Environmental surveillance for SARS-COV-2 to complement public health surveillance

Interim Guidance

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World Health Organization

Contents

1. Introduction

Management of the COVID-19 pandemic continues to prove challenging in the face of an evolving virus, and uncertainties in designing proportionate and evidence-based public health interventions. The primary source of evidence about the incidence of SARS-CoV-2 infection is PCR and rapid antigen diagnostic testing of upper respiratory tract samples.

In an increasing number of settings globally, routine COVID-19 surveillance programmes have augmented diagnostic testing with community-scale COVID-19 environmental surveillance (ES) of SARS-CoV-2 in wastewater samples. Similarly, ES have been done for other diseases and risks such as for polio *(1),* typhoid *(2)(3)* and antimicrobial resistance (AMR) *(4, 5, 6).*

The objective of ES is to provide early warning and additional evidence regarding the virus in circulation in the population, including its presence or absence, trends in concentrations, and variants of concern or interest. ES can help to inform decisions on, and help measure the effect of, interventions *(7)*.

Purpose

The purpose of this guidance is to provide globally applicable advice on the following questions:

- Why, or in what situations, does ES add value to public health decision making at different stages of the pandemic, and in different settings and contexts? (section 3)
- What are the minimum requirements for planning and coordinating an effective SARS-COV-2 ES programme in different resource settings? (section 4)
- How should data collection, analysis and interpretation and communication of results be carried out? (section 5)

Target audience

This guidance is targeted at public health officials and COVID-19 incident management team members who want to understand and integrate complementary ES, into their national, sub-national or local COVID-19 control strategy. 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 professionals make informed, evidence-based decisions on the value of ES for their context to help decide whether to implement such a programme;
- show how entities would set up a successful ES programme;
- support 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 utilisation of SARS-COV-2 ES results along with other COVID-19 surveillance modalities in means of public health decision making; and
- support sharing of lessons and case studies from implementation experiences for more efficient application of ES globally.

Scope

Air, surface and water matrices have been subjected to SARS-CoV-2 testing. However, only the testing of wastewater has been of value in assessing the levels of SARS-COV-2 circulating at the population scale.

This document discusses SARS-COV-2 ES of wastewater containing SARS-CoV-2 (RNA) shed in excreta and upper respiratory system secreta from symptomatic and asymptomatic COVID-19 cases in populations living, working or visiting in a defined catchment area. It describes use cases, planning and coordination and emerging best practice methods for data collection analysis and interpretation. This document does not provide specific recommendations on uses or standards methods for ES since approaches and details of the methods being used are evolving rapidly. However, there is sufficient experience to describe features and good practice in a range of contexts.

ES programmes normally draw wastewater samples from sewer systems at the inlet of wastewater treatment plants in setting with high coverage of sewers to gain a representative sample of people living in the catchment. This document also discusses SARS-COV-2 ES in areas that have limited sewer network coverage where this emerging and important work is being applied to environmental water (e.g., surface water or stormwater in open drains influenced by human excreta) *(8, 9)*.

Background

This interim guidance updates the World Health Organization (WHO) scientific brief *Status of environmental surveillance for SARS-CoV-2 virus: scientific brief, 5 August 2020*.

At the time of publication of the scientific brief, many countries including Italy *(10)*, Japan *(11)*, China *(12)*, India *(13)*, the United States of America *(14)*, and countries in Latin America and the Caribbean *(15),* had published or demonstrated proof of concept of ES for SARS-COV-2 by detecting SARS-CoV-2 in environmental samples. Since then, numerous SARS-COV-2 ES programmes have been established and become a routine component of national COVID-19 surveillance programmes *(16–21)*. SARS-COV-2 ES programmes began with SARS-CoV-2 detection, moved to increasingly reliable quantification, and some now include testing for targeted known variants *(22)* and finding novel variants *(23)*. Some countries (e.g., the Netherlands *(24)*, Hungary and the United Kingdom) have moved to some form of national SARS-COV-2 ES system and others are coordinating and consolidating data at a national level, and working with regional or state governments. Governance arrangements are diverse, and all involve complex multiple stakeholder arrangements.

