World Health Organization

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Contents

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Glossary of terms and acronyms

Term or acronym	Meaning as used in this guidance
Coliphage, bacteriophage	A virus that infects coliform bacteria (coliphage) or other bacteria (bacteriophage)
dPCR	Digital PCR
Enveloped virus	A virus that has a fatty lipid outer envelope (as distinct from naked viruses that have no such envelope)
ES	Environmental surveillance
Flocculation	Used to assist with precipitation and concentration of viruses and their RNA
Irradiated	Exposed to gamma radiation to modify the structure of genetic material (such as RNA) such that it will no longer be capable of producing an infectious virus
Lower limit of detection	The lowest concentration at which the method used can detect the target being analyzed.
Matrix	The liquid or solid material within which viruses and their large RNA fragments are being sought
Membrane filtration	Use of a thin layer of a material, termed a membrane, to capture small particles (including viruses and their large RNA fragments) and separate them from solutes
Normalization	Adjustment of data to allow for comparability.
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
Polyethylene glycol	Used to assist with flocculation, precipitation and concentration of viruses and their RNA
RAT	Rapid antigen test
RNA	Ribonucleic acid
RT-PCR	Reverse transcription PCR
qPCR	Quantitative PCR
Sewage	Wastewater that has been used for sanitation (e.g., for flushing away faecal matter), and is discharged via sewers or other sanitation systems
Skim milk	Used to assist with flocculation, precipitation and concentration of viruses and their RNA
Sludge	Solid or semi-solid materials settled from wastewater, in this case that refers to primary settled sludge
Spike	A control parameter added to a sample to provide a positive control
Ultracentrifugation	High-speed centrifugation to concentrate small particles (including viruses and large RNA fragments) and separate them from solutes
Ultrafiltration	Small size-class filtration to concentrate small particles (including viruses and their large RNA fragments) and separate them from solutes
VoCs	Variants of Concern
Wastewater	Water that has been in contact with people (e.g., for washing) or used for cleansing and sanitation (e.g., for flushing away faecal matter), and is discharged via sewers or other sanitation systems
WHO	World Health Organization

Key messages

This guidance is targeted at public health decision-makers wanting to understand and integrate environmental surveillance (ES) of wastewater into COVID-19 surveillance monitoring and control strategies.

The objective of ES is to provide an additional objective indicator of virus circulation at the population level. A benefit of ES is that it is not susceptible to biases inherent in clinical testing. If carried out ethically, ES can provide community-level data that is not reliant on clinical testing which has become increasingly important following the end of the COVID-19 emergency phase.

ES is a complementary activity that is not intended as a substitute for clinical surveillance. Public health leadership is critical for an effective ES programme to define ES objectives and for timely linking of data to public health response.

Demonstrated effective use cases (Table 1) of ES data for SARS-CoV-2 include:

- Cost effective and sensitive monitoring of spatial and temporal trends and changes in viral circulation, including for variants.
- Providing early indication of a change in incidence including when a peak has been reached, and as an input for modelling future infection trends.
- Guiding clinical testing and vaccination priorities to areas with high ES results, and tracking case clusters.
- Early warning of emergence in low prevalence or localized contexts (e.g., vulnerable or high-risk settings, isolated communities, transport vessels, mass gatherings), or in the absence of evidence from clinical testing.
- Communication of results to the public to reinforce risk reduction behaviours in the community.
- Monitor the impact of public health measures, including increasing or relaxing restrictions, awareness-raising campaigns, and vaccination programmes.

Limitations of ES are that it cannot precisely estimate the number of infected persons within a catchment nor link results to individual clinical care, and ES methods are not sufficiently sensitive during low viral shedding and high wastewater flow.

To decide whether to initiate, maintain, modify, pause, or stop an ES existing programme public health decision-makers should weigh benefits and costs compared to alternative methods (Section 4), and also plan and coordinate ES activities to ensure sufficient capacity, funding, and policy enablers (Checklist Box 2).

As yet, there is no universal standard method for ES for SARS-CoV-2. However, there are several communities of practice at the national, regional, and global scales along with many published protocols. Best practices for sampling, analysis, data interpretation and aggregation is summarized in Section 6.

1. Introduction

WHO declared COVID-19 a public health emergency of international concern (PHEIC) between 30 January 2020 and 5 May 2023. The causal agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), continues to evolve, yielding possible variants of concern, with the resulting pattern of pandemic COVID-19 being too unpredictable for the disease to have been declared endemic. COVID-19 remains a global pandemic but is expected to become globally endemic over time.

Proactive surveillance has remained a priority in many jurisdictions even beyond the PHEIC to help provide evidence about the incidence of SARS-CoV-2 infection and COVID-19 disease, inform decisions on proportionate and evidence-based public health preparedness, prevention and control strategies, and to measure the effectiveness of those strategies [1].

During the PHEIC period, the primary surveillance tool was testing of upper respiratory tract samples, initially primarily using polymerase chain reaction (PCR), and gradually shifting to greater use of rapid antigen tests (RAT). Genomic sequencing of residual PCR samples provided virologic surveillance to track emerging variants and model future waves. However, with the end of the PHEIC the extent of such testing, and just as importantly the systematic reporting and analysis of test results, is declining. Therefore, the principal sources of evidence on the incidence of SARS-CoV-2 infection and COVID-19 are now coming from hospital admission and mortality data [1] such that the actual incidence of SARS-CoV-2 infection and COVID-19 and virologic changes in the broader community are becoming less well understood.

Environmental surveillance (ES) of wastewater (from sewage, or environmental waters contaminated with human excreta and secreta) has been used for a variety of health threats, such as poliovirus [2-4], *Salmonella* Typhi [5-7], norovirus [8,9], hepatitis A virus [8], and antimicrobial resistance (AMR) [10-13].

From early 2020, global researchers began to demonstrate through multiple proof-of-concept studies in various countries that SARS-CoV-2 can be detected in wastewater, and further innovated to validate quantitative measures, both for SARS-CoV-2, and for specific variants using both targeted primers and probes as well as partial and whole genome sequencing [14]. Since that time, an increasing number of jurisdictions have augmented routine clinical testing with routine community-scale COVID-19 ES by monitoring SARS-CoV-2 in wastewater samples, as discussed in the body of this guidance.

The objective of ES is to provide evidence regarding circulation of the target in the population. The information can be reported in terms of the presence or absence of the target in individual samples or as a proportion of samples, its concentration, and specific variants. The intelligence can be used for early warning and to monitor trends to inform public health decisions and measure the effect of interventions [15], such as guiding clinical testing priorities and vaccination priorities, and tracking case clusters [16]. Communication of the results to the public can be relevant to the behaviours of individuals in the community. ES is a complementary activity that is not intended as a substitute for clinical surveillance.

1.1 Purpose

The purpose of this global guidance is to provide locally applicable advice to answer the following questions:

- How does ES fit within the broader public health surveillance context (section 2)?
- How does ES add value to public health decision-making in different settings and contexts, especially when there is competition for limited resources that can alternatively be allocated to other health-related priorities (section 3)?
- On what basis should an ES programme be initiated, maintained, modified, paused, or stopped, and by which agency (section 4)?
- How should an ES programme be planned and coordinated in different resource settings (section 5)?
- How should ES data collection, analysis, and interpretation and communication of results be carried out (section 6)?

1.2 Target audience

This guidance is targeted at public health decision-makers and professionals and COVID-19 pandemic management team members who want to understand and integrate complementary ES into their national, sub-national, or local COVID-19 and integrated surveillance monitoring and control strategies. The guidance also provides general information on coordination, capacity, and methods for laboratory scientists, and water and sanitation services providers. This document is intended to:

- help public health decision-makers and professionals make evidence-based decisions on the value of ES for their context to help decide whether to implement, continue, modify, pause, or stop such a programme;
- show how entities would generally set up a successful ES programme with reference to more specific information on the member states;
- support effective and accessible public communication of SARS-CoV-2 ES results;
- promote sharing and harmonization of SARS-CoV-2 ES methods and approaches between localities, countries and regions;
- guide utilization of SARS-CoV-2 ES results along with other SARS-CoV-2/COVID-19 integrated surveillance modalities to support public health decision-making; and
- share lessons from implementation experiences for more efficient application of ES globally.

1.3 Scope

The testing of wastewater can provide information on the presence, concentration, and variants of SARS-CoV-2 circulating at a population scale. SARS-CoV-2 and its RNA is found in wastewater because the virus is shed in excreta and upper respiratory system secreta. Shedding occurs from both symptomatic and asymptomatic SARS-CoV-2 infected individuals. Wastewater testing provides information on shedding among populations living in, working in, or visiting the defined catchment area from which that wastewater originates. This document discusses use cases, planning and coordination, and emerging best practice methods for data collection, analysis, and interpretation. This document does not provide specific recommendations on use cases or standard methods for ES for several reasons. Firstly, the approaches adopted, and fine details of the methods, are evolving. Secondly, there may be preferred and familiar methods that give the best results locally due to practitioner experience and that enable results to be compared to help assess patterns and trends.

Therefore, widespread method harmonization and standardization is not essential. However, there is sufficient experience to describe common features and good practices for a range of contexts.

ES programmes normally sample from the inlet of wastewater treatment plants in settings with high coverage of sewers, to gain a representative sample of persons present in the sewer catchment area. Sampling can also occur within sewerage networks covering varying spatial areas, including defined sewer sub-catchments, from a building, or group-of-buildings, at wastewater collection points that handle wastewater from decentralized systems, or from transport vessels and hubs. This document also discusses SARS-CoV-2 ES in areas that have limited sewer network coverage, where sampling of faecally-impacted environmental water occurs (e.g., surface water or water in open drains influenced by human excreta and secreta) [17,18].

1.4 Background

This Guidance updates the previous World Health Organization (WHO) 2020 scientific brief [19] and 2022 interim guidance [20]. Case studies that were included in the interim guidance are now summarized with reference links below.

Starting in early 2020, researchers demonstrated during proof-of-concept studies that SARS-CoV-2 can be detected in wastewater. At the time of publication of the first scientific brief in 2020, many countries, including the Kingdom of the Netherlands [21,22], Italy [23], Türkiye [24-28], Japan [29], China [30], India [31], Singapore [16,32], the United States of America [33,34], Australia [35], South Africa [36], and countries in Latin America and the Caribbean [37], had published or demonstrated proof of concept of ES for SARS-CoV-2 by detecting SARS-CoV-2 in environmental samples.

By 2022, numerous SARS-CoV-2 ES programmes had been established and become a routine component of national or subnational COVID-19 surveillance programmes [38-44]. SARS-CoV-2 ES programmes began with SARS-CoV-2 detection (presence vs. absence), then moved to increasingly reliable quantification (expressed as genome copies which were then estimated per unit volume of wastewater, mass of sludge, or an estimated equivalent for passive sampling), then evolved to include normalization and standardization of methods to improve comparability and allow aggregation across sites, and ultimately included testing for targeted known variants [45,46] as well as identifying novel variants [47]. Some countries (e.g., the Kingdom of the Netherlands [48], Switzerland [49], United States of America [50], South Africa [43], Hungary [51], the United Kingdom [52], Türkiye [28,53] and New Zealand [55]) moved to some form of national SARS-CoV-2 ES system that included public reporting. Others were coordinating and consolidating data at a national level, or working at regional, state or provincial subnational levels (e.g., the state of Victoria, Australia [56]). Governance arrangements are diverse, and all involve complex multi-stakeholder arrangements typically including health agencies, wastewater services providers, laboratory services providers and research organizations.

With the end of PHEIC and reduced population level impact of the COVID-19 pandemic, overall COVID-19 programmes have been reduced, with some SARS-CoV-2 ES programmes expanding in scope or scale, and others being reduced, modified, paused, or terminated, depending on availability of funding and its prioritization. However, multiple jurisdictions are continuing with their programmes. The nature of programmes that are continuing has been modified with, for instance, a more targeted approach being taken, such as reduced frequency, or sampling at airports [57].

Data and evidence available on methods and use of SARS-CoV-2 ES have greatly expanded in number and quality enabling this update. Advances in ES for SARS-CoV-2 have been documented in many journal articles, technical reports, expert opinion of SARS-CoV-2 ES programme managers [58,59] public health and COVID-19 pandemic management websites, global data-sharing platforms [60,61] and media communications. Collectively, they have demonstrated a variety of applications and their challenges, costs and limitations (section 2). Lessons have been learned to optimize planning, coordination and capacity for a credible and effective programme (section 4). Sampling techniques [62-68] and analytical methods have been validated and routinely used for detection and quantification of SARS-CoV-2 and its variants (see section 6). Innovations trialled or at proof-of-concept stage have been expanded, and formal research agendas have been prepared (see section 7).

2. Environmental surveillance in the broader public health surveillance context

2.1 Experiences with SARS-CoV-2 ES as part of public health surveillance

A growing body of experience and the specific added value of SARS-CoV-2 ES justifies inclusion of this surveillance method into routine COVID-19 surveillance and as part of integrated respiratory surveillance. ES is used to complement rather than replace public health surveillance methods that are based on compilation of individual clinical testing results (Figs. 1 - 3). Clinical testing can be undertaken for both diagnostic purposes and for community prevalence surveillance. ES provides another tool for assessing community prevalence. Therefore, this document should be read in conjunction with the WHO Interim Guidance on Public Health Surveillance for COVID-19 [69] which describes the range of COVID-19 surveillance methods.

There are several similarities and differences between ES and clinical testing methods and approaches, and summarising those can be useful for communicating ES to those familiar with clinical testing for diagnostic and public community prevalence surveillance.

Within the laboratory, the molecular detection methods used for SARS-CoV-2 ES are comparable, and in some cases identical, to those used for clinical testing. That is, the same reverse transcription PCR (RT-PCR) test kits are often used for the final testing component. What is different about SARS-CoV-2 ES in comparison to clinical testing programmes is the design and interpretation of the community-scale sampling programmes, as well as methods for the concentration and extraction of the RNA from the wastewater and environmental water samples [70-72].

An understanding of the wastewater dynamics within a catchment area, and the geographic and temporal relationship of samples with catchment populations and the communities represented by the sampling points, as compared with health reporting regions and local municipalities, is required to design, and interpret results from a representative SARS-CoV-2 ES programme. Experience with environmental samples, and often some minor adaptation of clinical molecular testing, is required to conduct reliable virus detection assays as part of a SARS-CoV-2 ES programme.

An important benefit of SARS-CoV-2 ES is that it is not susceptible to several biases inherent in clinical testing, which may include presence of symptoms, diagnostic test and health care-seeking behaviour, disease severity, health care and test accessibility (RAT or PCR), physician and personal disposition to test, and cost and reporting limitations. These biases change over time in ways that ES methods do not. In contrast, SARS-CoV-2 ES is independent of clinical testing practices and capacity and provides an objective indicator of virus circulation in the population.

SARS-CoV-2 ES has demonstrated its value in providing important evidence to inform the overall surveillance picture by providing an additional line of evidence to inform disease surveillance to support management programmes and other public health and social measures [73]. This has become increasingly important at a time when clinical testing has declined drastically but the threat of SARS-CoV-2 persists.

At its most basic, SARS-CoV-2 ES has been, and is being, used as a tool to monitor trends and change in viral circulation at a population level. SARS-CoV-2 ES is not a reliable tool to precisely estimate the number of infected persons contributing the virus within a sampled catchment, or to make specific estimates of incidence and prevalence of infections or COVID-19 in the community. Going forward, it

will be impractical to establish the precision of such correlations and relationships since there is likely to be a shortage in clinical testing data. However, reasonable correlations with hospitalizations have been shown in multiple settings [74], and these relationships are likely to improve as experience with SARS-CoV-2 ES grows.

The results from SARS-CoV-2 ES are particularly helpful in providing early indication of a change in COVID-19 incidence at a population level including when a peak has been reached [75]. Viral concentrations are highest early in the disease course, and RNA can be shed into wastewater before the onset of symptoms and before health care-seeking behaviour and associated clinical testing occurs and is reported. Therefore, ES results can inform public health agencies before symptoms, clinical test results, or hospitalizations are reported. As such, ES can provide earlier and more representative warning of trends [76] in COVID-19 incidence and the emergence of variants [46,52,77] than clinical testing. Both variant detection and quantitative ES data can also be used as an input for modelling future infection trends. This can, for instance, help plan for surges in demand for healthcare services and for identifying when such demand may have peaked.

In higher-prevalence contexts, SARS-CoV-2 ES is helpful at documenting trends [78-80], whilst in lower prevalence or localized contexts, or in the absence of evidence from clinical testing, ES provides early warning of SARS-CoV-2 emergence [81,82]. The role of ES in early warning of (re)emergence is more relevant as availability of passive clinical testing data and related sequencing to identify potential new variants has waned, with very low and variable case ascertainment.

Viral concentrations in sewage can be used to monitor the impact of public health measures, including increasing or relaxing restrictions, awareness-raising campaigns, and vaccination programmes. Results from SARS-CoV-2 ES can be used to augment risk assessments and provide information on virus (re)emergence to in turn inform decisions and communications on testing, quarantine, isolation, and vaccination.

With clinical testing becoming more targeted to specific sites and situations, ES is providing a means to cost-effectively monitor population-level trends and emergence. During low prevalence or situations with no known COVID-19 cases, ES methods can be a sensitive and cost-effective approach for early warning. ES methods are a particularly cost-effective and reliable means to track spatial and temporal trends in infection prevalence and test for variants when clinical testing capacities are overwhelmed during periods of elevated prevalence, or willingness or access to testing is low in certain times or areas.

SARS-CoV-2 ES has potential benefits of scalability and efficiency since a single sample can provide evidence of SARS-CoV-2 circulation at a population level in wastewater catchments ranging from small populations to populations of hundreds of thousands or millions of people, and if carried out ethically, can be a non-intrusive approach that also protects individuals' privacy [83]. SARS-CoV-2 ES provides community-level data that is not reliant on clinical testing.

