Santé publique France

INFECTIOUS DISEASES

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METHODS

VIROLOGICAL MONITORING OF SARS-COV-2 IN WASTEWATER IN FRANCE: PROTOCOL FOR IMPLEMENTATION FROM A PUBLIC HEALTH PERSPECTIVE – EPIDEMIOLOGICAL COMPONENT

English translation of a document published in French in December 2021

REGION
OCCITANIE

Preamble

Virological monitoring of SARS-CoV-2 in wastewater in France: protocol for implementation from a public health perspective – Epidemiological component

This document proposes a framework to facilitate the interpretation and use of data from surveillance of the SARS-CoV-2 genome in wastewater in France for public health and management purposes. Wastewater monitoring should be considered as a complement to surveillance in the population based on individual health data, but not as a substitute.

The following report presents the contribution of Santé publique France in response to the epidemiological component of a referral from the Directorate General of Health on the need to define a useful indicator for surveillance purposes. On this basis, it needs to be supplemented by a microbiological component (sampling, analysis) which will be handled by ANSES (French Agency for Food, Environmental and Occupational Health & Safety) as part of its mandate as national reference laboratory for the surveillance of SARS- CoV-2 in wastewater sewage sludge.

The protocol is based on experiments carried out in France and internationally, as well as on recommendations of the World Health Organisation and the Centers for Disease Control and Prevention of America, the Netherlands and the European Commission (EU 2021/472 of March 17, 2021). As wastewater monitoring is a rapidly developing discipline, the framing elements proposed in this document may evolve as knowledge advances.

KEY WORDS: MONITORING, SARS-COV-2, WASTEWATER, PUBLIC HEALTH

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1. INTRODUCTION

1.1 Definitions and concept

Virological surveillance of SARS-CoV-2 in wastewater through RNA detection began at the start of the pandemic and numerous research studies have been carried out in France, in Europe, and around the world [1-4].

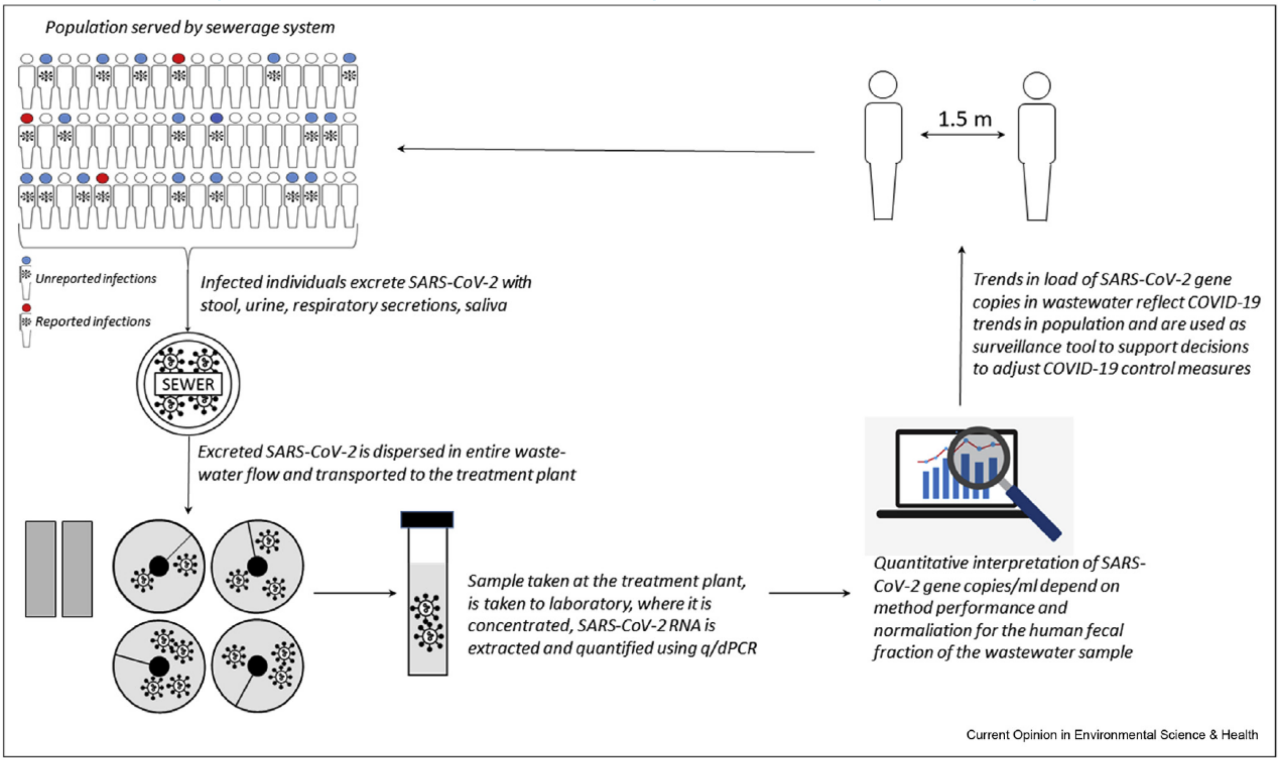
Wastewater is water that has been “polluted” through domestic and/or industrial use. Types of wastewater can generally be grouped into two categories: domestic, including grey water (which has been used for personal hygiene and washing clothes, dishes, floors) and black water (toilet water contaminated by faeces and urine), and non-domestic (rainwater runoff, industrial water, etc.). Wastewater thus constitutes a complex matrix incorporating a set of physical, chemical and biological contaminants of human, environmental and/or industrial origin, linked to the activities and uses of a set of people and/or establishments. In the case of public sanitation, wastewater is routed through sewers (centralised or separate depending on whether rainwater is routed jointly or not) to a wastewater treatment plant where it is treated before being discharged into the environment.

Wastewater surveillance is the process of monitoring wastewater quality and looking for contaminants. First, a sampling strategy (choice of points of interest and sampling plan) must be defined according to the target objectives. Secondly, the analysis and interpretation of the data collected (presence or concentration of pathogens, presence of chemical pollutants, physical and chemical measurements) need to be considered.

Applied to the field of infectious diseases, this surveillance can benefit public health by helping to detect and improve understanding of the spread of a disease within a population. In particular, it has proved useful in controlling the spread of the polio virus [5, 6]. More recently, the emergence of SARS-CoV-2 has provoked international interest in the opportunities offered by environmental monitoring of SARS-CoV-2 and research into this study method. The characteristics of SARS-CoV-2 hold potential for wastewater-based surveillance because the majority of infected people develop no or mild symptoms of the disease but shed a large viral load. This shedding occurs mainly in the respiratory fluids but also in faeces (in 43% of cases according to a recent meta-analysis [7]) and, less frequently, in urine. SARS-CoV-2 RNA remains detectable in wastewater for days or even weeks after the onset of infection in a case, depending on conditions [3].

