	++	Sequencing surveillance of WW samples should be considered complementary information to WGS of clinical samples
UK, London (Wilton et al., 2021)	12 months, January 20-January 21	NGS Detection of new variants (delta)	1 sample per month. RdRP and ORF8b NGS	1 WWTP 4 mio inhabitants	+++	In Future: Surveillance of VOCs
UK, London (Martin et al., 2020)	4 months January- May 20	NGS + quantification New variants	1 sample per month, RdRP and E-Sarbeco	1 WWTP 4 mio	++	In future : Change in virus variant predominance
UK, Wales, North, (Hillary et al., 2021)	5 months March-July 20	Quantification and V3 sequencing. Correlation	Weekly sampling N1 and MNV	6 WWTP 3 mio	++	In future: Measurment of the lockdown effective measure. Need to understand the factors affecting the SARS quantification in WW.

The Netherlands, (Medema, Heijnen, Elsinga, Italiaander, & Brouwer, 2020)	2 months 3 weeks before the first clinical case and then every weeks 2020	Quantification Sensitive tool to monitor the circulation of the virus Correlation with clinical cases	N1, N2, N3 and E genes	6 WWTP 3.723 mio	++	-
The Netherlands and Belgium, (Izquierdo-Lara et al., 2021)	70 days, March-June 20.	NGS, diversity of variants		20 WWTP	++	In future: Surveillance of diversity of variants
Sweden, Gotheburg,, (Saguti et al., 2021)	4.5 months Mid February-June 20	Quantification, Surveillance of peaks. Peaks in WW preceded 3 weeks the peaks of hospitalized patients Results showed that quantification of SARS-CoV-2 in WW maybe used to identify the prevalence of the virus in the population and predicted imminent hospitalization needs. Detection of local outbreak possible within a big city.	Daily samples pooled in weekly samples. RdRP gene	1 WWTP, 5 different sites 800'000 inhabitants	++	In the future: prediction imminent hospitalization and detection of local outbreak within a big city

Switzerland, Zurich, Lausanne, Alpine ski resort. (Jahn et al., 2021)	8 months July 20-February 21	Genomic sequencing of 122 samples. Searching of signature mutations B.1.1.7, B.1.351, and P1 variants. Estimate of the fitness advantage of the B.1.1.7 variant. Comparison with clinical samples. Detection of new variants in WW before observed in clinical samples		3 WWTP 700'000 inhabitants	+++	High sampling density across time and replicates sequencing are key elements to improve the signal-to-noise ratio of the sequencing data
Switzerland, Lausanne, Lugano and Zurich, (Fernandez-Cassi et al., 2021)	2 months March-April 20	Quantification. Correlation between SARS in WW and Clinical cases without precedence in Lausanne and with 6 days of precedence in Lugano	Daily samples, N1 and N2 genes	815'000 inhabitants	+++	-
Switzerland, Zurich and San Jose (USA). (Huisman et al., 2022)	4 months Sep 20 – January 21	Use of the dynamic of sars-Cov_2 RNA in WW to estimate Re (effective reproductive number) in near real-time, independent of clinical data and	Twice per week N1 and N2	2 WWTP	+++	In future: Deriving Re from WW offers an independent method to track disease dynamic

		without associated biases.				
Czech Republic, (Mlejnkova et al., 2020)	3 months April-June 20	Quantification Preliminary results Lower detection rate frequency than expected (compared to the clinical cases	Weekly Eligene COVID19 Basic A Rt kit.	33 WWTP 12.2 % of the total population	++	-
Slovak Republic, Bratislava, (Krivonakova et al., 2021)	7 months September 20- March 21	High correlation with positive individual numbers. Precedence of 2 weeks with positive infections number and of 4 weeks with number of death cases	50 samples in total (daily, or weekly or twice a week or every two weeks depending the period). E, RdRP and ORF1ab genes	2 WWTP 600'000 inhabitants	+++	Display mathematical correlation between tested WW samples, positive PCR tests and death cases. Elaboration of a mathematical modeling.
Hungary,Budapest, (Roka et al., 2021)	5 months June-October 20	Precedence of 2 weeks with the rise of positive infections number, then WW viral load correlated to the weekly new cases from the same week and the rolling 7-day average of active cases in the subsequent week	Weekly N1 gene	3 WWTP 1.8 mio	+++	In future: Efficient tool for early warning of outbreak detection. Correlation better during the uprising phase.

Austria, (Amman et al., 2022)	10 months Dec 20-September 21	Development and validation of a Variant Quantification in Sewage pipeline designed for Robustness (*VaQuERo*) Variant abundance, spatiotemporal dynamic of the dominant alpha and delta variants, detection of regional clusters	Weekly N1 gene amplification and sequencing	95 WWTP >60% Austrian population	+++	Framework to predict emerging variants *de novo* and infer variant-specific reproduction numbers from WW
Austria (Arabzadeh et al., 2021)	May 20 – Dec 20	Quantification + correlation active cases + identification of hotspots	24h composite samples, bi-weekly or higher nucleocapsid (N1)	23 WWTP ~2.3 mio hbts	++	the use of modelling for peak anticipation should be done with care
Liechtenstein (Markt et al., 2021)	September 20 to October 25, 2020	Quantification + SARS-CoV-2 whole genome sequencing	weekly basis, nucleocapsid (N1) gene	1 WWTP (11 communities)	++	-
Croatia (Vilibic-Cavlek et al., 2021)	December 2020 to February 2021	Quantification + correlation active cases	24 h composite samples, bi-weekly	1 WWTP		-

Croatia (Brnić et al., 2022)	December 2019 and July 2020	Quantification + correlation active cases + absence of detection in shellfish+ nsp14 sequencing	Grab samples, E, N and nsp14 protein genes	9 WWTP ~1.8 mio hbts	++	-
Spain (Carcereny et al., 2021)	December 2020 to the end of March 2021 (week 21_13)	Quantification + SNP Identification	Grab samples, 2019-*n*CoV RUO qPCR Probe Assay	32 WWTP,		-
Spain (Randazzo et al., 2020)	12 March to 14 April 2020	Quantification + Correlation to active cases	Grab samples, aluminum hydroxide adsorption-precipitation protocol, CDC RT-qPCR diagnostic panel assays	6 WWTP, 750000		-
Portugal (Monteiro et al., 2022)	April 27, 2020, and December 2, 2020	Quantification	24h composite samples, PEG precipitation E_Sarbecco, RdRp and N_Sarbecco	5 WWTP, 3x a week		-
Roumanie (Baicus, Cherciu, & Lazar, 2021)	January 20 ;	Viral detection	Grab sampling, two-phase	4 regions of Roumania		-

	october 20- January 21		separation method, Allplex 2019-nCoV assay, Seegene,			

Table 2 : List of the national reports on WBE of SARS-CoV-2 virus in Europe (see more details in the following text)