There are some limitations of SARS-CoV-2 ES as compared with other surveillance approaches [84]. The most significant limitation is the lack of individual sampling and test results, and thus an inability to link results to individual clinical care. Another limitation comes from the sensitivity of the ES methods meaning that during low viral shedding relative to volumes of wastewater the concentration of virus may be too low to reliably detect in that wastewater.

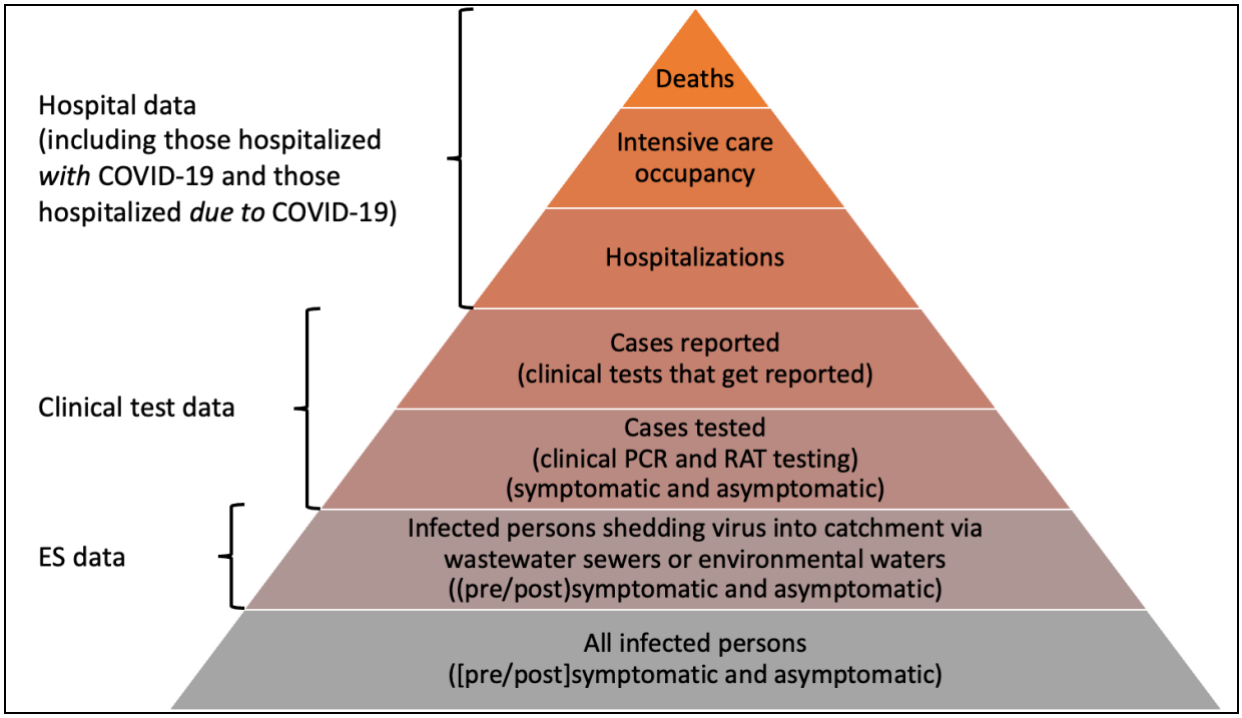


Fig. 1. Illustration of role of SARS-CoV-2 ES as a source of data on SARS-CoV-2 shedding in communities within a defined wastewater catchment. The surveillance pyramid adapted from Havelaar et al. *2007* [85].

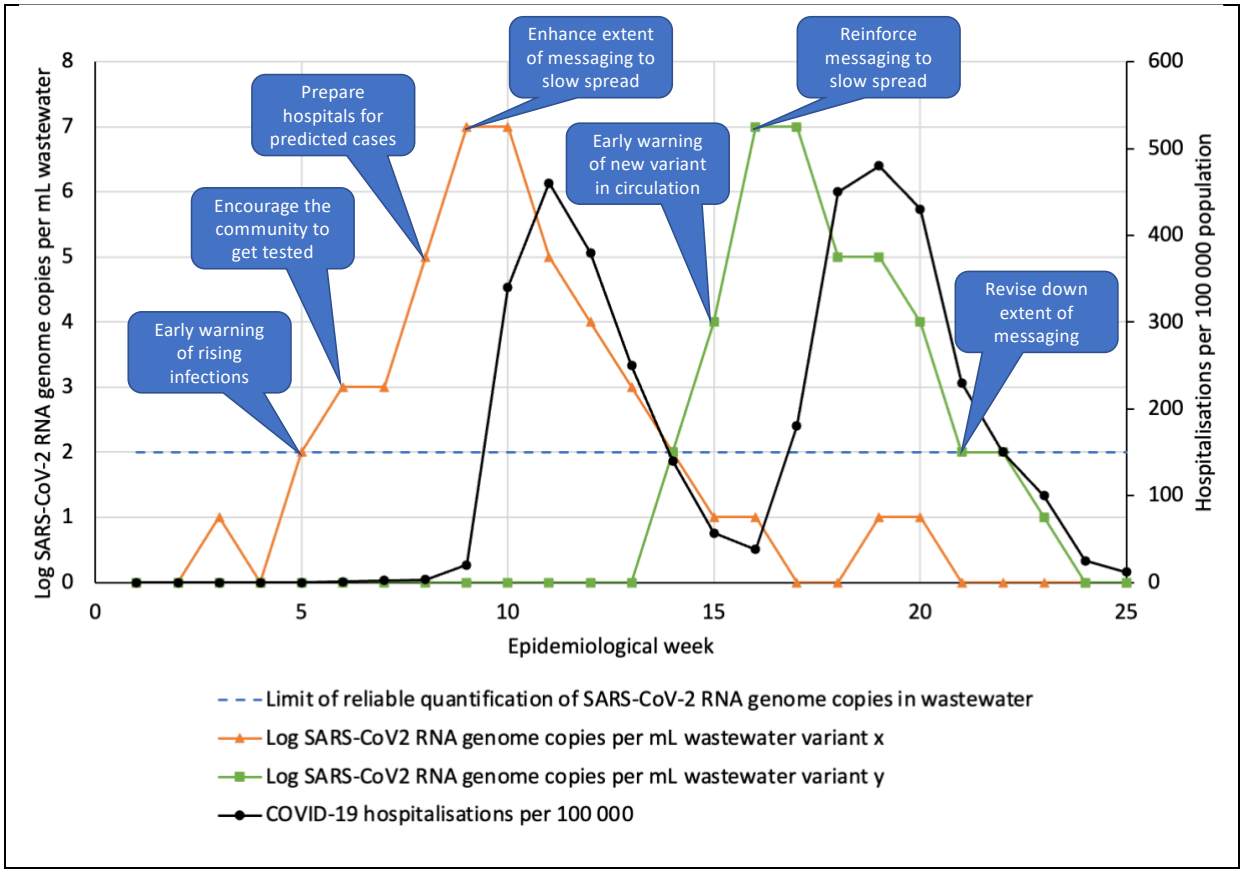
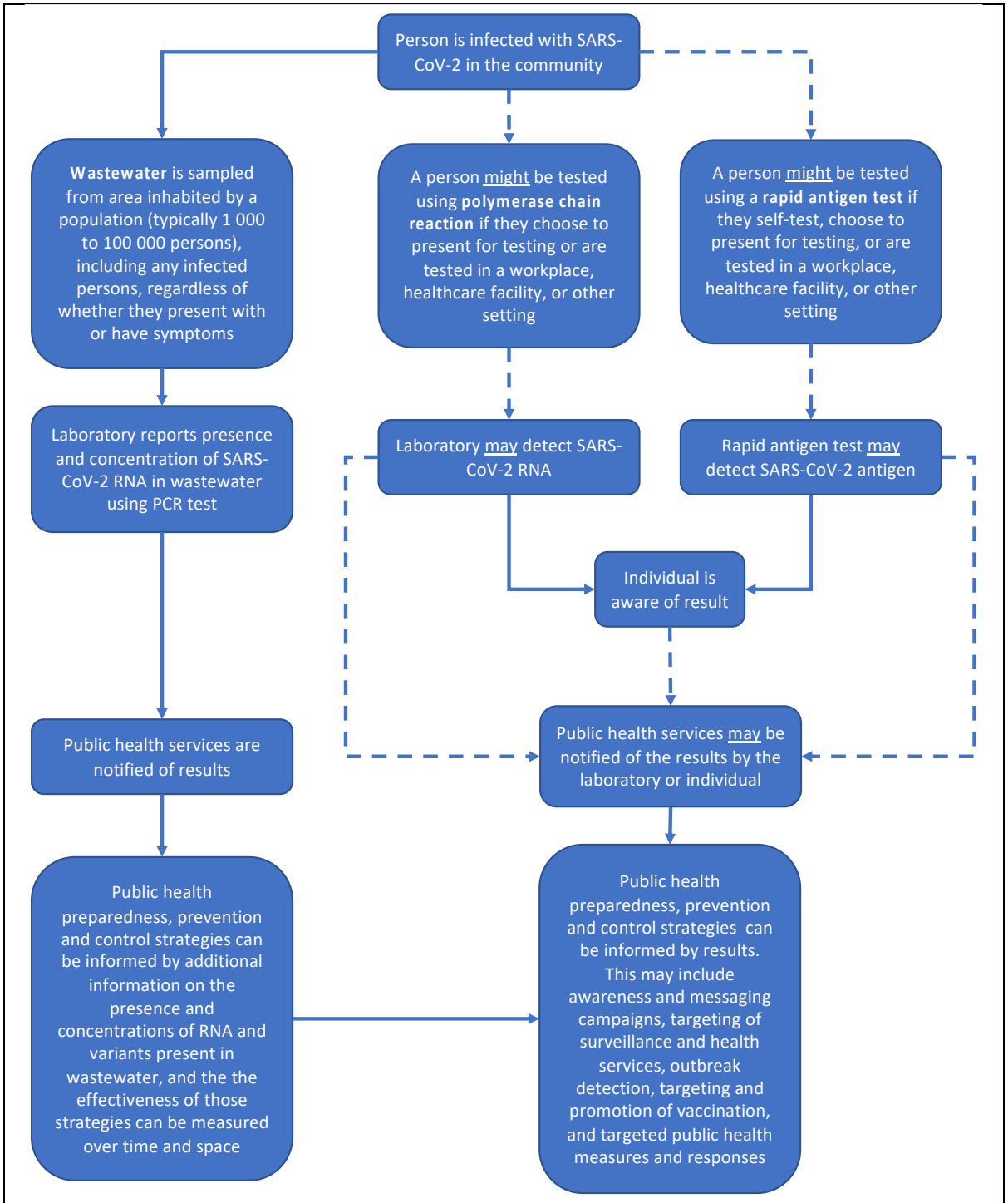


Fig. 2. Illustration of ES data compared to hospitalization data and potential use cases for public communication, public health decision-making, targeting public health preparedness, prevention and control strategies, and measuring the effectiveness of those strategies.



NB:

- *The emphasis on different test methods may vary during different phases of the pandemic.*
- *The timeframe from sampling to visualising test results is of the order 15 min for rapid antigen tests and approximately 0.5 to 2 days for both clinical and ES PCR tests (sometimes more depending on backlogs and turnaround times).*
- *The early warning offered by ES comes from its ability to detect virus in pre-symptomatic and asymptomatic persons in the community who shed the virus but who might not have (yet) presented for clinical testing.*
- *In some contexts, results are shared directly with the community at the same time as the public health agency.*

Fig. 3. Illustration comparing the use of surveillance methods based on personal and clinical testing (rapid antigen testing or PCR testing), with wastewater testing, from the perspective of a public health agency.

2.2 Experience of ES as part of public health surveillance relating to other diseases and targets

ES has been used for other health threats, including poliovirus [2-4], *Salmonella* Typhi [5-7], norovirus [8,9], hepatitis A virus [8], and AMR [10-13], with some of this work dating back more than 70 years. Most notable is poliovirus, with WHO having produced globally-applicable ES guidance for poliovirus in 2003 [2]. Many of the standard methods, approaches and global reporting processes for poliovirus ES are applicable or adaptable to SARS-CoV-2. Some countries, such as South Africa, have already built on their poliovirus experience and created comprehensive SARS-CoV-2 ES programmes to detect the presence and concentrations of SARS-CoV-2 [53,54] and in some cases its variants [46,52,77]. However, there are important differences relative to poliovirus ES:

- The main driver of ES for poliovirus has been to inform vaccination through two use cases: i) early detection of poliovirus infections at the community level; and ii) confirmation of the absence of circulation of wild-type and vaccine-derived poliovirus in a population [86]. Therefore, ES for poliovirus has not depended on quantitative data to look at trends in prevalence. In contrast, ES for SARS-CoV-2 has been driven more by a need for information on quantitative trends rather than to inform decisions on vaccination.
- Presence/absence use cases were relevant in the early stages of the COVID-19 pandemic, but are no longer a priority use case since SARS-CoV-2 is becoming globally endemic and within the foreseeable future will not be eradicated, as is the goal for poliovirus.
- Standard methods and oversight by WHO are available for poliovirus ES. This covers overall integration with public health surveillance, selection of ES sampling sites, through concentration of the virus from wastewater and environmental water samples, to poliovirus extraction and cultivation, and genetic characterization and results interpretation. However, as yet, the pace of innovation in ES for SARS-CoV-2 means that an equivalent set of standard methods has yet to be agreed. At this stage, standardising methods between different laboratories and sites is less important than having consistent methods and quality covering a geographic area of interest. Some studies and proficiency trials have begun to address questions such as sample type, quantifying sensitivity, specificity, other performance characteristics of the methods, and cost [84, 87-89], and an ISO initiative is underway at the time of writing [90].

In addition to pathogens, other targets for established ES programmes include testing for illicit drugs [91,92]. Experiences from these established programmes have been used help inform the development of SARS-CoV-2 ES programmes, as well as public health surveillance for other diseases and risks, such as chemicals of emerging concern [93]. Reciprocally, the experiences gained in SARS-CoV-2 ES have expanded ES options for both poliovirus and other targets.

3. Optimizing the integration of SARS-CoV-2 ES into public health surveillance

3.1 Public health leadership

Leadership by the agencies responsible for public health, and with overall responsibility for COVID-19 public health preparedness, prevention and control, is critical to SARS-CoV-2 ES programmes. Multidisciplinary, cross-sector coordination is required for SARS-CoV-2 ES programmes, involving key stakeholders, such as environment agencies, regional and local authorities, wastewater system operators and managers, and laboratories responsible for sampling and analysis, communication experts and research partners.

The public health sector is the end user of the information. Therefore, this sector needs to take the lead in designing surveillance programmes, merging and linking the SARS-CoV-2 ES data with other surveillance platforms, coordinating interpretation and communication of the findings, and using the findings to inform public health action. Public health agencies, working in partnership with a multidisciplinary team, should be responsible for leading SARS-CoV-2 ES initiation, coordination, and implementation to ensure a health-led and integrated decision-making process.

The public health agencies should ensure complementarity between the SARS-CoV-2 ES and other surveillance activities. The public health agency should typically fund the SARS-CoV-2 ES programme since it is not a water and sanitation sector function but rather about accessing the information encoded in wastewater to provide an unbiased indicator of SARS-CoV-2 in the community. In some cases, other parties responsible for defined populations and with an interest in understanding infections may (co-)fund the programme, such as at educational institutions, mining sites and military bases.

3.2 Defining how SARS-CoV-2 ES adds value to public health surveillance

Before initiating a SARS-CoV-2 ES programme, it is important to define how SARS-CoV-2 ES is anticipated to add value to health sector decision-making for COVID-19 public health preparedness, prevention, and control strategies, and to measure the effectiveness of those strategies. Broader considerations of how the ES program strengthens and is a cost-effective part of the overall public health system are also relevant. An example of possible use cases is given in Table 1.

The benefits of SARS-CoV-2 ES vary according to factors such as phase of the pandemic, the method used to collect wastewater liquid or solids samples, spatial coverage, sampling frequencies, analytical methods, and the interventions triggered in response to SARS-CoV-2 ES results.

From least to most advanced, SARS-CoV-2 ES programmes can provide the following evidence:

- At their most basic, SARS-CoV-2 ES programmes indicate whether SARS-CoV-2 is above (present) or below (absent) the limit of detection of the testing methods used at the level of the community. This is particularly relevant in low prevalence settings, to confirm absence of virus circulation or warn about (re)emergence of the virus. Where SARS-CoV-2 is endemic, this use case is only of limited value. Such presence-absence assessment may be useful for sampling from small populations, such as targeted sampling from isolated communities with significant vulnerability to poor COVID-19 outcomes, or in high-risk settings such as residential care facilities. Presence-absence assessment is also potentially useful to test for defined variants that are not known to be present in the target population.

- Most programmes involve quantification of results to identify increasing or decreasing SARS-CoV-2 trends and the timing of peaks at the population level. SARS-CoV-2 concentrations in wastewater do not precisely translate to the number of COVID-19 cases in the community [94]. There are four principal confounders that prevent precise correlation of SARS-CoV-2 concentrations in wastewater with numbers of shedders who contributed to the viral concentrations in wastewater: i) variability in rates and patterns of virus shedding into wastewater including potential differences between variants/subvariants; ii) concentration of human excreta and secreta in wastewater given water use patterns (e.g., flush volumes and variability in greywater and blackwater separation); iii) fluctuation in flow rates in wastewater systems (e.g. due to stormwater and groundwater inflows or industrial and commercial discharges); and iv) the movement of people between wastewater catchments. Studies examining the relative contributions from faeces, sputum, urine, and saliva to the SARS-CoV-2 concentrations in wastewater illustrate some of these complexities and have not found satisfactory means to quantitatively account for discrepancies [95]. Some of these confounders can be accounted for to some extent in programmes that use normalization methods that simultaneously measure markers of human waste (see section 6).
- In the most advanced cases, SARS-CoV-2 ES programmes monitor variants, including both known variants of interest or concern, and in some cases searching for new and emerging variants.
- Whilst beyond the scope of this guidance document, it is noted that other targets can be simultaneously monitored in wastewater samples that can be collected alongside those sourced for the SARS-CoV-2 ES programmes, and can be tested for RNA, DNA, microorganisms and/or chemicals.