I FIGURE 1 I

Conceptual diagram of SARS-CoV-2 monitoring in wastewater (source: [3])



1.2 International situation

From early on in the pandemic, international bodies began to mobilize a panel of experts from the fields of urban hydrology, wastewater treatment, analytical laboratories and public health in order to draft recommendations on the benefit of measuring the presence of the virus in wastewater [8], to position the surveillance of SARS-CoV-2 in wastewater from a public health perspective [9] and to discuss the initiatives and problems encountered by the different countries [1, 10]. An international collaborative portal has been set up on this topic in order to promote the dissemination of scientific work and exchanges between teams (COVID-19 WBE Collaborative - https://www.covid19wbec.org/). Finally, at present, the risk of SARS-CoV-2 contamination from wastewater is considered negligible both for workers exposed to wastewater and for the general population from wastewater discharged from treatment plants into the environment [4, 11].

Several countries have deployed nationwide SARS-CoV-2 wastewater surveillance strategies [12-15]. These examples highlight similar approaches to sampling strategies. Samples are generally taken at the entrance to the wastewater treatment plant, to form a composite sample over 24 hours, once or twice a week. Some countries, such as the Netherlands, link the volume sampled to the flow rate of the treatment plant. This approach makes it possible to compensate, at the time of sampling, for any dilution effects linked to variations in flow rate caused by rains, for example. Others choose to take rainfall into account when interpreting results. The indicators presented may also vary. For example, the Australians report the results as the presence/absence of the virus in the samples, while Luxembourg and the Netherlands report the number of copies or viral particles per 100,000 inhabitants. This last measure means that precise information about the structure of the network is required, including the number of equivalent persons connected (and any changes during periods of high population movements such as holidays) as well as data on the collection of water from industrial waste. The advantage is that this measure means that levels and trends for a given station and between plants can be quantitatively compared. It is also possible to calculate an indicator at regional or national level.

In terms of reporting results, all countries publish regular reports, with the Netherlands including regional and national indicators in the RIVM dashboard (Dutch National Institute for Public Health and the Environment, RIVM [16]). In Australia sewage monitoring is primarily used as a public communication tool [17]. This way the tool can be used to stress prevention messages (wearing face coverings, physical distancing, etc.) when a sample is positive in an area where there are already known to be cases, and to encourage the population to get tested when a sample is positive in an area where there have not been known cases in the population. In the other countries, wastewater monitoring is used to follow the temporal and spatial trends of SARS-CoV-2, to compare distribution from one region to another (number of particles/100,000 inhabitants) and to detect areas where there are early recoveries. According to a European consortium [18], the estimated costs for this monitoring would amount to 25,000 Euros per year per monitoring site with a frequency of two samples per week [4].

The CDC (Centers for Disease Control and Prevention) has published recommendations on its website to be taken into account for the implementation of virological surveillance of SARS-CoV-2 in waters in the United States and contribute to the national wastewater surveillance system [14]. Recently, the Netherlands also took stock of the opportunities and challenges of this surveillance by proposing recommendations for its implementation [3]. Finally, on 17 March 2021 the European Commission published recommendations concerning a common approach for the implementation of routine monitoring of the presence of SARS-CoV-2 and its variants in wastewater in the European Union [19].

1.3 Situation in France

In France, there is a group of stakeholders from public (mainly from research), and private arenas (industrial water treatment and analytical laboratories) who have been delivering monitoring of the SARS-CoV-2 genome in wastewater from many sites across the country since March 2020. Some of these stakeholders are partners of the “Obépine” network [20], a national network set up in April 2020 from a research consortium[1] driven by the Analysis, Research and Expertise Committee (CARE) of the Ministry of Higher Education, Research and Innovation. The main objectives are to set up reliable and reproducible analysis protocols, to establish a monitoring network in France, to evaluate the extent to which the viral load in wastewater predicts circulation of the virus in the general population and the evaluation of control measures, to assess the sensitivity of the detection system and to establish a mathematical model for the viral load in wastewater and the number of people infected in the corresponding area. The network currently uses four analysis methods which have been evaluated by inter-laboratory tests performed by seven laboratories known for these methods. At the same time, many other stakeholders (Marseilles marine fire-fighters, Marseilles Water Company, the Departmental Fire and Rescue Service (SDIS) of the Bouches-du-Rhône, along with regional public laboratories or private laboratories, Veolia, Suez , etc.) have also developed monitoring networks or commercial offers [21, 22] targeted at a range of leaders (local authorities, long-term care facilities, companies, etc.). The detection methods used differ. Currently, the Obépine network have only made reports public since January 2021 as open data in the form of graphs and maps [20], as well as data tables (www.data.gouv.fr). In addition, since the emergence of variants, the Obépine network and other stakeholders (National Centre for Scientific Research (CNRS) in Nice, University Hospital Institute on Infectious diseases (IHU) Marseilles) have also started projects aimed at identifying and sequencing the different variants of SARS-CoV-2 in wastewater.

[1] Eau de Paris, Sorbonne University, Inserm (Paris), University of Clermont-Auvergne, University of Lorraine, Armed Forces Biomedical Research Institute, Armed Forces Health Services, Ifremer – LSEM (Health, environment and microbiology Laboratory of the French Institute for Ocean Science), CNRS – EPHE (École Pratique des Hautes Études, Paris).

1.4 Benefits and limitations of surveillance

Monitoring SARS-CoV-2 in wastewater through RNA detection can meet objectives relating to research, as well as those relating to public health, making it a useful operational tool for management.

The public health benefits of SARS-CoV-2 surveillance in wastewater are:

- It covers almost the entire population in urban areas;
- It is a low-cost method of obtaining an integrated test that is representative of a population without calling for active participation;
- It contributes to monitoring the circulation of the virus within the population on a local and national scale, in particular when individual screening is implemented insufficiently or not at all and/or when SARS-CoV-2 is on the way to being eliminated;
- It takes all infected people into account (symptomatic and asymptomatic);
- It is not influenced by population-based screening strategies;
- It is able to detect renewed viral circulation early to predict a possible increase in the demand on health care in the following days, in particular when there is less screening in the population. To date, the objective of early warnings based on a signal from wastewater has been demonstrated retrospectively in several studies (presence of the SARS-CoV-2 genome in wastewater several days or even weeks before cases are identified in the population) [2, 4, 13] but rarely in real time. Nevertheless, this objective can be targeted and useful in unaffected areas when sequences of the virus or of a variant have been characterized in a few human cases (in France or in other countries) and the results can be reported within a short time after collection.