Country	Sampling period	Goals	Measured parameters	Network density	Quality of data	Derived measure/action
Denmark Del af Statens Serum Institut	July 21 -	Quantification Comparison to clinical incidence	24h composite samples, once a week, VIRseek test	198 sampling points	++	Estimation of the level of infection in the area where the wastewater originates
Estonia	May 2021 - December 2021	Quantifiation	24h composite samples, Once a week	56 cities	++	A tool helping the Health Board monitor changes in the outbreak dynamics and discover hidden outbreaks Gives early information to estimate the spread of the virus before clinical cases are detected
Ireland NWSP project	August 21 -	Detection	24-hour composite samples, biweekly sampling	68 WWTP (80% of the population)		Acting as a potential early warning system for the circulation, or the increased circulation, of SARS-CoV-2 in an area, Inform testing strategy and the initiation of preventive public health measures

Latvia	July 21 -	Quantification + comparison to cumulative cases	24h composites samples twice a week	13 cities		Highlight the dynamics of incidence in municipalities and identify trends and differences
Austria	August 21 -	Quantification + Comparison to active cases	24 h composite, the SARS-CoV-2 nucleocapsid (N1) gene	23 WWTP		
Croatia						
Spain Projet VATar COVID-19	July 20 -	Quantification		> 50 WWTP		
Italia SARI network	July 21 -	Quantification + detection of mutations		98 WWTPs	++	
Portugal project COVIDETECT	April 20 -				++	Early warning system for the presence of the SARS-CoV-2 virus
Slovenia	October 20 -	Quantification + comparison to active cases		7 cities	++	Help to understand the spread of the virus and in order to raise awareness, responsiveness, and check the effectiveness of the measures implemented to curb the virus

Table 3 : Characteristics of studies from America (USA, South America and Canada): Sampling period (first wave ; second wave ; and other waves).Outcomes (anticipation of clinical peak cases; correlation between SARS-Cov-2 RNA in WW and clinical cases). Quality of data

according judgement of experts: +++ = internal control and big sample size and sophisticated data interpretation; ++ = internal control + correct sample size; + = not very original or part of a sub-project.

Country	General experience/goals	Outcomes	Measured parameters	Network density	Quality of data	Derived measure/action
Ohio (Ai et al., 2021)	9 From July 2020 to January 2021	• Regression analyses (Spearman average r = 0.85) • Powerful tool in capturing the real-time infection trend; • Pinpointing epidemic hotspots;	Composite twice a week	2 > 900.000 population; 7 between 14.000-49.000	+++	Average of the most recent two samples with the average of the prior third and fourth sample to determine the percent change: > 100% substatial increase; 50% to 100% increase; -49% to 49% steady; <=-50% decrease.

Wisconsin (Feng et al., 2021)	12 From August 2020 to January 2021	• Spearman rank correlation (ρ = 0.59 to 0.87) • Wastewater-based epidemiology may need to be validated at individual plants • Powerful tool in capturing the real-time infection trend	Composite 2 or 3 samples per week	> 100.000	+++	Increasing trends are when the change from the prior seven-day period to the most recent seven-day period is greater than or equal to 10 percent and statistically significant
California (Greenwald et al., 2021)	6 From April 2020 to September 2020	• Kendall's Tau-b coefficients (t 0.43; p<0.0001) • Wastewater testing could provide insights about COVID-19 trends in the population when clinical testing capabilities are limited.	Composite once a week	> 100.000	+++	When COVID-19 case rates equaled or exceeded 2.4 daily cases per 100,000 people, 95% of wastewater technical replicates amplified via RT-qPCR for N1

Montana (Nemudryi et al., 2020)	1 From March 2020 to June 2020	• SARS-CoV-2 RNA concentration in the wastewater lagged behind symptom onset data by 8 days (Pearson's r = 0.989), and preceded laboratory test results for individuals by 2 days (r = 0.969). • When cases resurged in May, wastewater detection trailed symptom onset by 5 days (r = 0.92) and foreshadowed the increase in positive tests by 4 days (r = 0.953).	Composite (for 74 days)	50.000	+++	real-time measure of viral prevalence in the community track the outbreak and inform policy.
Minnesota (Melvin, Chaudhry, Georgewill, Freese, &	19 From May 2020 to August 2020	• Good trend correlation (r=0.38) • lag analysis showed that wastewater predicted	Composite	large (> 100k population), medium (<100,000 and	+++	

Simmons, 2021)		confirmed new cases by 17d (r107 =0.68, P < 0.001).		>10,000), and small (<10,000)		
Massachusetts (Xiao et al., 2022)	1 From March 2020 to May 2021	Before August 2020, the wastewater signal preceded clinical cases by approximately 6.2 days; after August 15, 2020, the wastewater signal was more in phase with clinical cases mean time lag 1.0 days	Composite	> 100.000	+++	Our results indicate that jurisdictions can use primary sludge SARS-CoV-2 concentrations as an additional basis for imposing or easing infection-control restrictions, especially in locations affected by limits in clinical testing capacity or delays in test reporting.
Utah (Weidhaas et al., 2021)	10 From April 2020 to May 2020	Non parametric Spearman rank correlation (**Spearman average r = 0.54**)	Composite	> 100.000	+++	
Texas (Stadler et al., 2020)	39 From May 2020 to October 2020	• Strong relationship between the wastewater viral load and positive cases, Spearman r = 0.92 • Detection of SARS-CoV-2 RNA in wastewater could serve as a	Composite once a week	From 5000 to > 100.000	+++	estimate positivity rate during periods when clinical testing data was scarce pinpoints areas where infections are most rapidly worsening or improving

		leading indicator of the positivity rate for community recorded infections.				
Virginia (Gonzalez et al., 2020)	9 From May 2020 to August 2020	• Good trend with clinical cases, • Pinpointing epidemic hotspots	Composite and grab	1.7 millions	++	
Louisiana (Sherchan et al., 2020)	2 From January 2020 to April 2020	Viral load (RNA)	Composite and grab	1 > 100.000; 1< 100.000	++	
Nevada (Gerrity, Papp, Stoker, Sims, & Frehner, 2021)	2 From March 2020 to May 2020	Model for viral load estimation	Composite and grab	a large 1 milion and a small 60.000	+	
Connecticut (Peccia et al., 2020)	1 From March 2020 to June 2020	Correlation	Grab (Primary sludge)	> 100.000	+	
Mexico (Carrillo-Reyes et al., 2021)	2 From April 2020 to July 2020	Trends	Grab	1 > 100.000; 1< 100.000	+	