The purpose of the programme influences its detailed design. For instance:

- More frequent sampling with more rapid turnaround of results provides more timely intelligence for action.
- The sampling frequency can be adjusted based on epidemiological trends.
- Finer spatial sampling scales (smaller wastewater catchments) allows better targeting of mitigation responses to those areas, if relevant.
- Safeguarding high risk settings can involve direct targeting of buildings such as long-term care facilities, dormitories, healthcare and correctional facilities, and displaced persons and refugee camps.
- Testing for cross-border introduction of variants can involve testing wastewater as it is received at land borders and sea and airports, including direct testing of transport vessels. Targeted testing, potentially including genomic sequencing, in arriving travellers can provide information about SARS-CoV-2 variant circulation in countries with limited domestic testing or that don’t share their testing information.

The sanitation and socioeconomic context of the programme influences the details of its design. For instance:

- ES programmes are technically relatively simple in areas where a high proportion of the population is connected to sewers, allowing sampling points to capture most of the

population resident in the sewered area. Most programmes described in the published literature cover such contexts.

- ES programmes are more challenging in areas with high proportions of individual on-site sanitation systems (e.g., septic tanks and pit toilets). However, successful applications have been developed using samples from open drains following lessons learnt from ES for poliovirus as part of polio disease eradication [17,18], or septic tanks of public toilets [96], and making use of passive samplers [97-100].

The value of SARS-CoV-2 ES varies according to the context. For instance:

- Information from SARS-CoV-2 ES has helped address information gaps in situations of limited or inconsistent levels of clinical testing and genomic surveillance data notified to the public health agency. As the levels of clinical testing are decreasing, and requirements to notify are being reduced, the relative value of ES is increasing as a means to provide intelligence on incidence of infection and disease peaks.
- As individual genomic surveillance is also reducing dramatically along with testing, ES population-level virologic evidence to detect incursion and geo-temporal spread is becoming a more important source of intelligence.
- SARS-CoV-2 ES can play a valuable role in remote areas in high-income countries, and in low- and middle-income countries, where access to clinical testing is limited, particularly if methods for areas not connected to sewerage systems can be implemented and the ES programme can be integrated with the broader public health surveillance system.

A summary of example possible use cases for SARS-COV-2 ES that have been demonstrated successfully and consistently in multiple contexts is provided in Table 1. Note that SARS-CoV-2 ES programmes often serve multiple purposes simultaneously. For instance, a SARS-CoV-2 ES programme primarily focused on observing trends can also be used for risk communication and targeting of public health surveillance and response resources. Efficiencies can be gained by intentionally designing SARS-CoV-2 ES programmes to meet multiple objectives and serve multiple use cases. However, SARS-CoV-2 ES programmes may also be designed to serve a single purpose. The flexibility of ES also allows purposive dynamic and adaptive design elements to optimize cost-benefit. For example, an ES program with routine monitoring at an airport and major population centers could include thresholds which trigger aircraft-level monitoring or more frequent sampling.

Table 1. Summary of use cases and their benefits in COVID-19 response strategies in various settings

Use case	Description	Benefits for COVID-19 response strategy							Setting or level where application has greatest benefit, and comments on benefits
		Provides early warning	Encourages clinical testing	Informs decisions on public health responses	Encourages compliance with public health advice	Informs decisions on hospital care capacity	Informs decisions on targeted clinical testing	Improves vaccine uptake	
Tracking trends (stable, increasing or decreasing) at community level to help ***target COVID-19 responses and interventions***	Observing increasing and decreasing trends (increasing or decreasing concentrations of viral genome copies, or percentages of positive samples) at community level to, once confirmed, provide an early indication (4–7 days) of changes in incidence and levels of virus circulation to assist with timely decisions on public health surveillance strategies, COVID-19 control interventions and responses.	++	+	+++		+++	+++		Subnational and local/city-level planning All prevalence levels Communities with low uptake of clinical testing or failing reporting system or increase in self-testing Larger population sizes
Finding outbreaks in places thought to be free of SARS-CoV-2 or a particular variant	Involves testing for SARS-CoV-2 in areas where it is not expected, to provide early warning of its emergence and enable earlier intervention.	+++		+++		++	+	+	Locations where SARS-CoV-2 is thought to have been eliminated or locations where COVID-19 cases have not been identified
Augmenting risk communications to help ***promote good behaviours***	Publicizing data on detection in wastewater reminds the community that the virus is circulating, encourages people to seek clinical testing, and reduces complacency about control interventions (e.g. masking, distancing, vaccination).	+	+++	+	++			++	Low to moderate prevalence
Cost-effective ***targeting of public health surveillance*** (clinical testing resources)	Allows deployment of scarce clinical testing resources in hotspot areas with higher SARS-CoV-2 ES signals.	+	++	++			+++		Spatially differentiated, low to moderate prevalence Larger population sizes
Cost-effective ***targeting of vaccination campaigns***	Allows deployment of scarce vaccination capacities to hotspot areas with higher SARS-CoV-2 ES signals.							+++	Spatially differentiated, low to moderate prevalence Larger population sizes
Informing early and localized restrictions in pockets of (re-) emergence by helping ***detect outbreaks***	Informs more targeted rapid interventions to minimize the extent and economic impact of restrictions (e.g., service closures, travel restrictions).	+	+++	+++					Spatially differentiated, low prevalence

Use case	Description	Benefits for COVID-19 response strategy							Setting or level where application has greatest benefit, and comments on benefits
		Provides early warning	Encourages clinical testing	Informs decisions on public health responses	Encourages compliance with public health advice	Informs decisions on hospital care capacity	Informs decisions on targeted clinical testing	Improves vaccine uptake	
Targeted surveillance for early warning of circulation:	Allows early warning to inform earlier intervention to help limit SARS-CoV-2 dissemination in targeted settings:								
• vulnerable or high-risk settings	- managed isolation facilities, aged care facilities, schools, prisons, informal settlements, refugees and displaced persons								Ensure equity and protect vulnerable groups
• isolated communities	- remote and indigenous communities; industrial, mining and research facilities; quarantine facilities; student residences	+++		+++			++	+	Enable clusters or groups to be contained. Augment data in areas with low uptake of clinical testing.
• transport vessels	- sullage tanks of ships and aircraft arriving at borders, or the waste collection vehicles or dump points								Provide evidence of variants arriving via travellers
• multi-day events and gatherings	- meetings, events, or festivals spanning days or weeks								Provide evidence to inform continuation of events and gatherings
Identifying existing, ***known variants*** of interest or concern	Involves testing for known gene targets where proportions of variants in circulation are uncertain or higher resolution of information is needed.	++		++		++	+		Locations where occurrence of variants have not been described
Detecting emergence of ***novel variants***	Involves whole-genome sequencing to identify novel variants circulating in the environment.	+++							Moderate to high prevalence
Biobanking and retrospective analysis	Involves retrospective analysis of data to provide intelligence on introduction, evolution, and dissemination of the virus, to inform future pandemics.			++					Global, but particularly in areas more vulnerable to future pandemics There has been feedback that retrospective sequencing can be prone to contamination, so for evidence of evolution, near-real-time sequencing may be preferable

Legend: +++ = primary benefit; ++ = secondary benefit; + = adjunct benefit

4. Key considerations for deciding whether to implement, maintain, modify, pause, or stop ES for SARS-CoV-2

Deciding whether to implement a new SARS-CoV-2 ES programme, or maintain, modify, pause, or stop an existing programme, requires weighing up the benefits against costs, as well as comparing the value of ES with that of alternative methods. In addition, there needs to be sufficient technical, organizational, and logistical capacity, security of funding, and regulatory and policy enablers, for the programme to occur. Therefore, it is necessary to undertake the following steps:

1. Determine the broader public health objectives.
2. Understand whether ES can, in principle, provide evidence to support the achievement of those public health objectives. This involves understanding what ES can achieve with current technologies. If ES cannot technically support the objective, it is not useful. Table 2 has been included to assist with this assessment and should be read in conjunction with Table 1 for more detail on potential use cases.
3. Where ES approaches are technically able to support the objective, compare the costs and benefits to other methods of sourcing the same evidence.
4. Weigh up the relative pros and cons to identify whether ES is potentially useful.
5. If ES is potentially useful, undertake an assessment of capability to deliver an ES programme in the relevant context. Box 1 has been provided to assist with this assessment.
6. Experience has found that the availability of a sufficient funding allocation has been the principal factor determining whether an ES programme is undertaken. Therefore, if a decision to implement or continue an ES programme is made in principle, it is likely to be necessary to complete a business case or funding proposal to seek appropriate resources. Box 2 helps provide an understanding of the steps involved in delivering an ES programme which may help form the contents for such a business case or funding proposal.

Note that these steps need repeating periodically for three reasons:

- Surveillance needs change over time.
- ES technologies are rapidly improving and becoming more cost-effective which may change the outcomes of a decision-making process.
- Most funding allocations have a limited timeframe, after which they will be reviewed.

Box 1. Checklist of considerations to inform decisions on initiating or maintaining SARS-CoV-2 ES

- ☐ **Organizational capacity.** Is there an agency capable of coordinating a SARS-CoV-2 ES programme, including sourcing funding, coordination, delivery, interpretation, and reporting?
- ☐ **Sanitation agency.** Is there a wastewater management or sanitation agency capable of assisting with sampling? Do they have sufficient and suitable staff, equipment, and health and safety systems?
- ☐ **Laboratory capacity.** Is there a laboratory or laboratories capable of analyzing environmental samples for SARS-CoV-2 using quantitative PCR (qPCR) or digital PCR (dPCR)? Do they have sufficient and suitable staff, equipment, and facilities? Ideally, but not essential, they would have the ability to undertake or source support for sequencing and bioinformatics.
- ☐ **Sustainability.** Is there a sustainable source of revenue to support the programme, a reliable supply chain of required laboratory consumables, tools, and equipment, and of other critical requirements, such as personnel?
- ☐ **Permits and authorizations.** Is it permitted legally and is it accepted ethically, to undertake ES in the jurisdiction, and with the intended ES objectives?

Table 2. Objectives to consider when deciding on implementing or continuing ES for SARS-CoV-2

Surveillance objective	Value of ES	Alternative
Trends in the proportion of individuals currently infected within a defined catchment.	Achievable, cost-effective, and non-invasive, provided proportions of positive samples, and/or genome concentrations, are determined. Normalization methods improve reliability and comparability.	Direct clinical surveillance of a representative sample of individuals.
Trends in the proportions of variants infecting individuals within a defined catchment.	Achievable, and non-invasive. Technically more challenging than simply detecting the presence of SARS-CoV-2 RNA or quantifying SARS-CoV-2 genome concentrations.	Direct clinical surveillance of a representative sample of individuals. Technically more challenging than simply detecting the presence of SARS-CoV-2 infection.
Proportion of individuals currently infected within a defined catchment.	Too approximate and indicative to be practically useful. Normalization methods reduce uncertainties.	Direct clinical surveillance of a representative sample of individuals.
Proportion of individuals currently infected in isolated locations whose wastewater does not flow to a defined catchment.	Not practically useful. Would require dedicated testing of a representative sample of decentralized wastewater management systems.	Direct clinical surveillance of a representative sample of individuals is likely to be just as practical and more clinically relevant.
Proportion of individuals currently infected in locations whose wastewater is treated in ways that remove the target RNA before discharge to the centralized system.	Not practically useful. Would require dedicated testing of a representative sample of wastewater at connected facilities prior to treatment.	Direct clinical surveillance of a representative sample of individuals is likely to be just as practical and more clinically relevant.
Number of individuals currently infected within a defined catchment.	Not possible.	Direct clinical surveillance of all individuals.
Number of individuals currently infected in isolated locations whose wastewater does not flow to a defined catchment.	Not possible.	Direct clinical surveillance of all individuals may be possible but is still unlikely to be reasonably practicable.
Number of individuals currently infected in locations whose wastewater is treated in ways that remove the target RNA before discharge to the centralized system.	Not possible.	Direct clinical surveillance of all individuals.
Number or proportion of individuals that have been previously infected within a defined catchment.	Not possible.	Serological testing of individuals can assist in this respect but specific studies are needed to discriminate infection from vaccination.
Immune status of number or proportion of individuals within a defined catchment.	Not possible.	As above
Detecting with certainty whether there is an infected individual within a defined catchment.	Not achievable. Whilst there is evidence that the lower limit of sensitivity is one infected individual in a sewer catchment, the extent and timing of shedding of the virus to the wastewater system needs to be sufficient and adequately coincident with the sampling to enable detection. Passive sampling may improve sensitivity. However, there may be one or more infected individuals within a wastewater catchment without SARS-CoV-2 being detected.	None. Even direct clinical surveillance of all individuals can miss infected persons since all assays have a limited (< 100%) sensitivity.

5. Key considerations for planning and coordination

After deciding to initiate ES for SARS-CoV-2, good planning, coordination, and capacity building is needed. Areas that need resourcing include ensuring quality of data collection, analysis, and interpretation of the data, and using the data to inform decision-making and risk communication [101]. This section summarizes the components of a wastewater surveillance programme and the requirements for establishing one that is credible and effective. In outline, the components of a SARS-CoV-2 ES programme include:

- Public health agencies and policy makers who use the information generated to inform decisions and frame the questions that the programme needs to answer ensuring legal and ethical considerations are addressed.
- Epidemiologists and data managers who collect, manage, and interpret data.
- Water, sanitation and environment agencies and municipal authorities responsible for wastewater management and (usually) for sampling that understand wastewater flows catchments and how they relate to the locations of specific populations and to public health districts.
- Laboratories that analyze the samples, and sometimes conduct the sampling, report the results, and undertake quality management. Ideally, these laboratories have expertise in handling wastewater samples and in molecular biology and bioinformatics, including qPCR or dPCR, and the use of variant-specific primers and probes, or even sequencing.
- Information technology and communications personnel that undertake spatial mapping and data interpretation, prepare data visualizations and reports, and maintain a user-friendly, accessible ES data system integrated with regular public health reporting.

A successful programme requires public health sector leadership and multisector coordination. Dedicated, specialized resources need to be committed to meet the organizational, technical, and financial requirements to implement a meaningful and effective SARS-CoV-2 ES programme at a meaningful scale. Scaling up to the required capacity may take several months. In addition to costs for setting up a programme, the costs could be hundreds of thousands of US dollars per year for a smaller jurisdiction (e.g., a city or region) and millions of dollars per year for a larger jurisdiction (e.g., a country). However, the benefits can outweigh the costs. Savings can potentially be made by reducing costs for other forms of public health surveillance, as well as health and economic benefits arising from using the information gained from ES. Synergies and efficiencies can be found by making use of existing capacity within other ES or wastewater sampling programmes.

Maximizing the value of the SARS-CoV-2 ES programme requires an ability to rapidly use the data at a local level, and to aggregate and report the data at the levels at which surveillance is required and intervention actions are undertaken. Harmonization of sampling and laboratory testing methods at local, national, regional, and potentially global scales would be beneficial since it would assist with quality assurance, proficiency testing, comparison between laboratories, and sharing of methods and approaches. In addition, there are important equity, ethical, and cultural considerations *(63)*. These include the equitable representation of populations, including considering how to target areas that are not sewered (e.g., septic tanks, pit toilets) or that lack sanitation services.

Box 2 provides a checklist of typical organizational and capacity requirements that need to be in place to establish and implement a successful SARS-CoV-2 ES programme.

Box 2. Checklist of steps to initiate, establish, and implement a SARS-CoV-2 ES programme

- ☐ **Identify the relevant stakeholders, and their needs, expectations, and willingness and ability to participate.** Outline what the ES programme should look like and the actors that need to participate at national, regional, and local levels. Assess which actors are already engaged. Understand the receptivity and interest of the necessary actors to participate. Actors include the primacy public health agency, the COVID-19 pandemic management and control agency, the wastewater management agency, and actors undertaking wastewater sampling, processing of samples and molecular genetic testing. Ideally, normative bodies will provide laboratory standards and review, and accreditation, as part of quality assurance. Existing ES actors engaged in poliovirus, or other testing and related research, should be considered. There may be private partners seeking to engage in public-private or philanthropic partnerships.
- ☐ **Identify a lead agency or collective that will be responsible for the ES programme**. The lead is typically a public health agency, a COVID-19 pandemic management and control agency, or a collective (in which the public health agency plays the major role).
- ☐ **Understand the technical, organizational, and financial capacity of the participating stakeholders**. An ES programme will be limited by these factors. It may be possible to scale up capacities, but this will take time. Capacity limitations on supporting services and supply chains should also be considered and managed – some laboratory reagents, equipment, and personnel can be in short supply or take time to arrive. Funding needs to be committed to the programme, both setting it up and maintaining it. Funding aspects need to be reviewed in response to changing circumstances, including in moving to endemic COVID-19, and applications of ES beyond COVID-19.
- ☐ **Explicitly define and communicate the objectives of the ES programme**. Primary objectives would typically include tracking trends in community SARS-CoV-2 RNA presence or concentrations, providing early warning of the emergence of COVID-19 cases, indications of changes in COVID-19 incidence, and incursion and spread of variants. This information might be used to define a semi-quantitative community COVID risk ranking and could provide an input to predictive models of future incidence. Secondary objectives might include providing information for research to inform responses to future pandemics, including novel SARS-CoV-2 mutations or other pathogens.
- ☐ **Identify the scale of the ES programme**. Typically, the ES programme is delivered at the same scale as the public health and COVID-19 public health surveillance and control services – for example, site, local/city government, national, transnational, or regional scale. In some cases, the ES programme can be tiered, with local or regional programmes being linked to national and transnational programmes.
- ☐ **Liaise with the COVID-19 management and control agency to maximize value.** Set up ongoing relationships with the COVID-19 pandemic management and control agency to enable two-way interaction to tailor the programme to meet information needs. Communicate the options, opportunities, and limitations of ES to the agency. Set up procedures to integrate and report ES data to the agency to support decision-making. Pre-plan health actions as response to ES results. Align sampling points with areas covered by clinical testing and hospitalization surveillance to the extent possible. Set up data dictionaries, data management systems and reporting systems (i.e., dashboards, or preferably integrated with public health systems) for coordination and data sharing.
- ☐ **Identify opportunities to build on existing capacities to ensure time and cost efficiencies.** Align sampling with existing sampling programmes, (e.g. the routine wastewater monitoring programmes undertaken by sanitation and wastewater providers and environmental agencies). Transport samples using existing channels (e.g., existing sampling points and points of analysis). Identify laboratories with experience in detecting viruses in wastewater and in molecular methods. Consider levering off other ES programmes (e.g., for polio, typhoid, or AMR). However, adverse community reactions can be expected from linking public health-focused ES programmes, such as for SARS-CoV-2, with those related to criminal activities, such as illicit drugs.