The main limitations identified are as follows:

- The number of cases of human infection cannot currently be estimated on the basis of measurements from wastewater. This type of estimation is currently at the research stage and requires a better understanding of the parameters of human viral shedding (proportion of infected people who shed virus in the faeces, period of shedding, quantity of virus shed, dynamics of shedding over time). It also requires knowledge of parameters related to the monitoring site (population covered including any changes during movements due to holidays or work, control of dilution effects, knowledge of the sanitation network) and/or the standardization of analytical results using a control for human activity (bacteriophage, pepper mild mottle virus - PMMoV)[4];
- It is difficult to determine the minimum number of infections needed in the population to detect the virus in wastewater because this depends both on the detection limit of the method, on the dilution of the wastewater (rain, etc.) and on the amount of virus excreted in the faeces, for which there is little data;
- The size of the sector whose wastewater is collected and routed to a wastewater treatment plant under surveillance does not allow a specific area or structure to be targeted (localization of cluster systems not possible);
- The coexistence of many analytical methods and the lack of standardization makes it difficult to compare results between separate sites monitored by different laboratories;

- The detection of the genome of a SARS-CoV-2 variant in a wastewater sample depends among other things on the sensitivity of the tests, the storage of the samples and the concentration of the variant. If a variant is not detected this does not mean that this variant is absent from the wastewater or the population (risk of false negative warnings);

- If the genome of a SARS-CoV-2 variant is present in wastewater this does not necessarily means we can draw conclusions about its epidemic potential, a change in contagiousness or severity or a change in sensitivity to vaccines in the absence of additional epidemiological data, collected in France or elsewhere (risk of false positive warnings).

2. PUBLIC HEALTH OBJECTIVES OF SARS-COV-2 GENOME MONITORING IN WASTEWATER

Based on current scientific knowledge, surveillance data for SARS-CoV-2 in wastewater can be used to meet two main objectives useful in public health to guide management measures:

2.1 Early detection of the presence of the SARS-CoV-2 genome and emerging variants in general population

This qualitative objective (detection/non-detection) relates to an early warning system and means it would be possible to trigger appropriate management measures. It is particularly useful at a time or in a geographical area where the level of circulation of the virus/variant is very low in the population. On the one hand, validated detection methods are required beforehand, and on the other, there needs to be a short time between sampling and reporting results.

A secondary objective may also be of interest depending on the context: detecting the presence of cases of infection on a targeted site that is believed to be "COVID-19-free", or where few tests are carried out (for example, long-term care facility, school, barracks, campus, prison or a district). This approach means that management and testing measures can be adjusted at a local level. It is more suitable for sites where the community to be monitored is resident.

In order to be able to meet the objective of early warning, the European Commission recommends that the result be recorded no later than 48 hours after sampling [23].

The detection of variants in wastewater throughout the territory in a short time, and irrespective of the level of circulation of known strains, can provide useful information to quickly describe the temporal and spatial distribution of these new variants before deployment of mass of sequencing capabilities in the general population. For new variants to be detected in wastewater and to be monitored to support management, the strain/sequence must first have been identified by the French National Reference Centre (CNR) for respiratory viruses in human samples or by other analytical laboratories in France or abroad and reported in GISAID[2]. Laboratories carrying out wastewater detection must therefore use qualified and validated methods for detecting variants of interest. The results of research programs carrying out sequencing of strains found in wastewater can also provide useful information about variants. Furthermore, given the rapid development of genomic surveillance, the detection of new emerging variants corresponding to the definition of variants currently being evaluated after advice from the CNR and the EMERGEN project[3] is to be considered. Forming a link between genomic surveillance in wastewater and the EMERGEN project could also be envisaged.

[2] Mutations or variants of the virus have been monitored since the start of the COVID-19 pandemic through the database of the *Global Initiative on Sharing Avian Influenza Data* (GISAID) on sequencing (https://www.gisaid.org/)

[3] The Emergen project was set up in January 2021 in order to increase sequencing capability in France. This is a SARS-CoV-2 genomic surveillance project for monitoring and research purposes, coordinated by the agency and the ANRS | Maladies Infectieuses émergentes (Emerging infectious diseases, (MIE) and Santé Publique France.

2.2 Monitoring viral circulation trends within a population

This objective is part of a strategy of routine monitoring and makes it possible to adapt the management measures in place and to check their effectiveness. It is particularly useful for identifying when viral circulation accelerates, reaches a plateau or decreases either in the population or in certain geographical areas. Given current knowledge on the duration of viral shedding in faeces, this objective is not particularly suited to dating an end to viral circulation in a given population (concept of end of the epidemic wave). Trends can be monitored on a local scale and more globally on a larger scale, provided that the laboratories involved use the same methods.

To date, in the absence of a sufficiently established link between the level of concentration in wastewater and the number of cases of infection in the population, it is not possible to produce a quantitative indicator of the number of cases in the population through wastewater surveillance.

3. SPECIFICATIONS FOR THE IMPLEMENTATION OF MONITORING

Wastewater monitoring can therefore complement other COVID-19 surveillance systems to inform decision makers. The wastewater sampling strategy must be suited to public health objectives, which may vary according to the phase of the epidemic (see above).

Appropriate public health interpretation of surveillance data on the SARS-CoV-2 genome in wastewater depends on a good understanding and knowledge of the urban hydrology associated with the sampling sites, the representativeness of the samples taken, compliance with good practices in terms of packaging and storing samples (from sampling to arrival at the laboratory) and all the stages of the analytical pathway up to the final interpretation of the results. Recommendations from the European Commission propose parameters to standardize data [23].

3.1 Selection of sites of interest and surveillance sampling plan

Sites of interest for the surveillance of the SARS-CoV-2 genome in wastewater must be chosen on the basis of the targeted public health objectives at national and local level, as well as available human and logistical resources. It follows that urban areas will represent a useful option for monitoring trends and detecting re-emergence because they cover the majority of the population in France. Similarly, tourist sites likely to welcome a large number of people over short periods may be of interest for the early detection of cases. Finally, in a more targeted manner, institutions accommodating vulnerable people (hospitals, nursing homes), schools, university campuses/residences, prisons or areas in which general population testing capacities are limited could also be useful points for surveillance. Out of these institutions, the ones that are places of residence (e.g., university residences, prisons, nursing homes) are to be preferred because toilets will be used for the excretion of faeces more routinely there than in other types of institution (school, workplace, etc.). The choice of sites of interest may change according to viral circulation and testing capacities in the general population.

To meet the main objectives of surveillance in the general population (detection and monitoring trends), splitting the country into a grid that allows for representation at a departmental scale could be considered. The capital towns of departments and/or metropolitan areas that bring together a significant part of the population of the department could constitute sentinel sites of interest. This geographical perspective is also consistent with the approach to surveillance data in the human population, which will have to be compared with the wastewater data. It is also consistent with EU recommendation 2021/472 of 17 March 2021, which stated that setting up wastewater surveillance for large urban areas with more than 150,000 inhabitants was a minimum requirement [23].

To meet the secondary objectives, additional sampling sites may be selected. The choice of sites depends on the target population that it is considered useful to monitor, and whether it is resident (detection in a given institution or district) or transient (tourist sites).