Brazil (Prado et al., 2021)	2 From April 2020 to August 2020	Confirmed presence of SARS-CoV-2 RNA 56 days ahead of the reports of COVID-19 cases in the continent and more than 90 days in the case of Brazil.	Composite	> 100.000	++	
Brazil (Fongaro et al., 2021)	1 From October 2019 to March 2020	Good trend with clinical cases,	Composite	5.000	++	
Argentina (Iglesias et al., 2021)	1 From June 2020 to September 2020	Pearson's correlation (Pearson's r=0.717) Outbreak-free periods vs outbreaks (Median IQR) (0 vs 200 copie/ml P < 0.5)	Composite once a week	16.000	++	
Canada (Acosta et al., 2021)	3 From August 2020 to December 2020	Correlation between hospitals admission and SARS-CoV-2 in wastewater	Composite twice a week (inside sewer access points outside of hospitals)	>2100 inpatient beds	+	
Canada (Hinz et al., 2021)	2 From September 2020 to December 2020	Correlation between hospitals admission and SARS-CoV-2 in wastewater	grab	> 100.000	+	

Canada (D'Aoust et al., 2021)	2 From April 2020 to June 2020	Trends	Composite primary clarified sludge	> 100.000	+	

Table 4 : Characteristics of studies from Africa: Sampling period (first wave ; second wave ; and other waves).Outcomes (; correlation between SARS-Cov-2 RNA in WW and clinical cases). Quality of data according judgement of experts: +++ = internal control and big sample size and sophisticated data interpretation; ++ = internal control + correct sample size; + = not very original or part of a sub-project.

Country (ref)	General experience/goals	Outcomes	Measured parameters	Network density	Quality of data	Derived measure/action
Tunisia (Jmii, Gharbi-Khelifi, Assaoudi, & Aouni, 2021)	*September–October 2020 and February–April 2021*	Target genes detection or quantification+ comparison to COVID cases	3 WWTP, 1 grab samples / week Allplex 2019-nCoV kit/ E, N qnd RdRp genes	unknown	-	
South Africa (Cape Town) (Street et al., 2021)	6 weeks, from 6 July 2020	Quantification (copies / mL) + correlation N1 – N2 + correlation SARS-CoV-2 and cases	23 WWTP, 1 grab sample /week, CDC N1 and N2 primer/probe	unknown	+	
South Africa (KwaZulu- Natal province) (Pillay et al., 2021)	July to October 2020	Quantification (copies / 100 mL) + comparaison active cases + estimation of infection numbers based on viral load	4 WWTP, 1 grab sample/week N2 gene	unknown	+	

Table 5 : List of the national reports on WBE of SARS-CoV-2 virus in Africa (see more details in the following text)

Country (ref)	General experience/goals	Outcomes	Measured parameters	Network density	Quality of data	Derived measure/action
South Africa SAMRC wastewater Surveillance and Research Programme	October 21 -	Quantification	Grab samples, once a week, 2019-nCoV CDC on N1 an N2 genes	95 WWTPs	++	provided to public health stakeholders within 48 hours of sample collection ; indication of COVID-19 case trends in a particular wastewater catchment area
Ghana	January to May 21	Quantification		3 WWTPs	-	
Malawi		Detection	CDC N1 gene	8 WWTPs	-	

2. National surveillance: Summary of the contain of websites and other reports

AUSTRIA: Coron-A project

Website: https://www.coron-a.at/

Methodology: Surveillance of 23 wastewater treatment plants (WWTPs) in Austria by taking 24 h composite volume proportional samples (CVVT: constant volume; variable time) from the inlet of the WWTPs. The sampling frequency is bi-weekly or higher. (see Markt et al. (2021)).

BELGIUM

Website: https://datastudio.google.com/embed/u/0/reporting/c14a5cfc-cab7-4812-848c-0369173148ab/page/p_ggbfgsqtmc

Methodology: 24h composite samples collected twice per week from the influent of 42 WWTPs (around 45% of the Belgian population is covered) are analyzed for the presence of SARS-CoV-2 RNA (3 targeted genes: E, N1, N2).

Goals	The wastewater surveillance serves as a proxy to follow-up the circulation of SARS-CoV-2 in the population. Report based on 3 alerting indicators highlighting the trends of concern at the levels of the treatment plant areas and the provinces
Important points	SARS-CoV-2 monitoring results in wastewater should be balanced with complementary indicators, such as clinically confirmed cases, hospitalizations, etc.

CROATIA

Website:	https://www.total-croatia-news.com/news/48417-project-launched-to-analyse-sewage-to-identify-covid-19-virus-traces DOI: doi.org/10.33602/mebm.3.2.2

More information not found (Croatian language)

DENMARK

Reports in Danish:	https://covid19.ssi.dk/overvagningsdata/overvaagning-af-sarscov2-i-spildevand https://files.ssi.dk/covid19/spildevand/ugeopgoerelse/spildevandsovervaagning-covid19-uge4-2022-ms43 https://covid19.ssi.dk/-/media/arkiv/subsites/covid19/overvaagningsdata/spildevand/notatssisarscov2-i-spildevand-evalueringsprojekt-juli-202121sept21.pdf?la=da

Methodology:	24h composites samples collected from 198 WWTP, extracted by flocculation method. Quantification done using the Kit test VIRseek (Eurofins Technologies), rapid variant detection by single point K417N and L452R mutations, NGS sequencing (Jørgensen, Gamst, Hansen, Knudsen, & Jensen, 2020).
Goals	Monitoring of virus spread Early detection of new variants (before clinical cases) to
Important points	Very early warning of the presence of the new variant Omicron allowing Danish authorities to take the necessary measures rapidly