- ☐ **Agree on sampling and analytical methods and procure equipment and consumables.** Depending on the setting and existing capacity of the lead ES agency, significant investment in equipment and capacity for sample collection, transport, analysis and interpretation may be needed. Decisions should be made on whether analysis of samples will be conducted at a single center or multiple centers for the geographic area of interest. In the latter case, interlaboratory comparison is beneficial. Standard operating procedures are needed for steps such as safe sampling and sample handling, collection, storage and transfer, location naming, and container labelling. Ideally, identify a central laboratory that can support training, consistent materials and supplies, harmonization of methods and result reporting, and undertake auditing, accreditation and certification services.
- ☐ **Train personnel.** Training approaches can include written protocols, procedural flow diagrams, videos, and in-person demonstrations, and competency assessments. For instance, wastewater treatment plant and other wastewater workers need to be properly trained to safely collect wastewater sample in compliance with local regulations. Training for laboratory personnel in safely handling wastewater samples, and appropriate analytical methods, needs to be tailored to the level of experience and expertise of the staff, and the tools and equipment available.
- ☐ **Clarify the coordination and data-sharing arrangements for end use of the data.** Where ES is conducted by an agency or entity other than the public health surveillance or COVID-19 control agency, clarity is needed at the outset on coordination mechanisms, data needs to fill gaps and uncertainties in public health surveillance, and timely mechanisms for sharing and interpretation of data for use in the response strategy.
- ☐ **Set up a database to collate and communicate relevant data and information.** Typical information captured for each sample includes method of sample collection, location, date, sample type, catchment represented, laboratory assay performed, and result. Ideally, the ES evidence is readily and directly linked to public health surveillance from the same period. Be clear about what information is to be captured within the database and how it is to be uploaded, quality assured, accessed, used, and presented. If multiple actors can access the database, include options to identify planned, in progress, and historical programmes. Ensure that information flow and communication channels allow timely, good-quality, fit-for-purpose, information to be transferred from the ES programme to the COVID-19 control agency.
- ☐ **Develop means to communicate the programme to stakeholders and the public.** Set up public reporting systems, such as spatial map displays, timeline graphs, summary tables, and dashboards, or preferably integrated public health reporting paired with public health advice that encourages adherence with public health measures in place. Set up processes to engage with the public, wastewater workers, plumbers, and the media. Provide training to persons involved in the program so that they understand SARS-CoV-2 ES, their role in the programme, and the value of the data provided. Be proactive with communications, such as allaying concerns about infectious virus being present, noting that only RNA, and not infectious virus, is being detected. Note that the data is not being used for individual identification, such as sequencing of human genetic information.
- ☐ **Ensure ongoing sustainability and reliability of the programme.** Gain formal commitment from relevant actors and ensure adequacy of resourcing (human resources, technical capability and competency, required facilities, and funding). Ensure ongoing training and maintenance of capacity, sourcing of revenue, and management of the data by the health and COVID-19 pandemic management and control agency. Ensure reliability of supplies and equipment (suppliers and supply chain). Ensure that results will be shared in a timely manner and will be used to inform public health action.
- ☐ **Evaluate and improve the programme.** Over time, ascertain the impact of the programme on public health. Identify and address gaps, weaknesses, and challenges in the programme to adapt and improve it.

6. Key considerations for data collection, analysis and interpretation

6.1 Overview of methods

There is no universal standard method or approach to ES for SARS-CoV-2. However, there are several communities of practice at the national, regional, and global scales, and several proficiency programmes, along with many published protocols [88,89,102-105]. The information below summarizes guidance on SARS-CoV-2 ES that is published or under development in these protocols. An overview of SARS-CoV-2 ES data collection and analysis workflow for wastewater testing is given in Fig. 5.

Similarities and differences between the various programmes have been summarized according to:

- Type of environmental sample: municipal or institutional sewage, raw liquid phase or settled solids, open drains, or surface water;
- Sample type and volume: grab, composite, passive [97-100];
- Virus and RNA concentration approach: membrane filtration, centrifugation, protein precipitation, affinity-capture magnetic hydrogel particles [106];
- RNA purification method: commercial kits or generally available reagents;
- RNA amplification and quantification method: choice of genetic primer and probe targets for the RT-PCR, coupled with a quantitation method using qPCR or dPCR;
- Characterization of virus present, e.g., variant determination using variant-specific primer and probe combinations for the qPCR or dPCR, or amplicon and/or whole genome sequencing; and
- Methods for quality assurance, quality control, inhibition controls, standardization, and normalization.

Methods and approaches need to be fit-for-purpose for their contexts. Decision trees can be used to help guide decisions on which methods or approaches are best suited to variations in sanitation systems, disease prevalence, speed of sample processing, ease of automation, local availability of supplies, skill levels, and other factors [107].

Most of the published guidance and implementation experience has come from settings with a high proportion of households connected to sewers, and relatively high financial resources and laboratory and organizational capacity. Some limited guidance is available for unsewered and lower-resource settings [17,18], particularly where SARS-CoV-2 ES programmes have been able to leverage existing capacity for poliovirus ES.

Where possible, the guidance below notes considerations for settings with low sewerage coverage and low financial resources and laboratory and organizational capacity and provides examples of potentially lower-cost and more readily available non-commercial methods that can be developed locally. For all settings, it is important to ensure that planning, coordination, and capacity requirements (section 5) are in place before a SARS-CoV-2 ES programme is initiated.

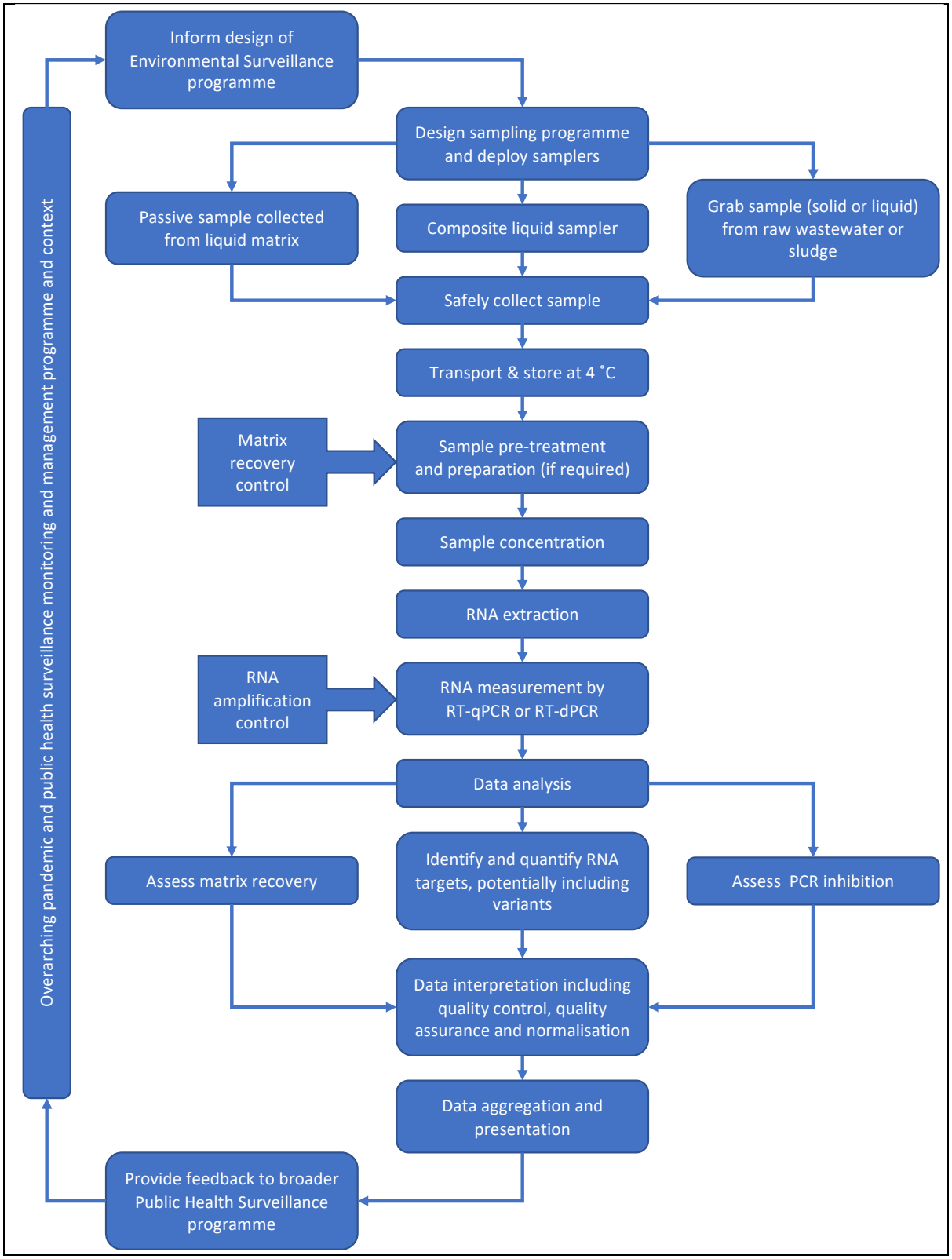


Fig. 4. Typical workflow for SARS-CoV-2 ES programmes

6.2 Selection of sampling sites

SARS-CoV-2 ES programmes should be optimized to prioritize sampling to gain the maximum value from the programme within financial and organizational capacity constraints. Prioritization may be adaptive – responding to what the SARS-CoV-2 ES and public health surveillance programmes require with changing contexts and goals. Adaptive programmes may define *a priori* thresholds which trigger a change of sampling frequency and/or sites [108].

In general, SARS-CoV-2 ES programmes are multi-tiered [109]. Sampling points representative of larger populations are covered first to efficiently obtain baseline and trend information, and potentially early warning, from larger proportions of the population. Smaller, more spatially targeted sampling points can then be selected at the next tier down, e.g., at major sewer or drainage points. In some cases, specific buildings, septic tanks or holding tanks from airplanes or ships can be selected for targeted sampling.

Sampling programmes should be designed to be representative of the target population. The frequency and spatial resolution of sampling should be adequate to meet the objectives of the use case. Seasonal variables may also be considered such as population displacements due to tourism and or seasonal work. Programmes should aim to achieve equitable coverage and prioritize geographic areas or populations based on anticipated health risk. For instance, they might target higher-risk communities, such as those with comorbidities, greater age, less access to healthcare services, or lower levels of SARS-CoV-2 clinical testing or lower vaccination levels.

The sampling points can be selected based on the size of the wastewater catchment and on what is actionable by public health agencies. Ideally, the wastewater catchment area would be linked to populations defined as part of the broader public health surveillance programme. In practice, sewer and drainage catchments are not always well-aligned with municipal or public health regional boundaries. For larger catchments, it is important to consider implications for spatial resolution and interpretation of results, as well as impacts on method sensitivity and specificity. For smaller catchments, sampling sites may need to consider more sophisticated composite sampling devices or passive samplers rather than just grab samples, since there is less opportunity for wastewater homogenization prior to the sampling point. Borders and points of entry can be targeted to assist in detecting spread between areas or to support quarantine arrangements. Ethical considerations, such as privacy and equity, should be addressed [83], particularly when sampling relatively small and well-defined buildings or confined areas such as prisons, refugee camps or schools.

Most SARS-CoV-2 ES programmes currently sample from piped wastewater systems or environmental waters that are heavily influenced by discharge from personal hygiene and sanitation activities. For practical reasons, and concerns over stigmatization, sampling of on-site sanitation systems used by individual dwellings has not been common, except where large numbers of people use a single system.

Wastewater should be sampled before it has been treated, as far as practicable. SARS-CoV-2 RNA is degraded in wastewater at ambient temperatures and by wastewater treatment processes [110]. Therefore, samples need to be collected from places such as wastewater collection vessels, pipes and inflows to treatment plants. Either raw liquid wastewater or raw, fresh, settled primary solids can be sampled.

Expertise on the hydraulics and usage patterns of the wastewater system to be sampled should be sought to inform the programme, especially which geographical areas contribute to the sampling point selected. This requires information from sources such as sewerage network maps or maps of open drains and canals, diagrams, geographical information systems, and sanitation agency personnel knowledge. To inform the best times and days of the week for sampling the nature of the flow patterns and inputs to the wastewater system (e.g., industrial effluents, discharges from hospital wastewater, dilution, infiltration, stormwater) should be understood.

Material from on-site sanitation systems, and industrial and other wastewater may be transferred periodically to centralized wastewater treatment systems. This needs to be taken into consideration in designing sampling programmes and interpreting results.

For SARS-CoV-2 ES programmes using sewer infrastructure, the principal sampling location is usually the entry point to the wastewater treatment plant, using grab, composite, or passive samplers, after primary pre-treatment screening, or grab samples from settled primary sludge solids before further treatment. This sample location is sufficient for applications seeking information at the whole-of-catchment scale. For other use cases, particularly for larger catchments, a finer scale of sampling is required. Commonly used locations include pump stations and sewer access points relevant for the sub-catchment area of interest, such as a specific sub-urban area or building.

In low-resource settings, programmes have monitored septage from specific locations not connected to sewers, including drainage network confluence points, as recommended by the poliovirus ES programme, or where on-site systems such as septic tanks are used, or sullage tanks on boats or aircraft. Some programmes have successfully demonstrated the use of SARS-CoV-2 ES in environmental waters that have captured excreta and secreta in unsewered areas [111-113].

Protection of sampler safety is critical when sampling from wastewater [114]. The same safety considerations apply for any wastewater sampling, with no additional safety measures being required for sampling as part of SARS-CoV-ES programmes beyond those that apply to any wastewater sampling activity. Sampler safety risk factors that apply to water or wastewater-related sampling activities include road and traffic safety; personal security; and physical safety from slipping, tripping, head strikes, entrapment, drowning, and exposure to toxic or explosive gases. Handling untreated wastewater presents risks due to a wide range of faecal–oral and respiratory pathogens, and sometimes chemicals. As a result, samplers are typically vaccinated against a range of infectious diseases, such as hepatitis A and B virus, and in many cases the use of personal protective equipment, such as gloves, is required.

Understanding the objectives of the SARS-CoV-2 ES programme influences its design. For instance, if early warning is an objective, sampling and analysis need to be organized in a timely fashion. Therefore, some sampling sites may be preferred over others for logistical reasons – to enable samples to be returned to labs within at a few hours.

To enable subsequent analysis of the results, key metadata is required for all samples. This includes the location, date, time, duration, and sampling method. Ideally other information, such as flow rate of the water sampled, or unusual observations made during sampling, should be noted.

6.3 Sampling methods

Sampling equipment and volume

Sampling equipment and volume depend on the use case and context [115]. To date most SARS-CoV-2 ES sampling collects liquid wastewater, starting with simple grab samples, but with increasing use of automated composite sampling devices where power and logistics permit, and passive samplers. In addition, for wastewater treatment plants, primary settled solids are increasingly being sampled to provide a natural concentrated, composite sample.

- Automatic composite sampling is generally preferred because the sample can be gradually filled over time (e.g., 24 hours), to reduce the probability that briefly shed material will not be detected. However, this method usually requires a secure site, and either a battery or power supply to operate motorized pumps and refrigerators. Time and volume proportionating sampling can be done to help with normalization – the latter is more representative under varying flow conditions.
- Passive sampling places a medium in the wastewater to capture viruses and their RNA [97-100]. These devices are typically deployed at daily or multi-day intervals to provide a time-composite sample. Although the volume of wastewater that passes over the unit is not known (making the calculation of concentrations uncertain), the devices have proven sensitive and cost-effective, particularly where it is not practicable to install composite samplers. Comparison of the concentrations of RNA estimated when using passive and conventional liquid sampling methods correlate well.
- Primary settled solids at wastewater treatment plants have been shown to concentrate SARS-CoV-2 [116] which permits a single grab sample of solids to be collected at a point in time and be indicative of a composite of the virus passing through the wastewater stream. This approach is applicable to wastewater treatment works but not to all locations within the sewage system.
- Grab sampling methods involve collecting samples of 100–250 mL. Multiple grab samples can be collected, then mixed, to provide a semi-composite sample. For instance, five samples can be collected every 30 minutes during the predicted peak period of viral presence in wastewater (the morning high-flow period), and these can be pooled to provide one composite sample. Alternatively, single grab samples with volumes typically ranging from 250 mL to 1 L, can be collected at an optimal time of day – albeit there is no universal period on when that is for all wastewater systems. Most programmes target sample collection during peak morning sewage flow for instance, partly because sampling in the morning enables laboratory analysis the same day. But the extent to which time of day influences method sensitivity and specificity is not understood and may well vary between locations. Nonetheless, it is useful to record flow data.