For each selected site, a good knowledge of the following parameters is needed for interpretation of the results: the average hydraulic residence time of the wastewater before it reaches the point where the sampling device is installed, the area and the size of the connected population, with any significant ad hoc changes in the connected population (travel during holiday periods, work-related travel, work on the wastewater treatment plant), industrial or rainwater inflows. It will then be possible to relate the measurement of viral RNA obtained to a population equivalent figure and to a possible period of shedding, subject to better understanding of human viral shedding in faeces (extent and duration).

Finally, for each site, there must be accessible sampling points representative of the wastewater flow. For sites linked to wastewater treatment plants, the points must not be impacted by any pretreatment processes, so if possible they should be installed on the raw water at the plant inlet. For sites monitoring targeted sub-populations, a precise technical analysis of the wastewater networks must be carried out beforehand. The points must in particular allow the sampling device to be securely installed and an assessment of the representativeness of the samples must be carried out so that any uncertainties can be estimated (including the probability of false positives). The availability of metadata that may be useful for interpreting the results is also a site selection criterion: inlet flow rate, physical and chemical parameters, indicator of faecal mass independent of SARS-CoV-2.

The sampling plan for monitoring the SARS-CoV-2 genome in wastewater should be guided by public health needs. It may need to be updated over time based on changing scientific knowledge and public health goals (Table 1). The sampling strategy must also take into account the testing capacity of competent laboratories.

3.2 Type of sample to be collected

To meet the main monitoring objectives, untreated wastewater can be sampled at the inlet of the wastewater treatment plant. To meet the secondary objective, wastewater can be collected upstream in the wastewater collection network or at the outlet of the institution.

In order to take into account the daily variability of faecal matter inputs to wastewater, composite samples are considered more representative of community faecal contributions than grab samples and should be preferred. These samples are collected over a set period of time - usually 24 hours.

In order to take dilution phenomena into account, mainly due to rainy weather, it is recommended that the samples between plant inlets should as far as possible be linked to the flow rate or by default taken only in dry weather.

For samples upstream in the network or at the outlet of an institution, a grab sample can be taken if the restrictions in the field justify it. In this case, only the detection objective can be addressed. At the scale of small communities, variations in concentrations as a function of time can also strongly influence the results of grab samples. In this case, the identified uncertainties should be clearly communicated.

I TABLE 1 I

Sampling strategy and indicators of interest according to the objectives of monitoring SARS-CoV-2 in wastewater

Monitoring objectives	Sampling sites	Sampling frequency	Type of sample	Indicators of interest
Early detection of the presence of SARS-CoV-2 and emerging variants in the general population	WWTP Urban areas* WWTP Tourist areas	Twice a week Sampling days can be adjusted to fit the context**	Untreated wastewater at WWTP inlet Composite 24-hour or grab sample**** following a standardized and reproducible protocol	Present/Absent Variants identified Sensitivity of the method Size of the population connected
To detect or monitor the presence of cases of infection on a targeted site believed to be “COVID-19-free” or where few tests are carried out	Wastewater outlet of hospitals, nursing homes, campuses, prisons, neighbourhood sewers. A precise technical analysis of the wastewater networks must be carried out beforehand	Once or twice a week Sampling days and time slots can be adjusted to fit the context**	Untreated wastewater in the collection network Composite 24-hour or grab sample**** following a standardized and reproducible protocol	Present/Absent Cycle threshold value Size of the population covered Composite/grab sample
To track trends in viral circulation within a population	WWTP Urban areas*	Once or twice a week following a protocol harmonized between the sites and reproducible from one week to the next***	Untreated wastewater at WWTP inlet Composite 24-hour sample following a standardized and reproducible protocol	Number of copies/Litre Number of copies/ 100,000 inhabitants per day

WWTP: wastewater treatment plant;
**one WWTP per department and/or per major metropolitan area;*
***in dry weather only if the sample cannot be linked to flow rate;*
****the sampling frequency can be adjusted according to the epidemiological situation and the sampling days according to the meteorological context*
*****this type of sampling is to be used as a last resort and the uncertainties linked to variations in concentrations depending on the time of sampling must be identified*

3.3 Sample processing

Several test methods are used to detect or quantify SARS-CoV-2 RNA in wastewater in France without there being any real harmonization between the methods (see statement from the French Agency for Food, Environmental and Occupational Health and Safety (ANSES) of 19 February 2021 on the spreading of sludge from the treatment of urban wastewater[4]). For the purposes of surveillance, in particular for monitoring trends, we must be able to compare the results obtained by different laboratories and at different sites. This means that variability within and between laboratories needs to be assessed and reduced to a minimum. This aspect will be implemented more specifically by the ANSES, following the designation of its Hydrology Laboratory in Nancy (LHN) as the National Reference Laboratory (LNR) for monitoring SARS-CoV-2 in wastewater and sludge from treatment plants.

In this respect, it appears that an analytical section to complete these specifications is needed, and this would define the criteria/steps to be carried out to obtain standardized, validated, reproducible results as well as their level of uncertainty (see ANSES LNR mandate).

The main steps identified for this part are [14]: sampling, transport, storage, preparation, concentration, RNA extraction, detection method, controls (recovery of the matrix, human faecal standardization to take into account the effects of dilution or change in the size of excretory population, inhibition tests and control of negatives).

3.4 Data to be collected and useful indicators for decision-making

A data set is needed to be able to interpret measurements of the SARS-CoV-2 genome in wastewater. These data are collected at several stages of the monitoring process (Table 2).

The indicators of SARS-CoV-2 genome in wastewater results that are useful for surveillance can be both qualitative (presence/absence of viral RNA in wastewater) and quantitative. The indicators then correspond the quantity of viral RNA in the wastewater to a volume of wastewater (number of copies/litre) or to the population connected (number of copies/100,000 inhabitants) per day. For each estimated indicator, the level of uncertainty linked to the intrinsic variability of the analysis method must be known and reproducible between the sites and the samples.

[4] https://www.anses.fr/fr/system/files/MFSC2020SA0137Ra.pdf

I TABLE 2 I

Data to be collected for surveillance

Stage	Information to collect
Sampling site	Size of the population equivalent (connected population), daily incoming flow range (min, max, average, median), connection of institutions such as nursing homes or care settings, connection of industrial water (if there are large-volume production channels)
Each time a sample is taken	Date, time slot covered by the sampling (if composite sample) or sampling time (if grab sample), sampling location, characteristics of the sampling point and the sampling device, volume of water sampled, any precipitation at the time of sampling Incoming wastewater flow during sampling
For each sample	Approaches to sample concentration methods, extraction and quantification methods, efficiency of viral recovery and molecular inhibition control methods, human faecal standardization, date of receipt and start of analysis, date of validation of results. Overall sample characteristics (BOD, COD, TSS, NH4, NTK, etc.)