Data and evidence available on implications of SARS-COV-2 ES have greatly expanded in amount and quality enabling new interim guidance. 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 *(25, 26)* public health and COVID-19 incident management websites, global data-sharing platforms *(27, 28)* and media communications. Collectively, they have demonstrated a variety of applications including challenges, costs and limitations (Section 3 and Annex 1), and lessons have been learned to optimize planning, coordination and capacity for a credible programme (Section 4). Techniques for sampling *(5, 6, 29, 30, 31, 32, 33, 34, 35)* and analytical methods have been validated and routinely used for detection and quantification of SARS-CoV-2 and, in some cases, its variants (see section 5). Innovations being trialled or at proof-of-concept stage have been expanded, and formal research agendas have been prepared (see section 6).

2. Environmental surveillance in the broader public health surveillance context

A growing body of experience and specific added value of SARS-COV-2 ES can justify inclusion of this surveillance method into routine COVID-19 surveillance. ES is used to complement rather than replace public health surveillance based on compilation of individual diagnostic testing results (Figs. 1 - 3). Therefore, this document should be read in conjunction with the WHO interim guidance on public health surveillance for COVID-19 *(36)* which describes the range of COVID-19 surveillance methods.

There are useful similarities and differences between ES and diagnostic testing methods and approaches for those familiar with diagnostic testing.

Within the laboratory, the molecular detection methods used for SARS-COV-2 ES are comparable, and in some cases identical, to those used for diagnostic testing. That is, the same RT-PCR test kits are often used for the final testing component. What is different about SARS-COV-2 ES in comparison to diagnostic testing programmes, is the design and interpretation of the community-scale sampling programmes, as well as concentration and extraction of the RNA from the wastewater and environmental water samples *(37–39)*. An understanding of the wastewater catchment and the communities represented by the sample points as compared with health reporting regions and local municipalities is required to design and interpret 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 biases inherent in diagnostic testing, which include health seeking behaviour, disease severity, health care and test accessibility, 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 diagnostic testing practices and capacity, and so far, provides an objective indicator of virus circulation in the population.

SARS-COV-2 ES has potential to play an important role in the overall surveillance picture by providing an additional line of evidence to inform pandemic and endemic disease surveillance to support management programmes and other public health and social measures *(40)*. Presently, SARS-COV-2 ES is a tool to observe trends and change in viral circulation at a population level, rather than to make firm conclusions about the incidence and prevalence of COVID-19 cases in the community, however correlation with hospitalizations has been show in several settings.

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 *(41)*. Viral RNA can be shed into wastewater before the onset of symptoms and before diagnostic testing. Therefore, results can inform public health agencies before diagnostic test results are reported. As such ES can provide earlier and more representative warning of trends *(42)* in COVID-19 incidence and the emergence of variants *(43, 44)* than diagnostic testing – albeit over time this may change for different variants. 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 *(45, 46, 47*), whilst in lower prevalence contexts or in the absence of evidence of clinical testing ES provides an early warning of SARS-CoV-2 emergence *(48, 49)*. The role of ES and the early

warning of (re)emergence is expected to become more relevant now interest in clinical testing is waning.

Viral loads in sewage can be used to monitor the impact of public health social measures including increasing or relaxing restrictions. Results from SARS-COV-2 ES can be used to augment risk communication warn communities about virus (re)emergence and to inform community behaviour with respect to testing, quarantine, isolation, vaccination, and healthcare seeking behaviours.

When diagnostic testing capacities are overwhelmed during periods of elevated prevalence, or willingness to test is low in certain times or areas, ES methods can provide a more cost-effective and reliable means to track trends and test for variants. Likewise, during low prevalence or no known case situations, ES methods can be cost-effective for early warning. As diagnostic testing becomes more targeted to specific sites and situations, ES can provide a means of cost-effectively monitoring population-level trends and emergence.

SARS-COV-2 ES also have 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 tens of thousands of people, and if carried out ethically can be a non-intrusive approach that doesn't target individuals (50). Disadvantages of SARS-COV-2 ES as compared with other surveillance approaches are the lack of individual sampling and test results, and thus the ability to link to clinical care, particularly during periods of limited shedding and few cases when method sensitivity becomes limiting *(51)*.

ES for other diseases

WHO has produced ES guidance for other diseases, including polio *(1)* and typhoid *(52, 3),* and AMR *(5, 6)*, some of which dates back more than 70 years. Many of the standard methods, approaches and global reporting processes for Polio ES are applicable or adaptable to SARS-CoV-2. Some countries, such as South Africa, have already built on that experience and created comprehensive SARS-COV-2 ES programmes for the presence and concentrations of SARS-CoV-2 *(53, 54)* and in some cases its variants *(43, 44)*. However, there are two important differences.