Collecting large-volume samples is of limited value since inhibitors from wastewater and solids need to be kept at concentrations that will allow detection of viral RNA using the PCR. Hence, a common sample size is in the range 250 mL to 1 L of wastewater, or 1 g of settled solids.

Sampling frequency

For use cases involving long-term tracking of virus circulation, weekly programmes are acceptable. However, for early warning, more frequent sampling is warranted – typically daily to twice or three times weekly. In an emerging area of SARS-CoV-2 ES, sampling for studying genetic diversity by virus

variants in urban wastewater, including detection of variants of concern (VoCs), requires a different design and implementation. For instance, studies in New Zealand and Italy have reported routine weekly, monthly or bi-monthly surveys for such variants [55,117]. Typical SARS-CoV-2 ES sampling frequencies are shown in Table 3.

Table 3. Examples of sampling frequencies for different use cases and background variability

Use case	***Considerations relating to frequency***	***Example frequency***
Early warning	*Aims to detect emergence or small changes in virus concentration or percentage of positive samples at early stages of the pandemic to inform public health actions. In high-risk settings or where concentrations are low, testing frequencies are likely to be higher.*	*Daily to three times weekly (depending on resource constraints, risk of setting and concentrations in previous samples)*
Trend analysis	*Aims to detect significant changes in concentration to show trends over time. The rate of change observed from previous samples and the scale of the wastewater catchment are influential. For slower rates of change in COVID-19 prevalence, or for larger wastewater catchments that are inherently slower to change due to averaging effects in larger populations, sampling frequencies are likely to be lower.*	*Twice weekly to fortnightly (depending on resource constraints, historical rates of change and wastewater catchment scale)*
Point of entry	*Aims to detect presence of SARS-CoV-2 RNA or variant of concern at point of entry of transport vessel or holding point. This provides intelligence on air or sea port incursion in country, or for global monitoring as part of a network of sea and air ports.*	*Once – at the time of arrival of vessels from locations of concern*

Sample transport

- For transport, samples need to be stored at refrigeration temperatures, targeting the range 2-8˚C, but avoiding freezing. This is typically achieved by using containers of liquid ice or cooled blocks within insulated containers and/or refrigerated units in vehicles, whatever means is most practicable. The purpose is to seek to prevent samples becoming warm, (which will accelerate the degradation of the viral RNA), whilst avoiding freezing, (since freeze–thaw processes significantly reduce the concentration of detectable RNA, probably by encouraging the release of RNAse enzymes and/or mechanical shear).

Sample storage

- Samples should be stored in a refrigerator (typically 2-8˚C) until they are ready for analysis as soon as practicable after collection. Delays allow degradation of RNA and increase the time until results are available to inform public health responses. Samples should only be frozen (typically at -70˚C) when they are being stored for longer-term studies. Ideally, if frozen, the freezing should take place after RNA concentration and extraction since much less degradation occurs after that point.
- If practicable, a quality control standard is added to samples before storage, if they are to be stored for longer than about 24 hours (see matrix recovery control, below).

6.4 Laboratory analysis

Protection of analyst safety is critical when handling wastewater [114]. The same safety considerations apply for any wastewater analysis, with no additional safety measures being required for analysis as part of SARS-CoV-ES programmes beyond those that apply to any wastewater analytical activity. Handling untreated wastewater presents risks due to a wide range of faecal–oral and respiratory pathogens, and sometimes chemicals. As a result, analysts are typically vaccinated

against a range of infectious diseases, such as hepatitis A and B virus, and in many cases the use of personal protective equipment, such as gloves, is required. Pasteurization of wastewater samples may be undertaken to make the wastewater handling safer; pasteurization does not preclude detection of SARS-CoV-2 RNA if done in accordance with proven protocols [118].

Choice of methods

- The choice of analytical methods used will be influenced by the surveillance goal, the availability of testing methods, supplies, and equipment, and the preferences of laboratory technicians and other key staff.
- The costs of labour and kits or the need for automation can also affect the choice.
- A variety of commercial kits and reagents and a range of methods are available for such testing and many have been found to be effective in comparative trials [88,89,106]. Credible, independent, third-party evidence and/or local trials to match the method to the context are recommended before committing to any one method [120].

Equipment and consumables required

- Equipment and consumables needed largely overlap with those used in clinical testing laboratories for molecular biology, and in environmental microbiology laboratories for wastewater handling and virus concentration. Clinical laboratories are often not equipped for processing environmental samples or will not accept them. Therefore, clinical testing and/or environmental testing laboratories could potentially undertake testing alone or in partnership.
- For ongoing longer-term programmes, it is likely preferable to set up a dedicated environmental microbiology laboratory and a central laboratory to support training, supply of reagents, and QA/QC protocols.
- Many routine SARS-CoV-2 ES programmes use sophisticated reagents and kits that can be prohibitively expensive and present supply chain challenges which may limit their use in lower-resource settings. However, there are some proof-of-concept analytical arrangements that have shown promise and do not require highly experienced laboratory technicians, and these may lead to expanded options at remote locations and in low resource contexts [118].

Sample recovery controls

- If practicable, a process control is added to the sample before sample processing and analysis to provide virus recovery data during the process. This is more important for more complex concentration processes. The process control typically consists of an enveloped virus (e.g., murine or bovine coronavirus, bovine respiratory syncytial virus, feline infectious peritonitis virus). The choice of process control is influenced by availability of such process control material, sample type and laboratory preferences. In principle, a coronavirus recovery process control would be expected to be a more representative control than the alternatives since coronaviruses might behave differently from phage or free RNA.

Pre-treatment

- Samples need to be mixed while still cool from storage immediately before analysis to suspend particles that settled during storage and transport. The mixing can be done using simple inversion and mechanical mixing, or a vortex or sonicator.

- Some pre-treatment may be necessary for samples that contains excessive oils or particulates to avoid these materials inhibiting detection methods and reducing sensitivity. Pre-treatment may reduce the concentration of viral RNA and reduce method sensitivity. Pre-treatment can be performed on one sample replicate and not another and the results compared. Pre-treatment options include:
    - allowing a brief period of sedimentation following initial mixing before decanting;
    - pre-filtration with larger pore size filter (e.g., 5 µm); and
    - removing large debris or skimming off fatty material before drawing off the liquid for analysis.

Concentration

- The virus and its RNA may need to be concentrated by reducing the volume (to approximately 1 mL). This typically involves using ultracentrifugation, ultrafiltration, membrane filtration, precipitation with polyethylene glycol (PEG), flocculation with skim milk, or affinity-capture magnetic hydrogel particles [36,106].
- The choice of concentration method depends on factors such as preferences / experience of laboratory staff, availability of laboratory equipment and reagents, desired sample processing time and the nature of the wastewater matrix. Some commercial kits can be faster and require less handling than some simpler methods.
- Simpler methods [121] may be preferred in contexts where labour costs are low but there are limited funds for commercial kits. Such methods are similar to WHO recommended or accepted poliovirus concentration methods familiar to many laboratories in low-resource contexts [2].

RNA extraction

- RNA is typically extracted using commercial RNA extraction kits developed for environmental samples, which include all necessary reagents and operating procedures. The reagents and kits are designed to protect RNA and extract, separate, and concentrate RNA from other substances, particularly inhibitors of PCR reactions. The choice of kit can be influenced by the nature of the wastewater matrix, cost, availability, and the laboratory equipment required to use the kit. Automated (using extraction robots) or manual extractions may be performed

RNA detection and quantification

- RNA detection methods are similar to those used for clinical testing and are typically provided as commercial kits. They utilize RT-qPCR or dPCR, with fluorescent probes. The choice of genetic target and RNA test kit used can depend on the variants of the virus dominating at the time, and experience from comparing different targets and kits. Some laboratories have developed their own assays, ordering primers and probes that target specific regions of the genome.
- Test results can be compared with a calibration control (RNA) run in separate aliquots. This enables back-calculation of the relationship between the number of PCR cycles, the strength of the associated signal and the starting concentration of RNA in the reaction mix. Such controls are typically provided as part of routinely used PCR assays and are ideally run alongside each batch of tests for each PCR run.

Inhibition tests

- Physical (e.g. pH), chemical and biological parameters may be present in wastewater that inhibit PCR reactions. The matrix recovery process control can provide an assessment of overall losses and inhibition. An additional control can be applied after RNA extraction and before the PCR reaction to separate out inhibition from the effects of recovery. These controls may be part of the PCR kit, which can include an internal positive control that serves as an inhibition test. In other cases, RNA (e.g., gamma-irradiated SARS-CoV-2, or RNA from another coronavirus) has been added to the PCR reactions to test for inhibition.
- Concentrating larger samples into smaller, manageable volumes for completing the RNA extraction and analysis might be less sensitive than concentrating smaller starting volumes or more dilute samples. Both an undiluted sample and a 1:10 dilution can, for instance, be tested to assess inhibition, albeit noting that dilution will reduce method sensitivity.
- A multiplex PCR reaction (e.g., testing for a coliphage such as MS2) can be carried out routinely as part of the final stage of the PCR kit – this can serve as a general inhibition control. However, such assays may affect detection at low concentration of SARS-CoV-2.

Carryover and false positive controls

- PCR reactions generate very high copy numbers of their target. Therefore, negative controls should routinely be used (e.g., using blank reagent water) with each batch of samples.

Analytic targets

- The choice of genetic targets influences sensitivity and specificity. Some gene targets are, for reasons that are not understood, more sensitive than others. The genetic target may be selected as part of the decision about which test kit to use. A wide variety of such targets have successfully been used.
- The lower limits of detection and quantification for specific genetic targets can vary in ways that are poorly understood. Factors influencing this variation include the specific gene targets and the presence of potentially competing and inhibiting materials. Therefore, considerations relating to the use case and need influence the choice of gene target.

Method quality control, quality assurance and controls

- As noted above, it is vital to include controls with every batch of samples tested, along with quality assurance samples (see Fig. 3).
- As a minimum, all methods used need to be evaluated as being adequate at the outset of the SARS-CoV-2 ES programme, and revised and updated over time. Depending on the purpose of the programme, the evaluation of methods may need to cover the method's limit of detection, limit of quantification, measurement uncertainty, accuracy, precision, recovery efficiency, sensitivity, and specificity. This can be particularly challenging if controls are not readily available. There is currently no consensus on minimum required criteria for these assay quality variables. However, it is important to understand and communicate that information and any associated limitations to data users.

Variant analysis

- Variants of interest or concern can be detected in wastewater using genetic targets specific for those variants [109,123]. SARS-CoV-2 variants with targeted single nucleotide polymorphisms (SNPs) (single nucleotide differences unique to specific variants) can be

detected using RT-PCR assays with variant-specific primers and probes [45]. However, more recently, sequencing approaches including whole genome sequencing of target regions, such as the spike protein [47], are more commonly used to identify variants present. Sequencing approaches potentially permit the detection of novel or emerging variants not yet identified as variants or interest or concern.

6.5 Data interpretation

The sensitivity of SARS-CoV-2 ES methods to detect the presence of infected persons in the wastewater catchment area varies depending on factors such as:

- the variant and time dependent quantity of virus shed by an infected person;
- the timing of personal hygiene and sanitation activities and the usage patterns (e.g., weekdays vs. weekends) of sewers or sanitation systems within the sampled catchment area relative to the time window represented by the sample and sampling method used;
- the extent of dilution and degradation of viral RNA in the wastewater matrix due to inflow and infiltration into the sewer (rainwater and runoff and stormwater ingress, groundwater ingress, industrial and commercial discharges), and the influence of wastewater quality and potentially some forms of treatment or chemical additives before the sampling point;
- PCR assay inhibition due to inhibitory substances in the water matrix; and
- the recovery efficiency of the method used.

As with clinical testing, where the absence of a detectable biological response does not mean that a person is not infected at some level, the absence of detectable RNA in a wastewater sample does not demonstrate that there are no infected persons in the sampled catchment. Valid interpretation of non-detect results requires an understanding of the lower limit of reliable detection and potential implications of inhibition or other forms of interference. However, as is the case for poliovirus and despite a low negative predictive value, SARS-CoV-2 ES can be used to confirm the absence of significant virus circulation and, through ongoing testing, detect if that situation changes.

Whilst the precise number of infected people in a wastewater catchment cannot be accurately estimated based on SARS-CoV-2 ES results, the use of internal standards is an optional process that can be used to provide some normalization to enable results to be used in a relative manner and to observe trends. When sewers are highly influenced by stormwater during rainfall, or low flow during drought, results can be adjusted to account for dilution when quantitative trends are to be followed over time and compared with public health surveillance data. The effects of dilution from non-sewage inputs can be hard to discriminate from changes in COVID-19 cases. Therefore, controls can be used to help normalize against human-derived inputs. Conventional and widely used bacterial faecal indicator organisms, such as *Escherichia coli* (*E. coli*), can be used as a low-cost and widely available normalizing marker. Likewise, ammonia conductivity and other chemical parameters, can provide some normalization indicators and can cost less. Industrial water, stormwater, snowmelt, greywater, and groundwater might contain some background concentrations of these indicators that need to be considered.

Assays for other targets routinely shed by humans can provide a normalization control. Such targets include:

- viruses that infect bacteria (bacteriophage, e.g., cross-assembly phage (crAssphage), and *Lachnospiraceae* (Lachno3));
- viruses from food plants that are routinely present in human faeces and wastewater (e.g., pepper mild mottle virus (PMMoV));
- bacteria that are more specific to human waste than *E. coli* (e.g., *Bacteroides* HF183); and
- human mitochondrial DNA [124].

These biological or chemical indicator measurements can assist with identifying any elevated non-sewage inputs, but doing so requires specialist interpretation. Interpretation of data in conjunction with public health surveillance data means different things in high-prevalence versus low-prevalence settings. For instance, in high-prevalence settings, elevated levels of SARS-CoV-2 RNA from SARS-CoV-2 ES are expected, and interpretation relates to variant and relative concentration, rather than simple detection or non-detection of the viral RNA. In contrast, in low- or no known prevalence settings, unexpected detection relates to presence or absence of SARS-CoV-2. Correlation between results from public health surveillance and SARS-CoV-2 ES sampling is approximate because of the nature of sanitation systems and mobility of people. For instance:

- infected people may move between wastewater catchments areas (e.g., between home and work; for shopping, tourism and recreation as well as in case of hospitalization);
- members of the population using on-site sanitation (e.g., septic tanks, pits) will not be captured in sewer-based sampling programmes;
- wastewater catchments may not be accurately defined and/or may not match the population area observed by epidemiological and clinical surveillance and;
- wastewater and sludge from on-site systems may be transferred to other systems at periodic intervals.

These correlations are made more challenging by factors that influence the consistency of public health surveillance, the willingness and ability of potentially infected people to get tested, and the collection and reporting of testing results.

Therefore, both ES and public health surveillance approaches have sources of uncertainty, which makes precisely correlating the two challenging. The two approaches are complementary as each has different strengths and limitations and provides independent data for decision-making.

6.6 Aggregation and presentation of data

Public health agencies can integrate data from public health surveillance and SARS-CoV-2 ES programmes and harmonize ES data across local, regional and national contexts to use aggregated data in COVID-19 response at the local and national scales. Important is setting the governance of responsibilities with respect to public health decisions and communication upfront of any ES results.

There can be challenges in comparing different methods between laboratories and work groups. Therefore, there are benefits in using one laboratory for a geographic area of interest and standardizing methods across multiple laboratories, where practicable. If this is not possible, consideration can be given to ways of comparing the results from the range of methods used (e.g. through interlaboratory comparisons and expert professional judgement).

While integration of ES data in the regular public health reporting and communication scheme is most appropriate and effective, dashboards can be used to present data at local and national levels paired with public health advice. Examples of such dashboards include those from: South Africa [43], Hungary [51], the Kingdom of the Netherlands [48], Switzerland [49], the United Kingdom [52], USA [105], Türkiye [125], and New Zealand [55]. Combining SARS-CoV-2 ES information with public health data and communication of public health advice helps with the COVID-19 response and health promotion. Specifically:

- Interpretation of ES results by public health agencies should include clinical testing response decision-support process flow diagrams or algorithms.
- Formulation and communication of public health advice should help to focus clinical testing and community messaging on areas with elevated viral presence and concentrations detected from SARS-CoV-2 ES; and provide early warning of trends in COVID-19 in the community to inform control initiatives.

The minimum information to make ES data useful to public health agencies and the public includes:

- results as trends over time (rising, falling or steady, expressed as concentrations of SARS-CoV-2 genome copies, or changes in the proportion of samples testing positive);
- population monitored as represented by each sample with reference to its geographic catchment area (spatial and name labels);
- with filter function to view single catchment area or aggregate 'like' results to larger geographic areas at local, subnational, national and regional levels;
- implications of results relative to a benchmark (e.g., using traffic light indicators to define risk of SARS-CoV-2 exposure or COVID-19 health system burden)
- additional specific information that is desirable such as residential population monitored as represented by each catchment;
- historical results from the same location;
- current and historical results from nearby and comparable locations; and
- reported clinical information from the same location for the same period as sample collection, such as number of clinical COVID-19 cases, number of persons testing positive for SARS-CoV-2 infection (if case ascertainment is judged to be moderate to high), percentage of persons tested for SARS-CoV-2 infection that return a positive result, and COVID-19 hospitalizations.

Additional useful information that is desirable to public health agencies and technical audiences includes:

- sample type;
- gene target;
- assay detection limits;
- population normalization marker;
- units of measurement (e.g., ratio of SARS-CoV-2 to normalization marker); and
- quality assurance and quality control process and performance on method sensitivity and specificity.
- variant analysis

7. Emerging research

A range of research projects and innovations are in progress to improve ES for SARS-CoV-2 and other health threats [126]. Low cost, easy-to-deploy sampling methods which expand the possible sampling applications for wastewater and other water bodies are one area of focus. In higher-resource contexts, these include new areas, such as attempts to test antigen levels, and improvements to genome sequencing and next-generation sequencing for novel variant detection [46,128]. This requires molecular biology, computational [127] and bioinformatics capability that is not readily available in many lower-resource contexts. ES has shown potential to detect novel variants that emerge, as well as to increase understanding of the ecology and zoonotic potential of SARS-CoV-2 that is not identified in human clinical samples [129,130]. Potentially, ES could be used to monitor wastewater or other water sources from animal rearing operations, and to monitor transport hubs to support global pandemic intelligence.