ESTONIA

Website:	https://ut.ee/en/node/114718
Methodology:	Waste water samples are collected at the beginning of every week in all Estonian county centres, cities with more than 10,000 inhabitants and, if necessary, in smaller settlements
Goals	A tool helping the Health Board monitor changes in the outbreak dynamics and discover hidden outbreaks
Important points	Gives early information to estimate the spread of the virus before clinical cases are detected

FINLAND

Website:	https://www.thl.fi/episeuranta/jatevesi/jatevesiseuranta_viikkoraportti.html (in finnish)
Methodology:	24h-composite samples collected once a week in 14 WWTPs (~49% of the population covered), viral quantitation and NGS sequencing of positive samples for variant detection
Goals	Use to assess changes at the population level Help authorities to set up coronavirus outbreak control measures
Important points	Describes the infection status of the population in the area of the sewage plant's sewage system during the measurement period Wastewater surveillance can provide an important early warning for a particular area if a coronavirus is detected in the wastewater of a locality, even if no infection was detected in the field tests

FRANCE

Website:	https://www.reseau-obepine.fr/carte-des-tendances/
Goals	• Organize a national network of research teams to build duly validated protocols for monitoring the virus in wastewater, sewage sludge and, where appropriate, surface water.

- Cross-reference the quantitative results on the viral signal obtained with epidemiological data and hydrological data.
- Propose an experimental network of all or part of the territory in collaboration with the water agencies, the French Office for Biodiversity for the Overseas Territories and the ARS with a hierarchical structuring of the agglomerations, from the metropolitan areas to the rural communes to have representative sampling, including the coverage of places subject to large population flows.
- Organize in this geographical framework with the communities and the managers of the treatment plants the methods of sampling, conservation, transport and analysis of the samples.
- Organize the collection of information necessary to characterize the importance of the activity and the health status of the populations monitored.
- Set up protocols for the combined analysis of all this information.
- Develop a system for sharing the results obtained with the relevant State service but also with local authorities.
- Design the modalities for a rapid evolution of the surveillance system in epidemic mode.
- Centralize the results in a secure way and make them available to the State with the aim of coordinating their use and their availability within the framework of an integrated health surveillance plan.

GERMANY

Website: https://bimsbstatic.mdc-berlin.de/akalin/AAkalin_pathogenomics/sarscov2_ww_reports/210528_wastewaterall_pigxsarscov2ww_default/index.html

Goals Propose a pipeline performs mutation analysis of SARS-CoV-2 and reports and quantifies the occurrence of *variants of concern* (VOC) and signature mutations by which they are characterised.

GHANA

Website: https://www.healio.com/news/infectious-disease/20211118/wastewaterbased-covid19-surveillance-offers-lowcost-rapid-testing-for-african-nations

Methodology: 208 samples were analyzed between January to May 21 in 3 WWTP and 6 non-sewered public toilets

Goals Results are used by the Ghana Health Service to inform COVID-19 response in vulnerable communities where active cases have not been reported and in institutions (schools, industrial settings…)

GREECE

Website: http://trams.chem.uoa.gr/covid-19/

Methodology: 24h-composites samples collected daily from the wastewater treatment plant of Athens were analyz ed for a series of analytical parameters, including SARS-CoV-2 genetic material with a validated qPCR method.

Goals Assessment of COVID-19 trends (reported and unreported) within the community contributing to the sewer system.

Important points Data from the wastewater surveillance help the state to understand, and respond to the COVID-19 pandemic

HUNGARY

Website: https://www.nnk.gov.hu/index.php/koronavirus/szennyvizvizsgalatok (in hungarian)

Methodology: Surveillance of WWTP of 21 cities over the country

Goals Colored-based warning indicator of signal according the level of SARS-CoV-2 quantified in WWTP

IRELAND

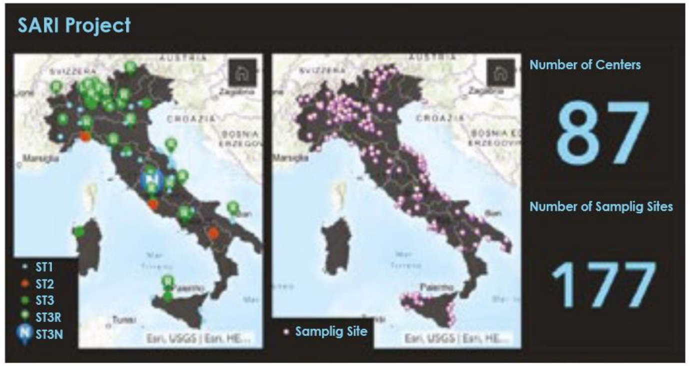
Website:	https://www.hpsc.ie/a-z/respiratory/coronavirus/novelcoronavirus/surveillance/wastewatersurveillanceprogramme/
Methodology:	68 wastewater catchment areas included in the NWSP cover 80% of the population connected to public wastewater treatment plants.
Goals	Aims to complement the case-based surveillance systems (i.e. monitoring the number of people testing positive or presenting to a healthcare provider with symptoms) Inform testing strategy and the initiation of preventive public health measures
Important points	Results shared with key stakeholders in the response to COVID-19/SARS-CoV-2 pandemic in Ireland, including the HSE Public Health Departments, the Irish Epidemiological Modelling Advisory Group (IEMAG), and the National Public Health Emergency Team (NPHET). Act as a potential early warning system for the circulation, or the increased circulation, of SARS-CoV-2 in an area. Results of greatest value when the circulation of SARS-CoV-2 and testing rates are lower, when a detection of SARS-CoV-2 in wastewater may be the first indication of circulation in a catchment area.

ITALIA: SARI network

Website: https://www.iss.it/cov19-acque-reflue

Methodology: 98 WWTPs using protocol “Sorveglianza di SARS-CoV-2 in reflui urbani - Protocollo progetto SARI - rev.3 (protocole not found), rt-PCR analsis with detection of characteristics mutations.