4. INTERPRETATION OF RESULTS

4.1 Consideration of temporality

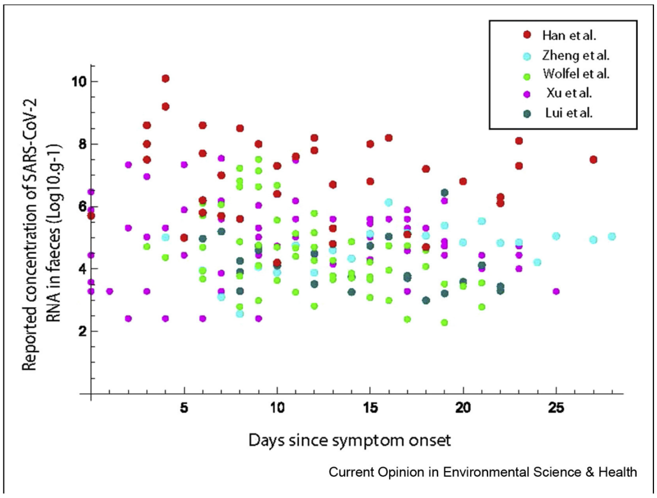
In order to be able to interpret the results of the concentrations of SARS-CoV-2 RNA measured in wastewater at a time t, the delay between taking the sample and reporting results needs to be taken into account, as well as the retention time in the wastewater collection system between the connected dwellings and the sampling point. Added to these delays is the duration of SARS-CoV-2 shedding in faeces, which can be several weeks, as was shown in a recent meta-analysis (figure 2) [3, 24, 25].

By way of comparison, detection in nasopharyngeal samples is possible from a few days before to a week after the onset of symptoms, based on surveillance in the human population (figure 3) [26]. The time taken to perform analysis and make the information available in the human virological surveillance database (SI-DEP) is around 24 to 48 hours. For symptomatic cases who are going to be tested, the total time is therefore around 3 to 4 days (the time between onset of signs and taking a sample is around 2 days, to which we add analysis and escalation time in SI-DEP). For symptomatic cases that require hospital treatment, the delay between the date of onset of signs and the date of hospitalization is around 1 to 2 weeks. Therefore, the early detection of the SARS-CoV-2 genome in wastewater is particularly useful when there is minimal screening in the general population or data is not collected (e.g., self-testing).

These delays are important to consider in order to compare wastewater monitoring data in real time with virological data in the population (incident cases). The objectives of detecting and tracking upward phase trends in viral circulation are not greatly affected by these lags, however, the downward phase trend may be delayed and slower in wastewater due to prolonged viral shedding.

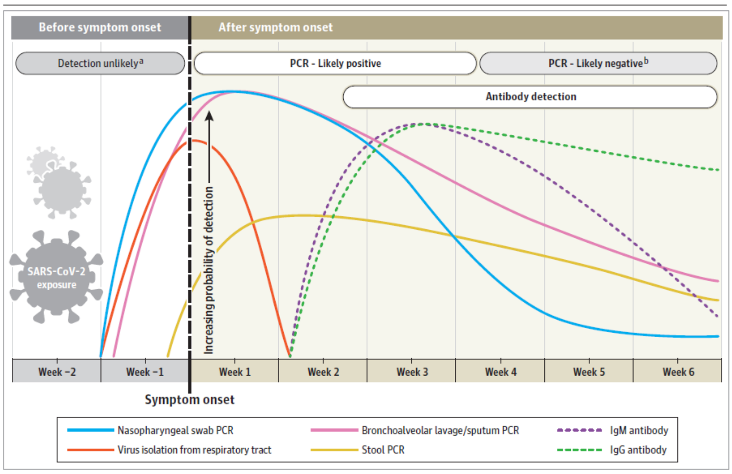
I FIGURE 2 I

Summary of reported concentrations of SARS-CoV-2 in faecal samples per day from onset of symptoms (note that data from Xu *et al.*, 2020 are reported in days after hospitalization) (source: [3])



I FIGURE 3 I

Estimation of variation over time of SARS-CoV-2 infection diagnostic methods in relation to date of onset of symptoms (source: [26])



Estimated time intervals and rates of viral detection are based on data from several published reports. Because of variability in values among studies, estimated time intervals should be considered approximations and the probability of detection of SARS-CoV-2 infection is presented qualitatively. SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2; PCR, polymerase chain reaction.

[a] Detection only occurs if patients are followed up proactively from the time of exposure.

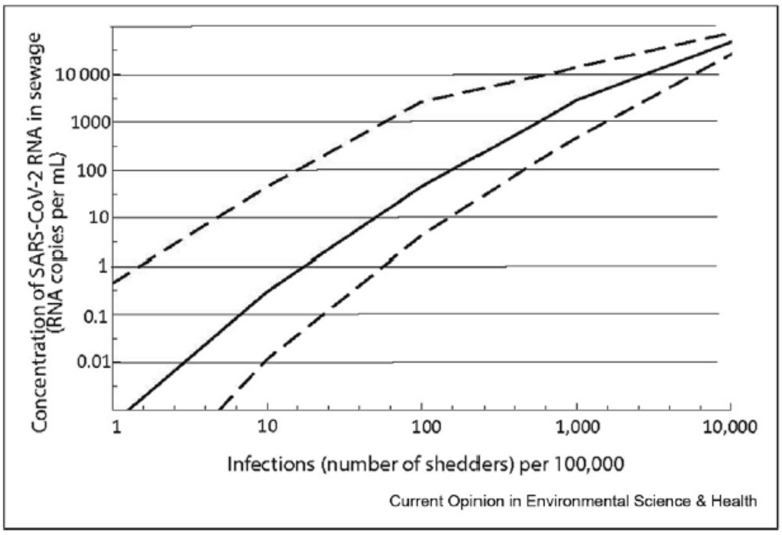
[b] More likely to register a negative than a positive result by PCR of a nasopharyngeal swab.

4.2 Estimation of the number of cases of infection in the population

Although recent studies suggest a relationship between the concentration of SARS-CoV-2 RNA in wastewater and the number of infections in the population (Figure 2) [3], current scientific knowledge is not sufficient to be able to estimate case numbers in the population on the basis of measurements of the SARS-CoV-2 genome in wastewater. It is specifically the lack of data on viral shedding in faeces that holds back this estimation: the proportion of cases shedding SARS-CoV-2 in faeces, the duration of shedding, the extent of shedding in relation to duration and the presence or absence of symptoms, as well as intra- and inter-individual variability. A review of studies on COVID-19 by Parasa *et al.* [27] reported that SARS-CoV-2 RNA was detected in the faecal specimens of 40.5% of patients with COVID-19 (95% confidence interval [CI] 27.4% to 55.1%). Another study reported that viral RNA was identified in 57% of infected people (55 of 96 patients) [25]. Finally, a recent meta-analysis estimated the prevalence of SARS-CoV-2 in the faeces of patients with COVID-19 at 43% (95% confidence interval [CI] 34%–52%) [7]. This same study also showed that the prevalence in faeces is higher in patients with gastrointestinal symptoms, especially diarrhoea, and that shedding of SARS-CoV-2 in faeces lasts about 3 weeks (compared to 2 weeks in nasopharyngeal swabs). In addition to parameters related to viral shedding in humans, those concerning wastewater sampling sites also need to be understood. This means that a good knowledge of the hydraulic conditions, the size of the population covered and any variations are needed to be able to estimate case numbers from the results of SARS-CoV-2 concentrations in wastewater.