Research needs for SARS-CoV-2 ES are being coordinated and promoted via the EU [131,132] and the Global Water Research Coalition that represents [133] to optimize the benefits of data sharing and coordinated research among SARS-CoV-2 ES programme managers, researchers and funding partners.

8. Details of guidance development

8.1 Search strategy

Multiple lines of evidence were used to inform this guidance.

- Step1: Precedence was given to evidence sourced from a review of refereed journal articles. Some pre-publication papers and technical reports were used where they addressed recent emerging findings in ES for SARS-CoV-2. Publications have been routinely extracted as they are published through the Publication Map covid19wbec.org and COVIDPoops19 covering over 4,500 sites in 72 counties covering all 6 WHO regions.
- Step 2: Experiences of practical implementation from grey literature were drawn upon, including:
    - European Commission – SARS-CoV-2 surveillance employing sewage – towards a sentinel system;
    - United States Centers for Disease Control and Prevention – National Wastewater Surveillance System (NWSS);
    - Water Research Foundation – COVID-19 guidance and resources;
    - South African Medical Research Council – Wastewater Surveillance and Research Programme;
    - South African Medical Research Council – Wastewater sampling guide;
    - South African Water Research Commission – National COVID-19 Water and Sanitation Surveillance Programme;
    - Water Research Australia – Collaboration on Sewage Surveillance of SARS-CoV-2 project for Australia, New Zealand and some of the Mekong countries and Fiji;
    - Canadian Water Network – COVID-19 Wastewater Coalition;
    - numerous public communication interfaces on wastewater surveillance;
    - global lessons from a survey undertaken by the University of Washington; and

- targeted expert interviews with participating members of the Global Water Research Coalition.

8.2 Evidence review and quality appraisal

Data was extracted from the individual papers and grey literature by consultant Dan Deere according to the five scoping questions described under the purpose sub-section in section 1 of this document. Unlike other areas such as rapid tests, there is a small number of methods and applications for COVID-ES that have been: a) described in the literature, b) are commonly used, and c) have been applied successfully in programmes at scale. As such the document summarizes evidence from published and grey literature that meets these three criteria as judged by the guidance development group.

Table 4. Summary of data extracted and quality assessment criteria

Data extracted	***Scoping topic***	***Quality assessment criteria***
• *Short description of the use case* • *Date* • *Location* • *Context: spatial context, sanitation context – sewer vs on-site systems, stage of pandemic, prevalence of infections, low, medium, high-income setting* • *Implementation lead* • *Benefit of use case for public health decision making* • *Sampling method* • *Analytical method(s) used* • *Capacity needs/challenges* • *Coordination structure* • *Data presentation* • *Comment on cost benefit* • *Implications for other prevalence settings* • *Implications for other resource settings*	1. *Fit within the broader public health surveillance (section 2)* 2. *Added value to public health decision-making (section 3)* 3. *Basis to initiate, maintain, modify, pause, or stop (section 4)?*	• *Published and grey literature included* • *Scale of application* • *Extent to which ES supports public health decision making*
	4. *Capacity, planning and coordination needs (section 5)*	• *Published and grey literature included* • *Degree to which ES supports public health decision making*
	5. *Methods for sampling, analysis, data interpretation (section 6)?*	• *Method described in published literature including description of methods or protocol* • *Method is commonly used* • *Method has been used in an at scale programme*

8.3 Evidence to decision-making process

Evidence was synthesized into guidance text based on quality assessment and evidence to decision criteria and presented to the guidance development group for decision by consensus via online meetings and email exchange. Decision criteria used were: feasibility for immediate implementation, resources requirements, intervention/option acceptable to all stakeholders, balance between benefits and harms, impact on equity. The revised draft was then circulated for external review and feedback compiled into the final document.

8.4 Plans for updates

WHO continues to monitor the situation closely for any changes that may affect this Guidance. Should any factors change, WHO will issue a further update.

8.5 Selection and declaration of interests

Guidance development group members and external reviewers were selected via research and practitioner networks working on COVID ES globally. Selection aimed for a balance of research and implementation experience, gender and regional representation. All members of the Guidance Development Group and External Review Group completed declarations of interest, which was reviewed by the Steering Committee in accordance with WHO principles and policies and assessed for any conflicts of interest. No conflicts of interest were identified that required individuals to abstain from consensus decision making.

References

1. Silk BJ, Scobie HM, Duck WM, Palmer T, Ahmad FB, Binder AM, Cisewski JA, Kroop S, Soetebier K, Park M, Kite-Powell A, Cool A, Connelly E, Dietz S, Kirby AE, Hartnett K, Johnston J, Khan D, Stokley S, Paden CR, Sheppard M, Sutton P, Razzaghi H, Anderson RN, Thornburg N, Meyer S, Womack C, Weakland AP, McMorrow M, Broeker LR, Winn A, Hall AJ, Jackson B, Mahon BE, Ritchey MD. COVID-19 Surveillance After Expiration of the Public Health Emergency Declaration - United States, May 11, 2023. MMWR Morb Mortal Wkly Rep. 2023 May 12;72(19):523-528. doi: 10.15585/mmwr.mm7219e1.
2. Guidelines for environmental surveillance of poliovirus circulation. Geneva: World Health Organization; 2003.
3. Asghar H, Diop OM, Weldegebriel G, Malik F, Shetty S, El Bassioni L, Akande AO, Al Maamoun E, Zaidi S, Adeniji AJ, Burns CC, Deshpande J, Oberste MS, Lowther SA. Environmental surveillance for polioviruses in the Global Polio Eradication Initiative. J Infect Dis. 2014 Nov 1;210 Suppl 1:S294-303. doi: 10.1093/infdis/jiu384.
4. Hovi T, Shulman LM, van der Avoort H, Deshpande J, Roivainen M, DE Gourville EM. Role of environmental poliovirus surveillance in global polio eradication and beyond. Epidemiol Infect. 2012 Jan;140(1):1-13. doi: 10.1017/S095026881000316X. Epub 2011 Aug 18.
5. Andrews JR, Yu AT, Saha S, Shakya J, Aiemjoy K, Horng L, Qamar F, Garrett D, Baker S, Saha S, Luby SP. Environmental surveillance as a tool for identifying high-risk settings for typhoid transmission. Clin Infect Dis. 2020 Jul 29;71(Suppl 2):S71-S78. doi: 10.1093/cid/ciaa513.
6. Uzzell CB, Troman CM, Rigby J, Mohan VR, John J, Abraham D, Srinivasan R, Nair S, Meschke JS, Elviss N, Kang G, Feasey N, Grassly NC. Environmental surveillance for Salmonella Typhi as a tool to estimate the incidence of typhoid fever in low-income populations. medRxiv. 2021;22 May 2021. doi: 10.1101/2021.05.21.21257547.
7. Typhoid: vaccine preventable diseases surveillance standards. Geneva: World Health Organization; 2018 (www.who.int/publications/m/item/vaccine-preventable-diseases-surveillance-standards-typhoid, accessed 19 June 2023).
8. Hellmér M, Paxéus N, Magnius L, Enache L, Arnholm B, Johansson A, Bergström T, Norder H. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. Appl Environ Microbiol. 2014 Nov;80(21):6771-81. doi: 10.1128/AEM.01981-14. Epub 2014 Aug 29.
9. Lun JH, Hewitt J, Sitabkhan A, Eden JS, Enosi Tuipulotu D, Netzler NE, Morrell L, Merif J, Jones R, Huang B, Warrilow D, Ressler KA, Ferson MJ, Dwyer DE, Kok J, Rawlinson WD, Deere D, Crosbie ND, White PA. Emerging recombinant noroviruses identified by clinical and waste water screening. Emerg Microbes Infect. 2018 Mar 29;7(1):50. doi: 10.1038/s41426-018-0047-8.
10. Global action plan on antimicrobial resistance 2016–2020. Geneva: World Health Organization; 2015 (https://www.who.int/publications/i/item/9789241509763, accessed 19 June 2023).
11. Food and Agriculture Organization of the United Nations, World Organisation for Animal Health (OIE), World Health Organization. Technical brief on water, sanitation, hygiene (WASH) and wastewater management to prevent infections and reduce the spread of antimicrobial resistance (AMR). Geneva: World Health Organization; 2020 (https://www.who.int/publications/i/item/9789240006416, accessed 19 June 2023).
12. WHO integrated global surveillance on ESBL-producing *E. coli* using a “One Health” approach: implementation and opportunities. Geneva: World Health Organization; 2021.
13. Global antimicrobial resistance surveillance system (GLASS) report: early implementation 2020. Geneva: World Health Organization; 2020. Licence: CC BY-NC-SA 3.0 IGO.
14. Diamond MB, Keshaviah A, Bento AI, Conroy-Ben O, Driver EM, Ensor KB, Halden RU, Hopkins LP, Kuhn KG, Moe CL, Rouchka EC, Smith T, Stevenson BS, Susswein Z, Vogel JR, Wolfe MK,

Stadler LB, Scarpino SV. Wastewater surveillance of pathogens can inform public health responses. Nat Med. 2022 Oct;28(10):1992-1995. doi: 10.1038/s41591-022-01940-x.
15. Amereh F, Jahangiri-Rad M, Mohseni-Bandpei A, Mohebbi SR, Asadzadeh-Aghdaei H, Dabiri H, Eslami A, Roostaei K, Aali R, Hamian P, Rafiee M. Association of SARS-CoV-2 presence in sewage with public adherence to precautionary measures and reported COVID-19 prevalence in Tehran. Sci Total Environ. 2022 Mar 15;812:152597. doi: 10.1016/j.scitotenv.2021.152597. Epub 2021 Dec 23.
16. Wong JCC, Tan J, Lim YX, Arivalan S, Hapuarachchi HC, Mailepessov D, Griffiths J, Jayarajah P, Setoh YX, Tien WP, Low SL, Koo C, Yenamandra SP, Kong M, Lee VJM, Ng LC. Non-intrusive wastewater surveillance for monitoring of a residential building for COVID-19 cases. Sci Total Environ. 2021 Sep 10;786:147419. doi: 10.1016/j.scitotenv.2021.147419. Epub 2021 Apr 29.
17. Bernard K, Davis A, Simpson IM, Hale VL, Lee J, Winston RJ. Detection of SARS-CoV-2 in urban stormwater: An environmental reservoir and potential interface between human and animal sources. Sci Total Environ. 2022 Feb 10;807(Pt 3):151046. doi: 10.1016/j.scitotenv.2021.151046. Epub 2021 Oct 18.
18. Pepe Razzolini MT, Funada Barbosa MR, Silva de Araújo R, Freitas de Oliveira I, Mendes-Correa MC, Sabino EC, Garcia SC, de Paula AV, Villas-Boas LS, Costa SF, Dropa M, Brandão de Assis D, Levin BS, Pedroso de Lima AC, Levin AS. SARS-CoV-2 in a stream running through an underprivileged, underserved, urban settlement in São Paulo, Brazil: A 7-month follow-up. Environ Pollut. 2021 Dec 1;290:118003. doi: 10.1016/j.envpol.2021.118003. Epub 2021 Aug 19.
19. World Health Organization. Status of environmental surveillance for SARS-CoV-2 virus: scientific brief, 5 August 2020. World Health Organization. (https://apps.who.int/iris/handle/10665/333670. License: CC BY-NC-SA 3.0 IGO, accessed 19 June 2023).
20. World Health Organization. Environmental surveillance for SARS-CoV-2 to complement public health surveillance – Interim Guidance, 14 April 2022. World Health Organization. (https://www.who.int/publications/i/item/WHO-HEP-ECH-WSH-2022.1, accessed 19 June 2023).
21. Medema G, Heijnen L, Elsinga G, Italiaander R, Brouwer A. Presence of SARS-Coronavirus-2 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 May 20:acs.estlett.0c00357. doi: 10.1021/acs.estlett.0c00357.
22. Lodder W, de Roda Husman AM. SARS-CoV-2 in wastewater: potential health risk, but also data source. Lancet Gastroenterol Hepatol. 2020;5(6):533-534. Doi:10.1016/S2468-1253(20)30087-X.
23. La Rosa G, Iaconelli M, Mancini P, Bonanno Ferraro G, Veneri C, Bonadonna L, Lucentini L, Suffredini E. First detection of SARS-CoV-2 in untreated wastewaters in Italy. Sci Total Environ. 2020 Sep 20;736:139652. doi: 10.1016/j.scitotenv.2020.139652. Epub 2020 May 23.
24. Alpaslan Kocamemi B, Kurt H, Hacıoglu S, Yaralı C, Saatci AM, Pakdemirli B. First Data-Set on SARS-CoV-2 Detection for Istanbul Wastewaters in Turkey. MedRxiv. 2020; 6 May 2020. doi:10.1101/2020.05.03.20089417.
25. Alpaslan Kocamemi B, Kurt H, Sait A, Sarac F, Saatci AM, Pakdemirli B. SARS-CoV-2 Detection in Istanbul Wastewater Treatment Plant Sludges. MedRxiv. 2020; 16 May 2020. doi: 10.1101/2020.05.12.20099358.
26. Alpaslan Kocamemi B, Kurt H, Sait A, Kadi H, Sarac F, Aydin I, Saatci AM, Pakdemirli B. Nationwide SARS-CoV-2 Surveillance Study for Sewage and Sludges of Wastewater Treatment Plants in Turkey. MedRxiv. 2020; 30 November 2020. doi: 10.1101/2020.11.29.20240549.
27. Alpaslan Kocamemi B, Kurt H, Sait A, Kadi H, Sarac F, Aydin I, Saatci AM, Pakdemirli B. Routine SARS-CoV-2 wastewater surveillance results in Turkey to follow Covid-19 outbreak. MedRxiv. 2020; 22 December 2020. doi: 10.1101/2020.12.21.20248586.

28. Tlhagale M, Liphadzi S, Bhagwan J, Naidoo V, Jonas K, van Vuuren L, Medema G, Andrews L, Béen F, Ferreira ML, Saatci AM, Alpaslan Kocamemi B, Hassard F, Singer AC, Bunce J T, Grimsley JMS, Brown M, Jones DL. Establishment of local wastewater-based surveillance programmes in response to the spread and infection of COVID-19 – case studies from South Africa, the Netherlands, Turkey and England. J Water Health. 20222; 20(2), 287-299. https://doi.org/10.2166/wh.2022.185.
29. Haramoto E, Malla B, Thakali O, Kitajima M. First environmental surveillance for the presence of SARS-CoV-2 RNA in wastewater and river water in Japan. Sci Total Environ. 2020 Oct 1;737:140405. doi: 10.1016/j.scitotenv.2020.140405. Epub 2020 Jun 20.
30. Zhao L, Atoni E, Nyaruaba R, Du Y, Zhang H, Donde O, Huang D, Xiao S, Ren N, Ma T, Shu Z, Yuan Z, Tong L, Xia H. Environmental surveillance of SARS-CoV-2 RNA in wastewater systems and related environments in Wuhan: April to May of 2020. J Environ Sci (China). 2022 Feb;112:115-120. doi: 10.1016/j.jes.2021.05.005. Epub 2021 May 14.
31. Kumar M, Patel AK, Shah AV, Raval J, Rajpara N, Joshi M, Joshi CG. First proof of the capability of wastewater surveillance for COVID-19 in India through detection of genetic material of SARS-CoV-2. Sci Total Environ. 2020 Dec 1;746:141326. doi: 10.1016/j.scitotenv.2020.141326. Epub 2020 Jul 28.
32. Mailepessov D, Arivalan S, Kong M, Griffiths J, Low SL, Chen H, Hapuarachchi HC, Gu X, Lee WL, Alm EJ, Thompson J, Wuertz S, Gin K, Ng LC, Wong JCC. Development of an efficient wastewater testing protocol for high-throughput country-wide SARS-CoV-2 monitoring. Sci Total Environ. 2022 Jun 20;826:154024. doi: 10.1016/j.scitotenv.2022.154024. Epub 2022 Feb 22.
33. Sherchan SP, Shahin S, Ward LM, Tandukar S, Aw TG, Schmitz B, Ahmed W, Kitajima M. First detection of SARS-CoV-2 RNA in wastewater in North America: A study in Louisiana, USA. Sci Total Environ. 2020 Nov 15;743:140621. doi: 10.1016/j.scitotenv.2020.140621. Epub 2020 Jun 30.
34. Wu F, Zhang J, Xiao A, Gu X, Lee WL, Armas F, Kauffman K, Hanage W, Matus M, Ghaeli N, Endo N, Duvallet C, Poyet M, Moniz K, Washburne AD, Erickson TB, Chai PR, Thompson J, Alm EJ. SARS-CoV-2 Titers in Wastewater Are Higher than Expected from Clinically Confirmed Cases. mSystems. 2020 Jul 21;5(4):e00614-20. doi: 10.1128/mSystems.00614-20.
35. Ahmed W, Angel N, Edson J, Bibby K, Bivins A, O'Brien JW, Choi PM, Kitajima M, Simpson SL, Li J, Tscharke B, Verhagen R, Smith WJM, Zaugg J, Dierens L, Hugenholtz P, Thomas KV, Mueller JF. 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 Aug 1;728:138764. doi: 10.1016/j.scitotenv.2020.138764. Epub 2020 Apr 18.
36. Pocock G, Coetzee L, Mans J, Taylor M, Genthe B. Proof of concept study: application of wastewater-based surveillance to monitor SARS-CoV-2 prevalence in South African communities. Research Report No. TT 832/20, ISBN 978-0-6392-0187-0. Water Research Commission, Pretoria, South Africa, September 2020.
37. Strengthening public health surveillance through wastewater testing: an essential investment for the COVID-19 pandemic and future health threats. Washington, DC: World Bank (https://openknowledge.worldbank.org/handle/10986/36852, accessed 19 June 2023).
38. Prado T, Fumian TM, Mannarino CF, Resende PC, Motta FC, Eppinghaus ALF, Chagas do Vale VH, Braz RMS, de Andrade JDSR, Maranhão AG, Miagostovich MP. Wastewater-based epidemiology as a useful tool to track SARS-CoV-2 and support public health policies at municipal level in Brazil. Water Res. 2021 Mar 1;191:116810. doi: 10.1016/j.watres.2021.116810. Epub 2021 Jan 5.
39. Thompson JR, Nancharaiah YV, Gu X, Lee WL, Rajal VB, Haines MB, Girones R, Ng LC, Alm EJ, Wuertz S. Making waves: Wastewater surveillance of SARS-CoV-2 for population-based health management. Water Res. 2020 Oct 1;184:116181. doi: 10.1016/j.watres.2020.116181. Epub 2020 Jul 13.