Data reported monthly on Istituto Superiore di Sanità (ISS) website



LATVIA

Website: https://bior.lv/lv/par-mums/jaunumi/notekudenu-monitorings-covid-19-izplatibas-noteiksanai

Methodology: Collection of 24h composites samples twice a week in 13 cities

	Comparison to cumulative incidence of clinical cases
Goals:	Highlight the dynamics of incidence in municipalities and identify trends and differences

LIECHTENSTEIN

Please see Insbruck University Coron-A project and Switzerland

LUXEMBOURG

Website:	https://www.list.lu/en/covid-19/coronastep/
Methodology:	Collections of 24h-composite samples once or twice a week in 13 WWTPs and quantification of SARS-CoV-2 E gene with a comparison to active cases
Goals	**Track the presence and evolution of the coronavirus in the Grand Duchy's wastewater**
Important points	Near real-time results are delivered weekly to the government for an informed decision
	Provides earlier information - approximately 24 to 48 hours - on the evolution of the virus circulation than clinical data
	Results included in a global report of the actors in the fight against the pandemic and published every Wednesday on the Ministry of Health website

NETHERLAND

Website:	https://coronadashboard.government.nl/landelijk/rioolwater
Methodology:	24h-composite samples collected twice a week in more than 300 WWTPs throughout the Netherlands (~17 million people covered)
Goals	Enable early detection in the event of localised outbreaks Detect and recognise new variants

PORTUGAL: project COVIDETECT

Website:	https://www.portugal.gov.pt/pt/gc22/comunicacao/comunicado?i=investigacao-em-aguas-residuais-pretende-criar-sistema-de-alerta-precoce-do-virus-sars-cov-2 (in portugese) https://www.adp.pt/en/business/innovation/covidetect/?id=222
Goals	Establish an early warning system for the presence of the SARS-CoV-2 virus

SCOTLAND

Website:	https://informatics.sepa.org.uk/RNAmonitoring/
Methodology:	Weekly collection in 106 pubic WWTP across the country, covering all 14 NHS Scotland health board areas (cover 70-80 percent of the Scottish population)
Goals	Help Scotland to understand the prevalence and distribution of the virus.
Important points	The project is a partnership working with Scottish Water and the University of Edinburgh's Roslin Institute The data is shared with Public Health Scotland, Scottish Government, the Office for National Statistics and the Joint Biosecurity Centre and is now being made publicly available

*S*LOVENIA

Website:	https://covid-19.sledilnik.org/en/stats#sewage-chart
Methodology:	Samples collected in 7 cities, quantification of SARS-CoV-2 particles and comparison to active clinical cases
Goals	Monitor the course of epidemic, the effectiveness of measures and analyze the presence of SARS-CoV-2 in individual facilities such as nursing homes, hospitals, schools and businesses.

SOUTH AFRICA: SAMRC wastewater Surveillance and Research Program

Website:	https://www.samrc.ac.za/wbe/
Methodology:	Each Monday morning, 1 grab sample is collected in 95 WWTPs. Each sample is analyzed at the SAMRC or partner university-based laboratories using RT qPCR on 2 target genes and presented as RNA signal copies per millilitres (copies/ml).
Important points	Results used as an early indicator of COVID-19 case trends within a community Results are provided to public health stakeholders across the country within 48 hours of sample collection and are also shared on our public dashboard Identification of COVID-19 hotspots and can guide action in preparedness strategies

SPAIN: Projet VATar COVID-19

Website:	https://miteco.maps.arcgis.com/apps/opsdashboard/index.html#/aab0e0653d694289b310f6485f9f2226
Methodology:	Determination of the presence of ARN fragments of the SRAS-CoV-2 in more than 50 WWTPs, mass sequencing of positive samples
Goals	Monitoring of SARS-CoV-2 epidemics Detection of new potential viral lineages

Important points Jointly developed with the Ministry of Health and with the support of the Spanish National Research Council(CSIC), attached to the Ministry of Science and Innovation, and CEDEX and the regional governments

SWEDEN

Website: https://crush-covid.shinyapps.io/crush_covid/

Goals Measure the virus content in the city of Uppsala

SWITZERLAND

Website: https://ibz-shiny.ethz.ch/wastewaterRe/

Methodology:

Goals Provide a longitudinal measurement of SARS-CoV-2 RNA in wastewater and estimates the effective reproductive number, Re

Important points These estimates provide an independent account of COVID-19 transmission dynamics, complementing existing Re estimates based on clinical data such as confirmed cases, hospitalisations and/or deaths.

Re estimates from wastewater that are part of the AbwasSARS-CoV-2 project in Switzerland are shown together with existing Re estimates from clinical data sources (for the corresponding cantons).

Sub-questions

1. Which parameters / factors are measured or collected? What indicators are calculated from them?

CDC have suggested sampling strategies and methodology for wastewater sample analyses. A sampling strategy should balance available resources and testing capacity with public health data needs, and it may need to be updated over time with changing scientific knowledge and public health needs.

Sample types: the **untreated water** that includes waste from household or building use (e.g., toilets, showers, sinks), which contains human faecal waste, as well as waste from non-household sources (e.g., rainwater, industrial use) and **primary sludge** that comprises suspended solids that settle out of wastewater during the first solids removal ("sedimentation") process. Changes in SARS-CoV-2 RNA concentrations in both wastewater samples have been shown to correlate with trends in reported cases.

Sampling methods. **Composite samples** are collected by pooling multiple grab samples at a specified frequency over a set time, typically 24 hours. Composite samples are considered more representative of community fecal contributions than grab samples. **Grab samples** can be collected rapidly and do not require automated equipment. Since this sample represent a single moment in time may be less representative of community fecal contributions than composite samples.

Sample processing. Sample clarification wastewater samples usually need to be clarified by filtration (0.45 µm filters) or centrifugation (7,140 g for 15 minutes at 4 °C) in order to eliminate debris and large particles. Then clarified samples were **concentrated** and different methods were available (listed below).