I FIGURE 4 I

Modelling the relationship between the number of infected people in the population and the concentration of SARS-CoV-2 RNA in wastewater (solid line = median; dashed lines = p5 and p95) (source: [3])



4.3 Detection of SARS-CoV-2 RNA in wastewater

The detection of SARS-CoV-2 RNA in wastewater indicates at least one case of infection (symptomatic or asymptomatic) in the population connected to the wastewater network during the last few days (max 2 weeks). During phases of low viral circulation, repeat sampling (for example twice a week) makes it possible to reduce the time between the appearance of a case in the community and detection in wastewater.

If no SARS-CoV-2 RNA is detected, this indicates a low level of circulation in the population of the area and it can be considered at a low risk of spread. One study showed that a signal (presence of viral RNA) in wastewater had been detected while the incidence in the population was around 1 case per 100,000 inhabitants [13].

At the small community scale (neighbourhoods or institution outlets), if no SARS-CoV-2 genome is detected in wastewater this should not lead to a relaxation in the monitoring of human cases suggestive of SARS-CoV-2 (possibility of false negatives), or the use of protective measures.

The potential for false alarms (false negatives or false positives) must be taken into account both when SARS-CoV-2 RNA is detected in wastewater and when it is not (Table 3).

The detection of the variants must be interpreted in terms of the epidemiological data which make it possible to characterize the epidemic threat and the seriousness of the mutations. Not all variants necessarily present a public health danger.

I FIGURE 4 I

Possible scenarios for the detection of the SARS-CoV-2 genome, or its variants, in wastewater and interpretation of the results

<table>
<tr><th colspan="2" rowspan="2"></th><th colspan="2">In the community</th></tr>
<tr><th>Virus/variants present</th><th>Virus/variants absent</th></tr>
<tr><td rowspan="2">In wastewater samples</td><td>Virus/variant detected</td><td>True positive samples

Testing and wastewater surveillance consistent with earlier data from the latter => possible alert
Usefulness:
- If testing not easily accessible
- Detection of variants known elsewhere but not (or partially) identified in the territory</td><td>False positive samples: possible if contaminated in the laboratory or artifact in the analytical method

“Variant false alerts” possible:
- In the event of erroneous identification, especially of variants to monitor
- If a variant is identified that has low or no epidemic potential</td></tr>
<tr><td>No virus/variant detected</td><td>“False negative” samples: sensitivity of the method, sampling or storage conditions, dilution</td><td>True negative samples
Unlikely considering the high probability of SARS-CoV-2 becoming endemic.
In the event of endemicity, perhaps a relatively high detection threshold would be useful to rule out the risk of local epidemic in the medium term</td></tr>
</table>

4.4 Quantification of SARS-CoV-2 RNA in wastewater

Quantitative results provide information on the trends and extent of viral circulation in the connected population.

Trends must be calculated on the basis of concentrations (number of copies/100,000 inhabitants or number of copies/L) by sampling date, obtained from composite samples (24 hours), which take into account possible dilution phenomena related to rainfall or variations in human or industrial inputs. Trends will preferably be interpreted on the basis of several measurements in order to confirm any changes. Interpretation will take into account the uncertainty of the measurement. The results of samples taken on rainy days will be discarded if the rainfall effect cannot be corrected.

Trend calculations as well as the representation of normalized standardized concentrations related to a population equivalent figure per day mean that different sites can be compared with each other.

Cross-referenced interpretation with health data can help to guide management measures (see part 5).

5. USE AND DISCLOSURE OF DATA

In order to be able to integrate measurements of SARS-CoV-2 in wastewater into the overall epidemiological surveillance strategy for COVID-19, surveillance partners must be able to rapidly collect and disseminate the data necessary for the interpretation in public health.

Sharing surveillance data is an essential source of collective progress in public health. This sharing of data should initially involve the operators of the wastewater treatment plants, the laboratories, the decision-makers, the regional health agencies and Santé Publique France, responsible for monitoring the state of health of the population. The format and frequency with which data is made available must be defined with users and be compatible with monitoring objectives.

Surveillance data for the SARS-CoV-2 genome in wastewater should not be interpreted in isolation. The joint interpretation of health data is necessary to appreciate the level of viral circulation in the population and the trend in order to guide management measures. Depending on the progress of knowledge in the precision of analytical methods and in the relationship between wastewater data and health data, wastewater surveillance could constitute in itself an alert system (if a variant of interest is identified or a quantitative alert threshold is exceeded).

Proposals for management measures can be drawn up taking into account the cross-referenced interpretation of population and wastewater surveillance data: recommendations for testing, raising awareness of wearing face coverings, recommendations for limiting contact within the population (physical distancing), etc. Several scenarios are considered for the interpretation of the data (Table 4).

I TABLE 4 I

Scenarios for wastewater surveillance and interpretation of the results according to the rate and extent of viral circulation in the general population (main objectives of wastewater surveillance) (source: adapted from Medema [3]) - 1/3

Wastewater monitoring points	Rate and extent of circulation in population	Monitoring indicator results: Population (SI-DEP or SIVIC) Variation of S-1 / S-2 indicators	Monitoring indicator results: Wastewater Variation indicators S-1 / S-2	Interpretation	Management proposals
WWTP Urban area Weekly or twice-weekly sampling	Weak circulation, between 2 epidemic waves *Low incidence rate* ($\leq 10/10^5$ inhabitants) / Few cases admitted to hospital** R ≤ 1****	Stability of incidence indicators	SARS-CoV-2 absent or at stable concentration	Consistent: confirms low circulation	No change, continue monitoring
		Stability of incidence indicators	SARS-CoV-2 detected or increase in concentration	Non-convergent: validate the variation, may indicate renewed circulation	Strengthen communication concerning prevention, strengthen testing, reinforce TAP, +/- alert healthcare institutions
		Increase in incidence indicators****	SARS-CoV-2 absent or at stable concentration	Non-convergent: validate the variation, may indicate renewed circulation	Strengthen communication concerning prevention, strengthen testing, reinforce TAP, +/- alert healthcare institutions
		Increase in incidence indicators****	SARS-CoV-2 detected or increase in concentration	Consistent: may indicate renewed circulation	Strengthen communication concerning prevention, strengthen testing, reinforce TAP, +/- alert healthcare institutions
	Moderate to high viral circulation, upward phase of an epidemic wave, reaching a plateau, downward phase *Moderate to high incidence rate* ($>20/10^5$ inhabitants) Moderate to large number of cases admitted to hospital** R > 1****	Increase in incidence indicators****	Increase in concentration	Consistent: further acceleration of viral circulation – management measures insufficient or not yet effective	Strengthen communication concerning prevention, strengthen testing, reinforce TAP, reinforce application of physical distancing and wearing face coverings - Prevention of pressure in hospitals
		Increase in incidence indicators****	Stabilised concentration	Non-convergent: validate the variation, may indicate a slowdown in circulation – effective management measures	No change, continuation of measures in place
		Increase in incidence indicators****	Decrease in concentration	Non-convergent: validate the variation, may indicate a slowdown in circulation – effective management measures	No change, continuation of measures in place