40. Kirby AE, Walters MS, Jennings WC, Fugitt R, LaCross N, Mattioli M, Marsh ZA, Roberts VA, Mercante JW, Yoder J, Hill VR. Using wastewater surveillance data to support the COVID-19 response - United States, 2020-2021. MMWR Morb Mortal Wkly Rep. 2021 Sep 10;70(36):1242-1244. doi: 10.15585/mmwr.mm7036a2.
41. Wu F, Xiao A, Zhang J, Moniz K, Endo N, Armas F, Bushman M, Chai PR, Duvallet C, Erickson TB, Foppe K, Ghaeli N, Gu X, Hanage WP, Huang KH, Lee WL, McElroy KA, Rhode SF, Matus M, Wuertz S, Thompson J, Alm EJ. Wastewater surveillance of SARS-CoV-2 across 40 U.S. states from February to June 2020. Water Res. 2021 Sep 1;202:117400. doi: 10.1016/j.watres.2021.117400. Epub 2021 Jul 2.
42. Wastewater surveillance testing methods. Atlanta, Georgia: United States Centers for Disease Control and Prevention; 2022 (https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/testing-methods.html, accessed 19 June 2023).
43. SAMRC SARS-CoV-2 Wastewater Surveillance Dashboard [website]. Durban: South African Medical Research Council (https://www.samrc.ac.za/wbe/, accessed 19 June 2023).
44. Geubbels ELPE, Backer JA, Bakhshi-Raiez F, van der Beek RFHJ, van Benthem BHB, van den Boogaard J, Broekman EH, Dongelmans DA, Eggink D, van Gaalen RD, van Gageldonk A, Hahné S, Hajji K, Hofhuis A, van Hoek AJ, Kooijman MN, Kroneman A, Lodder W, van Rooijen M, Roorda W, Smorenburg N, Zwagemaker F; National sewage surveillance group; RIVM COVID-19 epidemiology, surveillance team; de Keizer NF, van Walle I, de Roda Husman AM, Ruijs C, van den Hof S. The daily updated Dutch national database on COVID-19 epidemiology, vaccination and sewage surveillance. Sci Data. 2023 Jul 20;10(1):469. doi: 10.1038/s41597-023-02232-w.
45. Johnson R, Sharma JR, Ramharack P, Mangwana N, Kinnear C, Viraragavan A, Glanzmann B, Louw J, Abdelatif N, Reddy T, Surujlal-Naicker S, Nkambule S, Mahlangeni N, Webster C, Mdhluli M, Gray G, Mathee A, Preiser W, Muller C, Street R. Tracking the circulating SARS-CoV-2 variant of concern in South Africa using wastewater-based epidemiology. Sci Rep. 2022 Jan 21;12(1):1182. doi: 10.1038/s41598-022-05110-4.
46. Izquierdo-Lara RW, Heijnen L, Oude Munnink BB, Schapendonk CME, Elsinga G, Langeveld J, Post J, Prasad DK, Carrizosa C, Been F, van Beek J, Schilperoort R, Vriend R, Fanoy E, de Schepper EIT, Sikkema RS, Molenkamp R, Aarestrup FM, Medema G, Koopmans MPG, de Graaf M. Rise and fall of SARS-CoV-2 variants in Rotterdam: Comparison of wastewater and clinical surveillance. Sci Total Environ. 2023 May 15;873:162209. doi: 10.1016/j.scitotenv.2023.162209. Epub 2023 Feb 14.
47. Bar-Or I, Weil M, Indenbaum V, Bucris E, Bar-Ilan D, Elul M, Levi N, Aguvaev I, Cohen Z, Shirazi R, Erster O, Sela-Brown A, Sofer D, Mor O, Mendelson E, Zuckerman NS. Detection of SARS-CoV-2 variants by genomic analysis of wastewater samples in Israel. Sci Total Environ. 2021 October 1;789:148002. doi: 10.1016/j.scitotenv.2021.148002.
48. Coronavirus Dashboard: Virus particles in wastewater [website]. Government of the Netherlands (https://coronadashboard.government.nl/landelijk/rioolwater, accessed 19 June 2023).
49. SARS-CoV-2 in Wastewater, Department Urban Water Management, Switzerland [website]. (https://www.eawag.ch/en/department/sww/projects/sars-cov2-in-wastewater/, accessed 19 June 2023).
50. National Wastewater Surveillance System (NWSS). Atlanta, Georgia: United States Centers for Disease Control and Prevention, [website] (www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html, accessed 19 June 2023).
51. Concentration of SARS-CoV-2 virus measured in wastewater, National Public Health Centre, Hungary [website]. (https://www.nnk.gov.hu/index.php/koronavirus/szennyvizvizsgalatok, accessed 19 June 2023).
52. EMHP wastewater monitoring of SARS-CoV-2 in England: 1 June to 10 January 2022. London: United Kingdom Health Security Agency; 2022

(www.gov.uk/government/publications/monitoring-of-sars-cov-2-rna-in-england-wastewater-monthly-statistics-1-june-2021-to-10-january-2022/emhp-wastewater-monitoring-of-sars-cov-2-in-england-1-june-to-10-january-2022, accessed 19 June 2023).

53. Huang WL, Fann WB, Shen RJ, Chu Y, Yang JY. Surveillance of SARS-CoV-2 in Sewage Treatment Plants between January 2020 and July 2021 in Taiwan. Pathogens. 2021 Dec 10;10(12):1611. doi: 10.3390/pathogens10121611.
54. SARS-CoV-2 RNA levels in wastewater in the United States. Atlanta, Georgia: United States Centers for Disease Control and Prevention; 2022 (https://covid.cdc.gov/covid-data-tracker/#wastewater-surveillance, accessed 19 June 2023).
55. Institute of Environmental Science and Research, Wastewater Surveillance Dashboard, Government of New Zealand, https://www.esr.cri.nz/our-expertise/covid-19-response/covid19-insights/wastewater-surveillance-dashboard/, accessed 28 July 2023.
56. Wastewater testing. Government of Victoria, Australia [website]. (https://www.coronavirus.vic.gov.au/wastewater-testing, accessed 19 June 2023).
57. Deere DA, Jones DL, Ahmed W, Medema G, Kreuzinger N, Remmonay I, Lacroix S, Hewitt J, Tavazzi S, Gawlik BM. European Commission. Adhoc guidance: Wastewater sampling of aircrafts and airports for SARS-CoV-2 surveillance, 10 January 2023. 26 pp. European Commission Brussels 2023.
58. Rapid expert consultation on environmental surveillance of SARS-CoV-2 in wastewater: summary report. Virtual meeting, 23 July 2020. Copenhagen: World Health Organization Regional Office for Europe; 2020.
59. Expert consultation on public health needs related to surveillance of SARS-CoV-2 in wastewater: summary report. Virtual meeting, 30 November 2020. Copenhagen: World Health Organization Regional Office for Europe; 2021.
60. COVIDPoops19: Summary of global SARS-CoV-2 wastewater monitoring efforts by UC Merced researcher [online dashboard] (https://ucmerced.maps.arcgis.com/apps/dashboards/c778145ea5bb4daeb58d31afee389082, accessed 19 June 2023).
61. COVID-19 WBE Collaborative [website] (https://www.covid19wbec.org/, accessed 19 June 2023).
62. Scott LC, Aubee A, Babahaji L, Vigil K, Tims S, Aw TG. Targeted wastewater surveillance of SARS-CoV-2 on a university campus for COVID-19 outbreak detection and mitigation. Environ Res. 2021 Sep;200:111374. doi: 10.1016/j.envres.2021.111374. Epub 2021 May 29.
63. Corchis-Scott R, Geng Q, Seth R, Ray R, Beg M, Biswas N, Charron L, Drouillard KD, D'Souza R, Heath DD, Houser C, Lawal F, McGinlay J, Menard SL, Porter LA, Rawlings D, Scholl ML, Siu KWM, Tong Y, Weisener CG, Wilhelm SW, McKay RML. Averting an outbreak of SARS-CoV-2 in a university residence hall through wastewater surveillance. Microbiol Spectr. 2021 Oct 31;9(2):e0079221. doi: 10.1128/Spectrum.00792-21. Epub 2021 Oct 6.
64. Reeves K, Liebig J, Feula A, Saldi T, Lasda E, Johnson W, Lilienfeld J, Maggi J, Pulley K, Wilkerson PJ, Real B, Zak G, Davis J, Fink M, Gonzales P, Hager C, Ozeroff C, Tat K, Alkire M, Butler C, Coe E, Darby J, Freeman N, Heuer H, Jones JR, Karr M, Key S, Maxwell K, Nelson L, Saldana E, Shea R, Salveson L, Tomlinson K, Vargas-Barriga J, Vigil B, Brisson G, Parker R, Leinwand LA, Bjorkman K, Mansfeldt C. High-resolution within-sewer SARS-CoV-2 surveillance facilitates informed intervention. Water Res. 2021 Oct 1;204:117613. doi: 10.1016/j.watres.2021.117613. Epub 2021 Aug 28.
65. Aguiar-Oliveira ML, Campos A, R Matos A, Rigotto C, Sotero-Martins A, Teixeira PFP, Siqueira MM. Wastewater-based epidemiology (WBE) and viral detection in polluted surface water: a valuable tool for COVID-19 surveillance-a brief review. Int J Environ Res Public Health. 2020 Dec 10;17(24):9251. doi: 10.3390/ijerph17249251.

66. Kitamura K, Sadamasu K, Muramatsu M, Yoshida H. Efficient detection of SARS-CoV-2 RNA in the solid fraction of wastewater. Sci Total Environ. 2021 Apr 1;763:144587. doi: 10.1016/j.scitotenv.2020.144587. Epub 2020 Dec 18.
67. Rosiles-González G, Carrillo-Jovel VH, Alzate-Gaviria L, Betancourt WQ, Gerba CP, Moreno-Valenzuela OA, Tapia-Tussell R, Hernández-Zepeda C. Environmental surveillance of SARS-CoV-2 RNA in wastewater and groundwater in Quintana Roo, Mexico. Food Environ Virol. 2021 Dec;13(4):457-469. doi: 10.1007/s12560-021-09492-y. Epub 2021 Aug 20.
68. Ahmed W, Bertsch PM, Angel N, Bibby K, Bivins A, Dierens L, Edson J, Ehret J, Gyawali P, Hamilton KA, Hosegood I, Hugenholtz P, Jiang G, Kitajima M, Sichani HT, Shi J, Shimko KM, Simpson SL, Smith WJM, Symonds EM, Thomas KV, Verhagen R, Zaugg J, Mueller JF. Detection of SARS-CoV-2 RNA in commercial passenger aircraft and cruise ship wastewater: a surveillance tool for assessing the presence of COVID-19 infected travellers. J Travel Med. 2020 Aug 20;27(5):taaa116. doi: 10.1093/jtm/taaa116.
69. World Health Organization. Public health surveillance for COVID-19: Interim guidance. 22 July 2022. World Health Organization. (https://www.who.int/publications/i/item/WHO-2019-nCoV-SurveillanceGuidance-2022.1, accessed 19 June 2023).
70. Philo SE, Keim EK, Swanstrom R, Ong AQW, Burnor EA, Kossik AL, Harrison JC, Demeke BA, Zhou NA, Beck NK, Shirai JH, Meschke JS. A comparison of SARS-CoV-2 wastewater concentration methods for environmental surveillance. Sci Total Environ. 2021 Mar 15;760:144215. doi: 10.1016/j.scitotenv.2020.144215. Epub 2020 Dec 10.
71. Zhou NA, Tharpe C, Meschke JS, Ferguson CM. Survey of rapid development of environmental surveillance methods for SARS-CoV-2 detection in wastewater. Sci Total Environ. 2021 May 15;769:144852. doi: 10.1016/j.scitotenv.2020.144852. Epub 2021 Jan 14.
72. Sharma DK, Nalavade UP, Kalgutkar K, Gupta N, Deshpande JM. SARS-CoV-2 detection in sewage samples: Standardization of method & preliminary observations. Indian J Med Res. 2021 Jan & Feb;153(1 & 2):159-165. doi: 10.4103/ijmr.IJMR_3541_20.
73. Shah S, Gwee SXW, Ng JQX, Lau N, Koh J, Pang J. Wastewater surveillance to infer COVID-19 transmission: A systematic review. Sci Total Environ. 2022 Jan 15;804:150060. doi: 10.1016/j.scitotenv.2021.150060. Epub 2021 Sep 8.
74. van Boven M, Hetebrij WA, Swart A, Nagelkerke E, van der Beek RF, Stouten S, Hoogeveen RT, Miura F, Kloosterman A, van der Drift AR, Welling A, Lodder WJ, de Roda Husman AM. Patterns of SARS-CoV-2 circulation revealed by a nationwide sewage surveillance programme, the Netherlands, August 2020 to February 2022. Euro Surveill. 2023 Jun;28(25):2200700. doi: 10.2807/1560-7917.ES.2023.28.25.2200700.
75. Claro ICM, Cabral AD, Augusto MR, Duran AFA, Graciosa MCP, Fonseca FLA, Speranca MA, Bueno RF. Long-term monitoring of SARS-CoV-2 RNA in wastewater in Brazil: A more responsive and economical approach. Water Res. 2021 Sep 15;203:117534. doi: 10.1016/j.watres.2021.117534. Epub 2021 Aug 8.
76. Li L, Mazurowski L, Dewan A, Carine M, Haak L, Guarin TC, Dastjerdi NG, Gerrity D, Mentzer C, Pagilla KR. Longitudinal monitoring of SARS-CoV-2 in wastewater using viral genetic markers and the estimation of unconfirmed COVID-19 cases. Sci Total Environ. 2022 Apr 15;817:152958. doi: 10.1016/j.scitotenv.2022.152958. Epub 2022 Jan 10.
77. Kirby AE, Welsh RM, Marsh ZA, Yu AT, Vugia DJ, Boehm AB, Wolfe MK, White BJ, Matzinger SR, Wheeler A, Bankers L, Andresen K, Salatas C; New York City Department of Environmental Protection; Gregory DA, Johnson MC, Trujillo M, Kannoly S, Smyth DS, Dennehy JJ, Sapoval N, Ensor K, Treangen T, Stadler LB, Hopkins L. Notes from the Field: Early Evidence of the SARS-CoV-2 B.1.1.529 (Omicron) Variant in Community Wastewater - United States, November-December 2021. MMWR Morb Mortal Wkly Rep. 2022 Jan 21;71(3):103-105. doi: 10.15585/mmwr.mm7103a5.
78. Fernández-de-Mera IG, Rodríguez Del-Río FJ, de la Fuente J, Pérez-Sancho M, Hervás D, Moreno I, Domínguez M, Domínguez L, Gortázar C. Detection of environmental SARS-CoV-2

RNA in a high prevalence setting in Spain. Transbound Emerg Dis. 2021 May;68(3):1487-1492. doi: 10.1111/tbed.13817. Epub 2020 Sep 24.