- Ultrafiltration
- Filtration through an electronegative membrane with sample pre-treatment by addition of $MgCl_2$ or acidification

- Polyethylene glycol (PEG) precipitation
- Skim milk flocculation
- 4S-silica column method (Whitney et al. 2021)

In the US studies presented in question 4, authors used same sample type (untreated water) and composite sampling method, while concentration methods were different with the preference of PEG precipitation (table 9)

Table 9. Sample strategies used in studies presented in question 4. For the respective country, references were the same of the tables 7 and 8

Country	**Sample type**	**Sampling method**	**Sample pre-treatment**	**Sample concentration**
Ohio	Untreated water	Composite	Debris removal by filtration	Concentrating pipette
Wisconsin	Untreated water	Composite	None	Filtration
California	Untreated water	Composite	None	4S-silica column method
Minnesota	Untreated water	Composite	Debris removal by filtration	PEG-precipitation
Texas	Untreated water	Composite	Debris removal by centrifugation	PEG-precipitation and electronegative membrane filtration
Utah	Untreated water	Composite	Debris removal by centrifugation	Electronegative membrane filtration
Montana	Untreated water	Composite	Debris removal by filtration	Ultrafiltration
Massachusetts	Untreated water	Composite	Debris removal by filtration	PEG-precipitation

RNA extraction. Nucleic acid extraction and purification is an essential step in isolating SARS-CoV-2 RNA from the sewage mixture. Sewage is a complex mixture with materials known to interfere with molecular viral quantification methods, so consider the following when selecting an extraction method:

- Select an extraction protocol designed to produce highly purified nucleic acid extracts from environmental samples. Commercial kits are available for environmental sample extraction.
- Use an extraction kit or a protocol that is designed specifically to purify RNA and includes RNase denaturants prior to lysis.
- Avoid degradation of extracted RNA due to multiple freeze-thaw cycles by aliquoting extracts into separate tubes and storing them at -70°C or below.

Molecular target and RNA measurement. Molecular targets suggested by CDC were **N1** and **N2** (nucleocaside) and **E** (envelope), less common but also used were N3 (nucleocapside) (D'Aoust et al. 2021), S (spike protein) (Carrillo-Reyes, Barragán-Trinidad, and Buitrón 2021; Iglesias et al. 2021), and RdRp (RNA-dependent RNA polymerase) (Gerrity et al. 2021; Fongaro et al. 2021).

Process control and human faecal normalization. Process controls were used to understand the amount of virus lost (percentage of recovery) during sample processing. This control is important for comparing concentrations resulting from different testing methods and over time. It is important to quantitatively assess recovery because wastewater is chemically and biologically complex and variable, and often contains constituents that can interfere with sample concentration, nucleic acid extraction, or molecular quantification methods. It is important to include a matrix recovery control in method validation also for determine the sensitivity (limit of detection and quantification) of methods (Kantor et al. 2021). Candidates for matrix recovery controls are enveloped viruses with single-stranded RNA genomes, including murine coronavirus (MHV), bovine coronavirus (BCoV), and bovine respiratory syncytial virus (BRSV) (Prado et al. 2021; Gonzalez et al. 2020). Have been used also, human coronavirus (OC43) and heat-inactivated 2019-nCoV-2 or bacteriophage (Phi6 and MS-2) (Prado et al. 2021; Sherchan et al. 2020; Hinz et al. 2021) (Table 4). Normalizing SARS-CoV-2 concentrations by the amount of human faeces in wastewater can be important for interpreting SARS-CoV-2 concentrations and comparing concentrations between sewage samples over time. **Human faecal normalization** controls are organisms or compounds specific to human faeces that can be measured in wastewater to estimate its human faecal content. Human normalization controls include, but are not limited to Pepper Mild Mottle virus (PMMoV), crAssphage and Bacteroides HF183. Data normalization could be also done using daily flow rate and number of inhabitants connected to the WWTP (table 10).

Table 10. Methods for RNA extraction, target quantification, positives controls and percentage of gene recovery used in the US studies presented in question 4. For the respective country, reference were the same of the tables 7 and 8. *Extraction controls were used to assess consistency of extractions rather than recovery (Kantor, Nelson, Greenwald, & Kennedy, 2021)

Country	**RNA extraction methods**	**Quantification method**	**Target/s**	**Controls (% of Recovery)**	**Human faecal indicators/normalization**
Ohio	RNeasy PowerMicrobiome Kit	Droplet digital RT-PCR	N1, N2, E	BCoV, OC43, MS2 (50%)	PMMoV
Wisconsin	RNeasy PowerMicrobiome Kit	Droplet digital RT-PCR	N1, N2	BCoV (4.9%)	PMMoV, Hf183; per capita
California	ZymoPURE	RT-qPCR	N1	BCoV*	PMMoV, Hf183; crAssphage
Minnesota	Qiazol Lysis Reagent	RT-qPCR	N1, N2	Heat-inactivated 2019-nCoV-2 (95%)	PMMoV
Texas	Viral Mini RNA Kit, AllPrep PowerViral DNA/RNA Kit, Maxwell RSC PureFood GMO kit.	RT-qPCR, Droplet digital RT-PCR	N1, N2	None	Daily flow normalization
Utah	RNeasy Power Water extraction kit	RT-qPCR	N1, N2	MHV (26%)	Per capita normalization
Montana	RNeasy Mini Kit	RT-qPCR	N1, N2	None	Daily flow normalization
Massachusetts	RNeasy Mini columns	RT-qPCR	N1, N2	MHV (30%)	PMMoV

2. How comparable/repeatable are the results? How was the quality of the measurements assessed?

Since different laboratory used different methods for SARS-Cov-2 monitoring in wastewater, standardize a single method would be interesting but not realistic. So validate a method with recovery controls (see point 6) allowed to improve the quality of results. Wastewater is a complex matrix due to the variation in many factors, such as individual viral shedding amount and duration, RNA degradation rates, flow rate, rainfall and many environmental elements that could influence the real quantity of SARS-CoV-2 RNA. In order to smooth data and make them close to reality, it is possible to normalize results with human fecal indicators organisms, daily flow of WWTP and amount of population served (see point 6). Even if, some authors have demonstrated that normalization with fecal indicators gave different results (sometimes improve correlation between wastewater data and cases (Ai et al. 2021; Greenwald et al. 2021), sometimes does not affect results and sometimes produces a weak correlation (Feng et al. 2021). Also daily flow rate and per capita normalization could mislead the calculation of SARS-CoV-2 concentration. In fact, rainfall influence both daily flow, that increase, and number of SARS-Cov-2 gene target copies, that decrease due to dilution so, normalizing data with daily flow, does not allow monitoring the dilution and leads to overestimation (personal communication from Ottavio Beretta Ufficio del medico cantonale, Dipartimento della sanità e della socialità Divisione della salute pubblica Ticino Canton). Based on these considerations, wastewater-based epidemiology for SARS-CoV-2 should be validated at individual wastewater treatment plants (Feng et al. 2021). Pecson and colleagues (Pecson et al. 2021) have compared 36 standard operating procedures form different US laboratories with the aim to evaluate whether the existing methods provide sufficient reliability and reproducibility to track trends between SARS-CoV-2 recovery in wastewater and clinical cases. The study demonstrated that all methods were able to quantify the SARS-CoV-2 genetic signal in raw wastewater with a high degree of reproducibility.