I TABLE 4 I

Scenarios for wastewater surveillance and interpretation of the results according to the rate and extent of viral circulation in the general population (main objectives of wastewater surveillance) (source: adapted from Medema [3]) - Continued 2/3

Wastewater monitoring points	Rate and extent of circulation in population	Monitoring indicator results: Population (Sidep or Sivic) Variation indicators S-1 / S-2	Monitoring indicator results: Wastewater Variation of S-1 / S-2 indicators	Interpretation	Management proposals
WWTP Urban area Weekly sampling	Moderate to high viral circulation, upward phase of an epidemic wave, reaching a plateau, downward phase *Moderate to high incidence rate* (>20/10^5 inhab.) Moderate to large number of cases admitted to hospital** R = 1****	Stabilization of incidence indicators****	Increase in concentration	Non-convergent: validate the variation, may indicate a renewed rise in circulation – management measures not sufficiently effective	Strengthen communication concerning prevention, strengthen testing, reinforce TAP, reinforce application of physical distancing and wearing face coverings - Prevention of pressure in hospitals
			Stabilised concentration	Consistent: may indicate a slowdown in circulation – effective management measures	No change, continuation of measures in place
			Decrease in concentration	Non-convergent: validate the variation, may indicate a slowdown in circulation – effective management measures	No change, continuation of measures in place

I TABLE 4 I

Scenarios for wastewater surveillance and interpretation of the results according to the rate and extent of viral circulation in the general population (main objectives of wastewater surveillance) (source: adapted from Medema [3]) - Continued 3/3

Wastewater monitoring points	Rate and extent of circulation in population	Monitoring indicator results: Population (Sidep or Sivic) Variation indicators S-1 / S-2	Monitoring indicator results: Wastewater Variation of S-1 / S-2 indicators	Interpretation	Management proposals
WWTP Urban area Weekly sampling	Moderate to high viral circulation, upward phase of an epidemic wave, reaching a plateau, downward phase *Moderate to high incidence rate** *(>20/10^5 inhab.)* *Moderate to large number of cases admitted to hospital*** *R < 1****	Decrease in incidence indicators****	Increase in concentration Stabilised concentration	Non-convergent: validate the variation, may indicate a renewed rise in circulation – management measures not sufficiently effective	Strengthen communication concerning prevention, strengthen testing, reinforce TAP, reinforce application of physical distancing and wearing face coverings - Prevention of pressure in hospitals
			Stabilised concentration Decrease in concentration	Consistent: may indicate a slowdown in circulation – effective management measures	No change, continuation of measures in place
Wastewater outlet of a small population community believed to be “COVID-19-free” (nursing home, school, university campus, etc.) Twice-weekly sampling	Absence of circulation	No cases reported in the community	SARS-CoV-2 not detected in wastewater	Consistent	No change, continue monitoring
			SARS-CoV-2 detected in wastewater	Presence of at least one infection in the community	Investigation in the community: testing and detecting cases Strengthen physical distancing measures

Sidep data; **SIVIC data; ***Sources: Oscour, Sidep, SIVIC; ** Since the incidence is dependent on the positivity rate and the testing rate, variations in this indicator must be interpreted in terms of the testing strategies in the populations; TAP: Test Alert Protect*

6. A PARTNERSHIP APPROACH

The implementation of surveillance of the SARS-CoV-2 genome in wastewater for public health action requires a multidisciplinary approach that includes:

- Those in charge of wastewater management and treatment (public/private operators) for access to sites, knowledge of wastewater networks +/- taking samples;

- Persons delivering sampling, analyses, validation and reporting of results (study agencies, analytical laboratories) for routine surveillance;

- Persons in charge of epidemiological surveillance at a local and national level (Santé Publique France) to define surveillance objectives and for an understanding of population surveillance data and evaluation of the system;

- Stakeholders with expertise in microbiology (ANSES, research teams, analytical laboratories, CNR for respiratory viruses) to define the analysis protocols and evaluate the methods;

- Persons responsible for advancing knowledge (research teams);

- Stakeholders in management (regional health agencies, Directorate General for Health, prefectures, local authorities) to integrate the results of wastewater measurements into management decisions;

- Financers: costs are estimated at €25,000 per year per monitoring site at a frequency of two samples per week [4].

In addition, facility managers (long-term care facilities, campus, prison, etc.) should be able to benefit from information on the benefits and limitations of wastewater surveillance in order to elucidate the management/monitoring measures for which they are responsible.

Delivering surveillance requires the creation of coordination structures on a national and local scale in order to guarantee the application of a sampling strategy, protocol and analysis techniques and the reporting of consistent results throughout the territory that are compatible with public health objectives. This type of structure is consistent with European Union Recommendation 2021/472 in which "*Member States are encouraged to put in place adequate structures involving health and wastewater competent authorities with the objective of merging and linking relevant data and coordinating the interpretation and communication of results*" [23].

7. EVALUATION OF THE MONITORING SYSTEM

At this stage of development, the added value of wastewater surveillance results compared to health data in terms of detection sensitivity, early detection and trend monitoring needs to be assessed. For the qualitative objective, a comparison with the health data (hospitalizations from the Sivic database by sewer basin and cases reported on the Sidep portal by sewer basin) will mean that the delay between the changes observed in wastewater and those observed in health data can be pinpointed. The time needed to report wastewater results and to make health data available must be considered in this analysis.

8. REGULATORY ASPECTS

If SARS-CoV-2 genome detection in wastewater is carried out in a narrow geographical area or even a facility or building that may be in the private domain, care must be taken over data protection in line with the anonymity criteria of the National Commission for Information and Liberties (CNIL). In particular, it will be necessary to ensure that there is no correlation with another file which would allow a person to be identified by cross-referencing. Furthermore, the very extensive dissemination of the results files could be interpreted as potentially infringing the privacy of the persons concerned.

9. KEY RECOMMENDATIONS

9.1 For surveillance

It seems necessary to recommend that:

- An analysis method that is standardized and harmonized between the laboratories monitoring different sampling sites should be available;
- A standard and reproducible sampling protocol should be used, applicable both to the water sampled at wastewater treatment plant inlets and samples taken upstream in the wastewater network (sewers, outlets of facilities of interest);
- A protocol should be available for deploying a method that can rapidly detect the spatial and temporal spread throughout the territory of emerging variants after identification by the respiratory viruses national reference centre;
- A frame of reference for analytical interpretation should be available that can judge true variations between two samples (decreases or increases) taking into account uncertain aspects of the measurement related to sampling and analysis;
- A centralized dashboard of standardized indicators and mapping of these indicators should be available.