79. Barua VB, Juel MAI, Blackwood AD, Clerkin T, Ciesielski M, Sorinolu AJ, Holcomb DA, Young I, Kimble G, Sypolt S, Engel LS, Noble RT, Munir M. Tracking the temporal variation of COVID-19 surges through wastewater-based epidemiology during the peak of the pandemic: A six-month long study in Charlotte, North Carolina. Sci Total Environ. 2022 Mar 25;814:152503. doi: 10.1016/j.scitotenv.2021.152503. Epub 2021 Dec 23.
80. Hemalatha M, Kiran U, Kuncha SK, Kopperi H, Gokulan CG, Mohan SV, Mishra RK. Surveillance of SARS-CoV-2 spread using wastewater-based epidemiology: Comprehensive study. Sci Total Environ. 2021 May 10;768:144704. doi: 10.1016/j.scitotenv.2020.144704. Epub 2021 Jan 6.
81. Randazzo W, Truchado P, Cuevas-Ferrando E, Simón P, Allende A, Sánchez G. SARS-CoV-2 RNA in wastewater anticipated COVID-19 occurrence in a low prevalence area. Water Res. 2020 Aug 15;181:115942. doi: 10.1016/j.watres.2020.115942. Epub 2020 May 16.
82. Galani A, Aalizadeh R, Kostakis M, Markou A, Alygizakis N, Lytras T, Adamopoulos PG, Peccia J, Thompson DC, Kontou A, Karagiannidis A, Lianidou ES, Avgeris M, Paraskevis D, Tsiodras S, Scorilas A, Vasiliou V, Dimopoulos MA, Thomaidis NS. SARS-CoV-2 wastewater surveillance data can predict hospitalizations and ICU admissions. Sci Total Environ. 2022 Jan 15;804:150151. doi: 10.1016/j.scitotenv.2021.150151. Epub 2021 Sep 7.
83. Gable L, Ram N, Ram JL. Legal and ethical implications of wastewater monitoring of SARS-CoV-2 for COVID-19 surveillance. J Law Biosci. 2020 Jun 24;7(1):lsaa039. doi: 10.1093/jlb/lsaa039.
84. Manuel DG, Amadei CA, Campbell JR, Brault JM, Zierler A, Veillard J. Strengthening public health surveillance through wastewater testing: an essential investment for the COVID-19 pandemic and future health threats. Washington, DC: World Bank; 2021.
85. Havelaar AH, Bräunig J, Christiansen K, Cornu M, Hald T, Mangen MJ, Mølbak K, Pielaat A, Snary E, Van Pelt W, Velthuis A, Wahlström H. Towards an integrated approach in supporting microbiological food safety decisions. Zoonoses Public Health. 2007;54(3-4):103-17. doi: 10.1111/j.1863-2378.2007.01036.x.
86. Asghar H, Diop OM, Weldegebriel G, Malik F, Shetty S, El Bassioni L, Akande AO, Al Maamoun E, Zaidi S, Adeniji AJ, Burns CC, Deshpande J, Oberste MS, Lowther SA. Environmental surveillance for polioviruses in the Global Polio Eradication Initiative. J Infect Dis. 2014 Nov 1;210 Suppl 1:S294-303. doi: 10.1093/infdis/jiu384.
87. Black J, Aung P, Nolan M, Roney E, Poon R, Hennessy D, Crosbie ND, Deere D, Jex AR, John N, Baker L, Scales PJ, Usher SP, McCarthy DT, Schang C, Schmidt J, Myers S, Begue N, Kaucner C, Thorley B, Druce J, Monis P, Lau M, Sarkis S. Epidemiological evaluation of sewage surveillance as a tool to detect the presence of COVID-19 cases in a low case load setting. Sci Total Environ. 2021 Sep 10;786:147469. doi: 10.1016/j.scitotenv.2021.147469. Epub 2021 May 1.
88. Griffiths KF, Emslie KF, Burke DG, Baoutina A, Bhat S, Forbes-Smith M, Hall F, Lynch D, McLaughlin J, Partis L, Pinheiro LB, Deere D, Collaboration on Sewage Surveillance of SARS-CoV-2, Inter-laboratory Study: SARS-CoV-2 in Wastewater, Water Research Australia Project 2071 Final Report, ISBN 978-1-921732-01-0, 2021 (https://members.waterra.au/Members/Projects/Project-Documents/2071.aspx?PO=2071&WebsiteKey=a0060ed1-e5f1-45d0-abaa-2a1b7ce2a6e6, accessed 19 June 2023).
89. Pecson B, Fevig S, Interlaboratory and Methods Assessment of the SARS-CoV-2 Genetic Signal in Wastewater, Water Research Foundation Project 5089, (https://www.waterrf.org/research/projects/interlaboratory-and-methods-assessment-sars-cov-2-genetic-signal-wastewater, accessed 19 June 2023).
90. ISO/AWI 7014, Water quality: detection and quantification of SARS-CoV-2 in wastewater. Geneva: International Organization for Standardization; under development.

91. European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), Wastewater-based epidemiology and drugs topic page, (https://www.emcdda.europa.eu/topics/wastewater_en, accessed 28 May 2023).
92. Australian Criminal Intelligence Commission, National Wastewater Drug Monitoring Program reports, (https://www.acic.gov.au/publications/national-wastewater-drug-monitoring-program-reports, accessed 28 May 2023).
93. Mohapatra S, Bhatia S, Senaratna KYK, Jong MC, Lim CMB, Gangesh GR, Lee JX, Giek GS, Cheung C, Yutao L, Luhua Y, Yong NH, Peng LC, Wong JCC, Ching NL, Gin KY. Wastewater surveillance of SARS-CoV-2 and chemical markers in campus dormitories in an evolving COVID - 19 pandemic. J Hazard Mater. 2023 Mar 15;446:130690. doi: 10.1016/j.jhazmat.2022.130690. Epub 2022 Dec 28.
94. McMahan CS, Self S, Rennert L, Kalbaugh C, Kriebel D, Graves D, Colby C, Deaver JA, Popat SC, Karanfil T, Freedman DL. COVID-19 wastewater epidemiology: a model to estimate infected populations. Lancet Planet Health. 2021 Dec;5(12):e874-e881. doi: 10.1016/S2542-5196(21)00230-8.
95. Crank K, Chen W, Bivins A, Lowry S, Bibby K. Contribution of SARS-CoV-2 RNA shedding routes to RNA loads in wastewater. Sci Total Environ. 2022 Feb 1;806(Pt 2):150376. doi: 10.1016/j.scitotenv.2021.150376. Epub 2021 Sep 16.
96. Emory University researchers awarded NIH grant to conduct COVID-19 surveillance through detection of SARS Coronavirus-2 in wastewater [media release]. Emory University, 24 June 2021 (https://news.emory.edu//stories/2021/06/radx_wastewater_testing_atl/index.html, accessed 19 June 2023).
97. Schang C, Crosbie ND, Nolan M, Poon R, Wang M, Jex A, John N, Baker L, Scales P, Schmidt J, Thorley BR, Hill K, Zamyadi A, Tseng CW, Henry R, Kolotelo P, Langeveld J, Schilperoort R, Shi B, Einsiedel S, Thomas M, Black J, Wilson S, McCarthy DT. Passive Sampling of SARS-CoV-2 for Wastewater Surveillance. Environ Sci Technol. 2021 Aug 3;55(15):10432-10441. doi: 10.1021/acs.est.1c01530. Epub 2021 Jul 15.
98. Cha G, Graham KE, Zhu KJ, Rao G, Lindner BG, Kocaman K, Woo S, D'amico I, Bingham LR, Fischer JM, Flores CI, Spencer JW, Yathiraj P, Chung H, Biliya S, Djeddar N, Burton LJ, Mascuch SJ, Brown J, Bryksin A, Pinto A, Hatt JK, Konstantinidis KT. Parallel deployment of passive and composite samplers for surveillance and variant profiling of SARS-CoV-2 in sewage. Sci Total Environ. 2023 Mar 25;866:161101. Doi: 10.1016/j.scitotenv.2022.161101. Epub 2022 Dec 26.
99. Acer PT, Kelly LM, Lover AA, Butler CS. Quantifying the Relationship between SARS-CoV-2 Wastewater Concentrations and Building-Level COVID-19 Prevalence at an Isolation Residence: A Passive Sampling Approach. Int J Environ Res Public Health. 2022 Sep 7;19(18):11245. doi: 10.3390/ijerph191811245.
100. Liu P, Ibaraki M, VanTassell J, Geith K, Cavallo M, Kann R, Guo L, Moe CL. A sensitive, simple, and low-cost method for COVID-19 wastewater surveillance at an institutional level. Sci Total Environ. 2022 Feb 10;807(Pt 3):151047. doi: 10.1016/j.scitotenv.2021.151047. Epub 2021 Oct 18.
101. Pandey D, Verma S, Verma P, Mahanty B, Dutta K, Daverey A, Arunachalam K. SARS-CoV-2 in wastewater: Challenges for developing countries. Int J Hyg Environ Health. 2021 Jan;231:113634. doi: 10.1016/j.ijheh.2020.113634. Epub 2020 Oct 7.
102. Best practices for collection and storage of wastewater samples to support wastewater surveillance of the COVID-19 signal in sewersheds. Denver: Water Research Foundation; 2020 (www.waterrf.org/sites/default/files/file/2020-06/COVID-19_FieldSampleCollectionForm.pdf, accessed 19 June 2023).
103. SAMRC COVID-19 Prevention Research Programme: wastewater surveillance for SARS-CoV-2 – wastewater sampling guide. Cape Town: South African Medical Research Council; 2021 (www.samrc.ac.za/wbe/SARS-CoV-2WastewaterCollectionManual.pdf, accessed 19 June 2023).

104. Gawlik B, Tavazzi S, Mariani G, Skejo H, Sponar M, Higgins T, Medema G, Wintgens T, SARS-CoV-2 Surveillance employing Sewage - Towards a Sentinel System, EUR 30684 EN, Publications Office of the European Union, Luxembourg, 2021, ISBN 978-92-76-36887-8, doi:10.2760/909651, JRC125065.
105. National Wastewater Surveillance System (NWSS). Atlanta, Georgia: United States Centers for Disease Control and Prevention, [website] (www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html, accessed 19 June 2023).
106. Liu P, Guo L, Cavallo M, Cantrell C, Hilton SP, Nguyen A, Long A, Dunbar J, Barbero R, Barclay R, Sablon O 3rd, Wolfe M, Lepene B, Moe C. Comparison of Nanotrap® Microbiome A Particles, membrane filtration, and skim milk workflows for SARS-CoV-2 concentration in wastewater. Front Microbiol. 2023 Jul 5;14:1215311. doi: 10.3389/fmicb.2023.1215311.
107. High level guidance document on sewage surveillance of SARS-CoV-2 in wastewater. Adelaide: Global Water Research Coalition; in preparation.
108. Wang Y, Moe CL, Dutta S, Wadhwa A, Kanungo S, Mairinger W, Zhao Y, Jiang Y, Teunis PF. Designing a typhoid environmental surveillance study: A simulation model for optimum sampling site allocation. Epidemics. 2020 Jun;31:100391. doi: 10.1016/j.epidem.2020.100391. Epub 2020 Apr 17.
109. Wang Y, Liu P, VanTassell J, Hilton SP, Guo L, Sablon O, Wolfe M, Freeman L, Rose W, Holt C, Browning M, Bryan M, Waller L, Teunis PFM, Moe CL. When case reporting becomes untenable: Can sewer networks tell us where COVID-19 transmission occurs? Water Res. 2023 Feb 1;229:119516. doi: 10.1016/j.watres.2022.119516. Epub 2022 Dec 20.
110. Robinson CA, Hsieh HY, Hsu SY, Wang Y, Salcedo BT, Belenchia A, Klutts J, Zemmer S, Reynolds M, Semkiw E, Foley T, Wan X, Wieberg CG, Wenzel J, Lin CH, Johnson MC. Defining biological and biophysical properties of SARS-CoV-2 genetic material in wastewater. Sci Total Environ. 2022 Feb 10;807(Pt 1):150786. doi: 10.1016/j.scitotenv.2021.150786. Epub 2021 Oct 5.
111. Priyadarshini S. India's sewage surveillance for SARS-CoV-2 going down the drain. Nature India. 21 May 2021 (https://www.nature.com/articles/nindia.2021.75, accessed 19 June 2023).
112. Pocock G, Coetzee L, Mans J, Genthe B, Pillay S. Development of a framework for water quality-based COVID-19 epidemiology surveillance framework for non-sewered communities. Research Report No. 3062/1/22, ISBN 9978-0-6392-0370-6. Water Research Commission, Pretoria, South Africa, March 2023.
113. Dhaka COVID tracker: environmental surveillance for SARS-CoV-2 [online dashboard] (https://dhakacovidtracker.research.virginia.edu/, accessed 19 June 2023).
114. World Bank, International Labour Organization, WaterAid, World Health Organization. Health, safety and dignity of sanitation workers: an initial assessment. Washington, DC: World Bank; 2019.
115. Wastewater surveillance for SARS-CoV-2: wastewater sampling guide. Cape Town: South African Medical Research Council – SAMRC COVID-19 Prevention Research Programme; 2021.
116. Kim S, Kennedy LC, Wolfe MK, Criddle CS, Duong DH, Topol A, White BJ, Kantor RS, Nelson KL, Steele JA, Langlois K, Griffith JF, Zimmer-Faust AG, McLellan SL, Schussman MK, Ammerman M, Wigginton KR, Bakker KM, Boehm AB. SARS-CoV-2 RNA is enriched by orders of magnitude in primary settled solids relative to liquid wastewater at publicly owned treatment works. Environ Sci (Camb). 2022 Feb 15;8(4):757-770. doi: 10.1039/d1ew00826a.
117. La Rosa G, Brandtner D, Mancini P, Veneri C, Bonanno Ferraro G, Bonadonna L, Lucentini L, Suffredini E. Key SARS-CoV-2 mutations of alpha, gamma, and eta variants detected in urban wastewaters in Italy by long-read amplicon sequencing based on nanopore technology. Water. 2021;13(18):2503. doi:10.3390/w13182503.
118. Islam G, Gedge A, Lara-Jacobo L, Kirkwood A, Simmons D, Desaulniers JP. Pasteurization, storage conditions and viral concentration methods influence RT-qPCR detection of SARS-CoV-

2 RNA in wastewater. Sci Total Environ. 2022 May 15;821:153228. doi: 10.1016/j.scitotenv.2022.153228. Epub 2022 Jan 25.
119. Pecson BM, Darby E, Haas CN, Amha YM, Bartolo M, Danielson R, Dearborn Y, Di Giovanni G, Ferguson C, Fevig S, Gaddis E, Gray D, Lukasik G, Mull B, Olivas L, Olivieri A, Qu Y; SARS-CoV-2 Interlaboratory Consortium. Reproducibility and sensitivity of 36 methods to quantify the SARS-CoV-2 genetic signal in raw wastewater: findings from an interlaboratory methods evaluation in the U.S. Environ Sci (Camb). 2021 Jan 20;7:504-520. doi: 10.1039/d0ew00946f.106
120. Cutrupi F, Cadonna M, Manara S, Foladori P. Surveillance of SARS-CoV-2 in extensive monitoring of municipal wastewater: key issues to yield reliable results. Water Sci Technol. 2021 Dec;84(12):3508-3514. doi: 10.2166/wst.2021.469.
121. Philo SE, Keim EK, Swanstrom R, Ong AQW, Burnor EA, Kossik AL, Harrison JC, Demeke BA, Zhou NA, Beck NK, Shirai JH, Meschke JS. A comparison of SARS-CoV-2 wastewater concentration methods for environmental surveillance. Sci Total Environ. 2021 Mar 15;760:144215. doi: 10.1016/j.scitotenv.2020.144215. Epub 2020 Dec 10.
122. Itarte M, Bofill-Mas S, Martínez-Puchol S, Torrell H, Ceretó A, Carrasco M, Forés E, Canela N, Girones R, Rusiñol M. Looking for a needle in a haystack. SARS-CoV-2 variant characterization in sewage. Curr Opin Environ Sci Health. 2021 Dec;24:100308. doi: 10.1016/j.coesh.2021.100308. Epub 2021 Nov 6.
123. Martin J, Klapsa D, Wilton T, Zambon M, Bentley E, Bujaki E, Fritzsche M, Mate R, Majumdar M. Tracking SARS-CoV-2 in Sewage: Evidence of Changes in Virus Variant Predominance during COVID-19 Pandemic. Viruses. 2020 Oct 9;12(10):1144. doi: 10.3390/v12101144.
124. Wang Y, Liu P, VanTassell J, Hilton SP, Guo L, Sablon O, Wolfe M, Freeman L, Rose W, Holt C, Browning M, Bryan M, Waller L, Teunis PFM, Moe CL. When case reporting becomes untenable: Can sewer networks tell us where COVID-19 transmission occurs? Water Res. 2023 Feb 1;229:119516. doi: 10.1016/j.watres.2022.119516. Epub 2022 Dec 20.
125. Nationwide Tracking of Covid-19 Spread in Türkiye by SARS CoV-2 Wastewater Surveillance Dashboard: (https://covid19.tarimorman.gov.tr/, accessed 21 July 2023).
126. Alhama J, Maestre JP, Martín MÁ, Michán C. Monitoring COVID-19 through SARS-CoV-2 quantification in wastewater: progress, challenges and prospects. Microb Biotechnol. 2022 Jun;15(6):1719-1728. doi: 10.1111/1751-7915.13989. Epub 2021 Dec 14.
127. Hart OE, Halden RU. Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges. Sci Total Environ. 2020 Aug 15;730:138875. doi: 10.1016/j.scitotenv.2020.138875. Epub 2020 Apr 22.
128. Farkas K, Pellett C, Williams R, Alex-Sanders N, Bassano I, Brown MR, Denise H, Grimsley JMS, Kevill JL, Khalifa MS, Pântea I, Story R, Wade MJ, Woodhall N, Jones DL. Rapid Assessment of SARS-CoV-2 Variant-Associated Mutations in Wastewater Using Real-Time RT-PCR. Microbiol Spectr. 2023 Feb 14;11(1):e0317722. doi: 10.1128/spectrum.03177-22. Epub 2023 Jan 11.
129. Smyth DS, Trujillo M, Gregory DA, Cheung K, Gao A, Graham M, Guan Y, Guldenpfennig C, Hoxie I, Kannoly S, Kubota N, Lyddon TD, Markman M, Rushford C, San KM, Sompanya G, Spagnolo F, Suarez R, Teixeiro E, Daniels M, Johnson MC, Dennehy JJ. Tracking cryptic SARS-CoV-2 lineages detected in NYC wastewater. Nat Commun. 2022 Feb 3;13(1):635. doi: 10.1038/s41467-022-28246-3. Erratum in: Nat Commun. 2022 Mar 30;13(1):1836.
130. Statement from the Advisory Group on SARS-CoV-2 Evolution in Animals. Paris: World Organisation for Animal Health (OIE); 2022 (www.oie.int/app/uploads/2022/01/statement-agve-omicron.pdf, accessed 19 June 2023).
131. Commission recommendation (EU) 2021/472 of 17 March 2021 on a common approach to establish a systematic surveillance of SARS-CoV-2 and its variants in wastewaters in the EU. European Commission; 2021 (Official Journal of the European Union, L 98/3).

132. Communication from the Commission to the European Parliament, the European Council, the Council, the European Economic and Social Committee and the Committee of the Regions. Introducing HERA, the European Health Emergency preparedness and Response Authority, the next step towards completing the European Health Union. European Commission; 2021 (COM/2021/576 final).
133. About Us. Adelaide: Global Water Research Coalition; 2023 (http://www.globalwaterresearchcoalition.net/about-us/, accessed 28 July 2023).

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