3. How dense is the network of wastewater treatment plants (population catchment)? What criteria were used to select them?

CDC have suggested some criteria to select a WWTP for surveillance (https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/developing-a-wastewater-surveillance-sampling-strategy.html#anchor_1602855341209).

- Cover a certain percentage of the population.
- Provide data on communities at higher risk for COVID-19 or at increased risk for severe illness from COVID-19.
- Provide data on communities where timely COVID-19 clinical testing is underutilized or unavailable.
- Represent several sewersheds that serve a larger interconnected population, such as in dense urban areas

The population served by the US monitored WWTP (wastewater treatment plants) range from 5000 to 100.000 inhabitants and more (table 11).

Table 11. Number of monitored wastewater treatment plants (WWTP), sampling period and number of inhabitants served by each plants. For the respective country, reference were the same of the tables 7 and 8.

Country	**N° of WWTP monitored**	**Monitoring period**	**Served population**
Ohio	9	From July 2020 to January 2021	2 > 900.000 population; 7 between 14.000 - 49.000
Wisconsin	12	From August 2020 to January 2021	> 100.000
California (San Francisco, Bay)	6	From April 2020 to September 2020	> 100.000
Montana	1	From March 2020 to June 2020	49.800
Minnesota	19	From May 2020 to August 2020	large (> 100k population), medium (<100,000 and >10,000), and small (<10,000)
Texas	39	From May 2020 to October 2020	From 5000 to > 100.000
Utah	10	From April 2020 to May 2020	> 100.000
Massachusetts	1	From March 2020 to May 2021	> 100.000

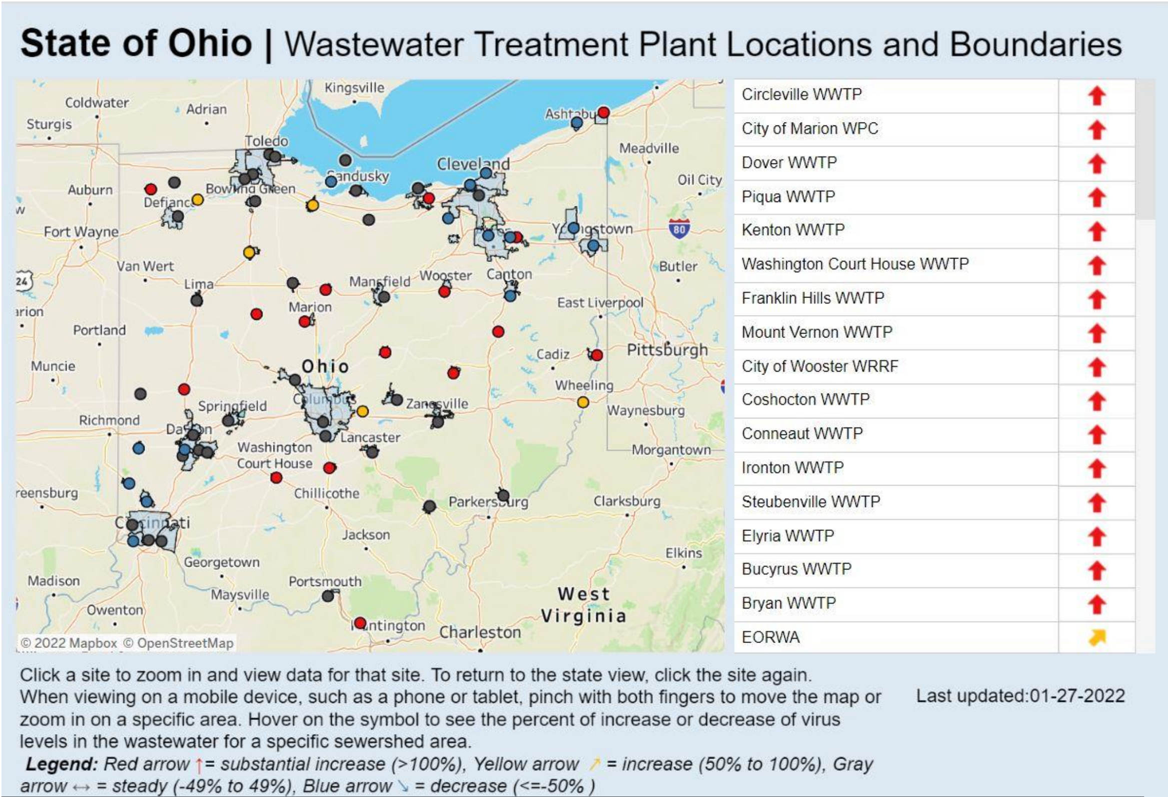
4. Are the results included in the assessment of the epidemiological situation or in the statistics? If yes, how? Predictively or retrospectively?

   All the results presented for USA SARS-CoV-2 wastewater monitoring were included in the assessment of epidemiological situation. For the details please referred to question number four and tables 7, 8, and synoptic tables number 1, 2, 3 and 5

5. Are measures or interventions derived from the results? If yes which measures are taken based on the results? E.g. are there specific threshold calculations in place that would trigger an intervention. What kind of interventions are taken (examples)

   Three US states (Ohio, Wisconsin and Utah) have included generating alerts to local jurisdictions, allocating mobile testing resources, evaluating irregularities in traditional surveillance, refining health messaging, and forecasting clinical resource needs. Below were presented some examples:

- **Ohio:** Ai et.al. have fixed threshold to evaluate increase or decrease trend between SARS-Cov-2 quantity and clinical cases. Average of the most recent two samples with the average of the prior third and fourth sample to determine the percent change: > 100% substatial increase; 50% to 100% increase; -49% to 49% steady; <=-50% decrease (Ai et al. 2021) (figure 4).



https://public.tableau.com/views/COVIDWastewater/Dashboard2?:embed=y&:showVizHome=no&:host_url=https%3A%2F%2Fpublic.tableau.com%2F&:embed_code_version=3&:tabs=no&:toolbar=yes&:display_count=yes&:language=en&:loadOrderID=0