9.2 For research

It can be recommended that:

- Methods for the analysis and sequencing of SARS-CoV-2 variants in wastewater should be promoted together with the respiratory viruses national reference centre in order to strengthen their monitoring;
- The acquisition of knowledge on detection and quantification thresholds in wastewater should be fostered and this should be compared with incidence data in the general population;
- The development of knowledge on viral shedding in faeces should be fostered;
- The acquisition of knowledge on the relationship between the amount of SARS-CoV-2 RNA in wastewater and the number of infected people in the general population should be fostered.

10. REFERENCES

1. World Health Organization. Expert consultation on public health needs related to surveillance of SARS-CoV-2 in wastewater - Summary report 30 November 2020 [1-27]. Available from: https://www.euro.who.int/en/health-topics/environment-and-health/water-and-sanitation/publications/2021/expert-consultation-on-public-health-needs-related-to-surveillance-of-sars-cov-2-in-wastewater-summary-report-virtual-meeting,-30-november-2020.
2. Wurtzer S, Marechal V, Mouchel JM, Maday Y, Teyssou R, Richard E, et al. Evaluation of lockdown effect on SARS-CoV-2 dynamics through viral genome quantification in waste water, Greater Paris, France, 5 March to 23 April 2020. Euro Surveill. 2020;25(50).
3. Medema G, Been F, Heijnen L, Petterson S. Implementation of environmental surveillance for SARS-CoV-2 virus to support public health decisions: Opportunities and challenges. Curr Opin Environ Sci Health. 2020;17:49-71.
4. European commission. Tracking COVID-19 employing waste waters: a reliable indicator for supporting the prevention and management of the pandemic - MEMO 2021 [Available from: https://ec.europa.eu/environment/water/water-urbanwaste/info/pdf/Waste%20Waters%20and%20Covid%2019%20MEMO.pdf.
5. Global Polio Eradication Initiative 2019-2024 [Available from: https://polioeradication.org/who-we-are/polio-endgame-strategy-2019-2023/.
6. Asghar H, Diop OM, Weldegebriel G, Malik F, Shetty S, El Bassioni L, et al. Environmental surveillance for polioviruses in the Global Polio Eradication Initiative. J Infect Dis. 2014;210 Suppl 1:S294-303.
7. Zhang Y, Cen M, Hu M, Du L, Hu W, Kim JJ, et al. Prevalence and Persistent Shedding of Fecal SARS-CoV-2 RNA in Patients With COVID-19 Infection: A Systematic Review and Meta-analysis. Clin Transl Gastroenterol. 2021;12(4):e00343.
8. The Water Research Fundation. Wastewater Surveillance of the COVID-19 Genetic Signal in Sewersheds - Recommendations from Global Experts Denver, USA2020 [1-27]. Available from: https://www.waterrf.org/news/water-research-foundation-announces-new-covid-19-research-effort.
9. World Health Organization. Status of environmental surveillance for SARS-CoV-2 virus - Scientific brief 7 august 2020 [1-4]. Available from: https://www.who.int/news-room/commentaries/detail/status-of-environmental-surveillance-for-sars-cov-2-virus.
10. World Health Organization. Rapid expert consultation on environmental surveillance of SARS-CoV-2 in wastewater - Summary report 23 July 2020 [1-19]. Available from: https://www.euro.who.int/en/health-topics/environment-and-health/water-and-sanitation/publications/2020/rapid-expert-consultation-on-environmental-surveillance-of-sars-cov-2-in-wastewater-summary-report-2020.
11. World Health Organization. Water, sanitation, hygiene, and waste management for SARS-CoV-2, the virus that causes COVID-19 29 July 2020 [Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-IPC-WASH-2020.4.
12. Ahmed W, Angel N, Edson J, Bibby K, Bivins A, O'Brien JW, et al. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. Sci Total Environ. 2020;728:138764.
13. Medema G, Heijnen L, Elsinga G, Italiaander R, Brouwer A. Presence of SARS-Coronavirus2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands. Environ Sci technol Lett. 2020;7:511-6.
14. Centers for Disease Control and Prevention of the united States. Wastewater Surveillance - A new public health tool to understand COVID-19 spread in a community 2020 [Available from: https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance.html.
15. Luxembourg Institute of Science and Technology. Coronastep 2020 [Available from: https://www.list.lu/fr/covid-19/coronastep/.
16. RIVM - Institut national de la santé publique et de l'environnement néerlandais. Coronavirus monitoring in sewage research [Available from: https://www.rivm.nl/en/covid-19/sewage.
17. Water Research Australia. ColoSSoS : Collaboration on Sewage Surveillance of SARS-CoV-2 [Available from: https://www.waterra.com.au/research/communities-of-interest/covid-19/.

18. European commission. SARS-CoV-2 Surveillance employing Sewers EU Umbrella Study – Status Update 2020 [Available from: https://ec.europa.eu/jrc/en/science-update/sars-cov-2-surveillance-employing-sewers-eu-umbrella-study-status-update.
19. européenne C. Recommandation (UE) 2021/472 concernant une approche commune pour la mise en place d'une surveillance systématique de la présence du SARS-CoV-2 et de ses variants dans les eaux usées de l'Union européenne 17 mars 2021 [Available from: https://eur-lex.europa.eu/legal-content/FR/TXT/HTML/?uri=CELEX:32021H0472&from=FR.
20. Réseau Obépine. [Available from: https://www.reseau-obepine.fr/.
21. Suez. Résurgence de l'épidémie Covid-19 : une nouvelle offre SUEZ pour détecter les marqueurs du virus SARS-COV-2 dans les réseaux d'eaux usées et anticiper les mesures sanitaires des collectivités [Available from: https://www.suez.fr/fr-fr/actualites/covid-city-watch.
22. Veolia. Avec Vigie-Covid-19, Veolia surveille la présence du SARS-CoV-2 dans les eaux usées [Available from: https://www.veolia.com/fr/actualites/covid-19-detection-sars-cov2-eaux-usees.
23. European commission. Recommandation (UE) 2021/472 on a common approach to establish a systematic surveillance surveillance of SARS-CoV-2 and its variants in wastewaters in the EU. 17 mars 2021 [Available from: https://eur-lex.europa.eu/legal-content/FR/TXT/HTML/?uri=CELEX:32021H0472&from=FR.
24. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. Lancet Microbe. 2021;2(1):e13-e22.
25. Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January-March 2020: retrospective cohort study. BMJ. 2020;369:m1443.
26. Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. JAMA. 2020;323(22):2249-51.
27. Parasa S, Desai M, Thoguluva Chandrasekar V, Patel HK, Kennedy KF, Roesch T, et al. Prevalence of Gastrointestinal Symptoms and Fecal Viral Shedding in Patients With Coronavirus Disease 2019: A Systematic Review and Meta-analysis. JAMA Netw Open. 2020;3(6):e2011335.