Figure 4. Ohio dashboard presenting trend between SARS-Cov-2 quantity and clinical cases

- **Wisconsin** Feng and colleagues reported an increasing trends when the change from the prior seven-day period to the most recent seven-day period is greater than or equal to 10 percent and statistically significant (Feng et al. 2021) (Fig. 5)

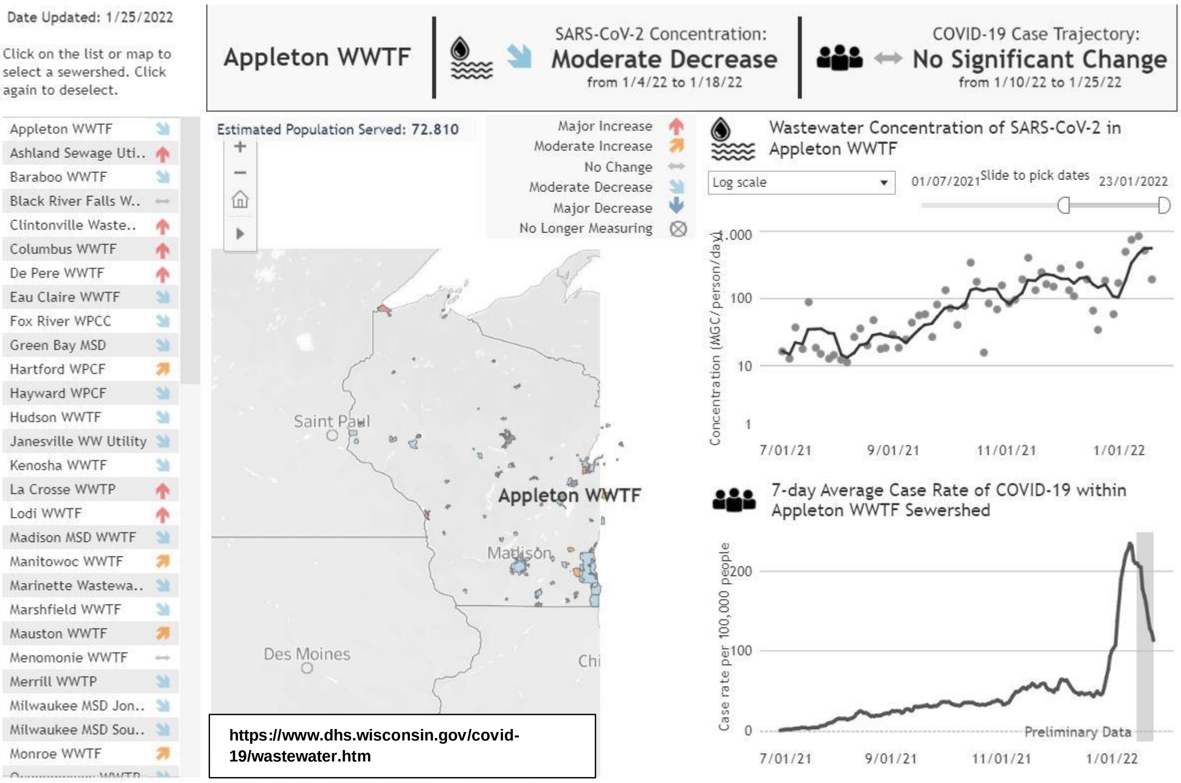


Figure 5. Wisconsin dashboard presenting trend between SARS-Cov-2 quantity and clinical cases

- **Utah** figure 6, shown results from ongoing SARS-CoV-2 sewage monitoring in Utah along with observed infection rates in each corresponding sewershed. Color-codes represented trends in gene copies in sewage over the four most recent samples (Figure 6).

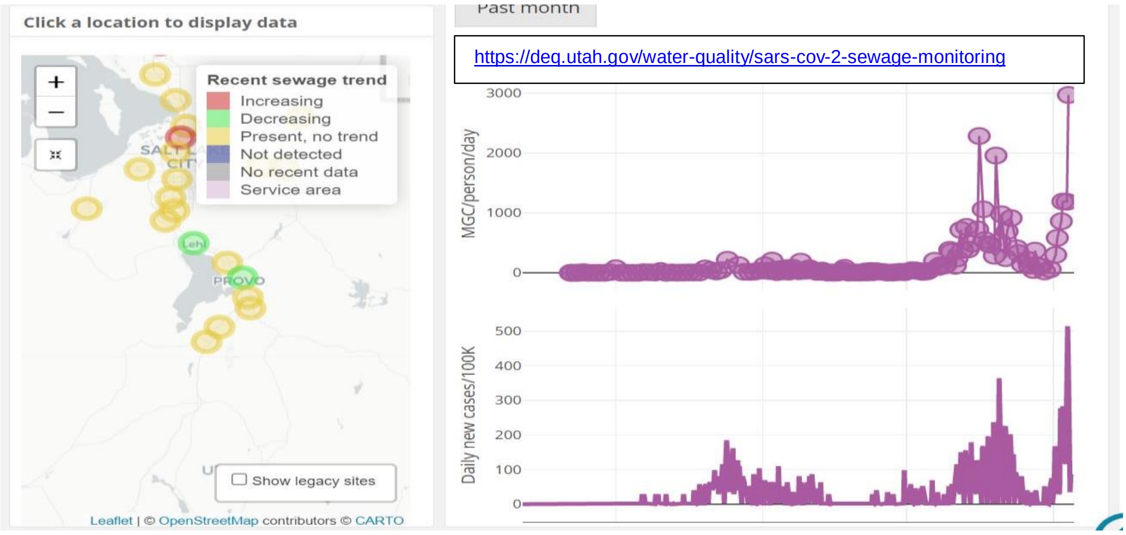


Figure 6. Utah dashboard presenting trend between SARS-Cov-2 quantity and clinical cases

In Europe, wastewater testing is mainly a monitoring tool for outbreak dynamics and to discover hidden outbreaks for a better anticipation. This surveillance complements the other health surveillance systems already in place. The national surveillance systems were implemented under the impetus of the European Union's recommendation by merging the experimental monitoring networks. In order to create a strong European surveillance network, a systematic and comparable methodology between existing networks needs to be developed. In some countries such as Denmark, Finland and Ireland, results based on wastewater testing help national authorities to set up measures but no additional information have been found.

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