- The main use cases of ES for polio are early detection of an outbreak and confirmation of the absence of circulation of wild-type and vaccine-derived poliovirus in a population *(55)*. Therefore, ES for polio has not depended on quantitative data to look at trends in prevalence. Presence/absence use cases were relevant in the early stages of the COVID-19 pandemic, but are less relevant in the situation of global spread and high incidence.
- Standard methods from selection of sites to sewage concentration and poliovirus genetic characterization, are available for Polio ES but as yet, there is not enough experience with ES for SARS-COV-2 to specify equivalent standard methods since the approaches and details of the methods being used are evolving rapidly. At this stage standardising methods between different laboratories and sites is less important than having consistent methods and quality at any one site. Some studies have begun to address questions such as sample representativeness, quantifying sensitivity, specificity, other performance characteristics of the methods and cost *(51, 56)* and there is also an ISO initiative to address them *(57)*.

Learning from existing ES programmes has the potential to inform public health surveillance for other diseases and risks such as chemicals of emerging concern, antimicrobial resistance, illicit drugs, or understanding of populations and their movements and behaviours.

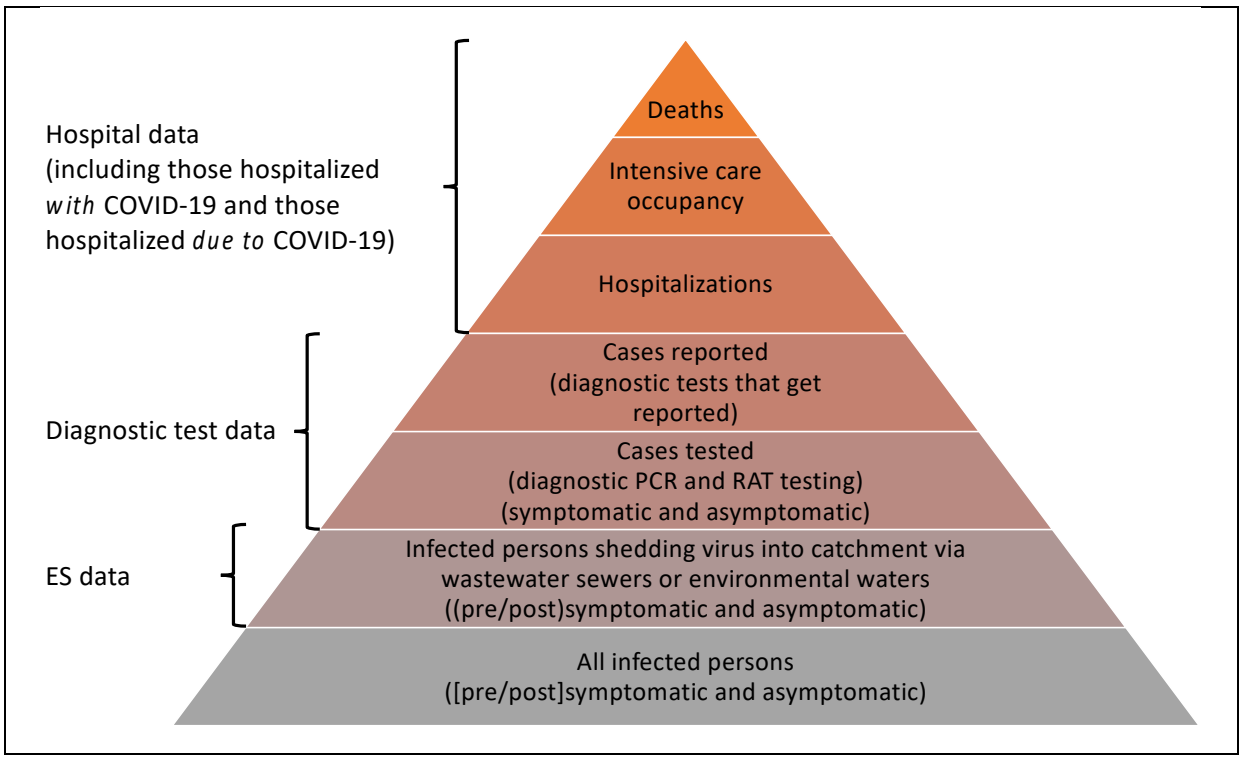


Fig. 1. Illustration of the role of SARS-COV-2 ES as a source of data on COVID-19 and SARS-CoV-2 shedding in communities via a defined wastewater catchment.

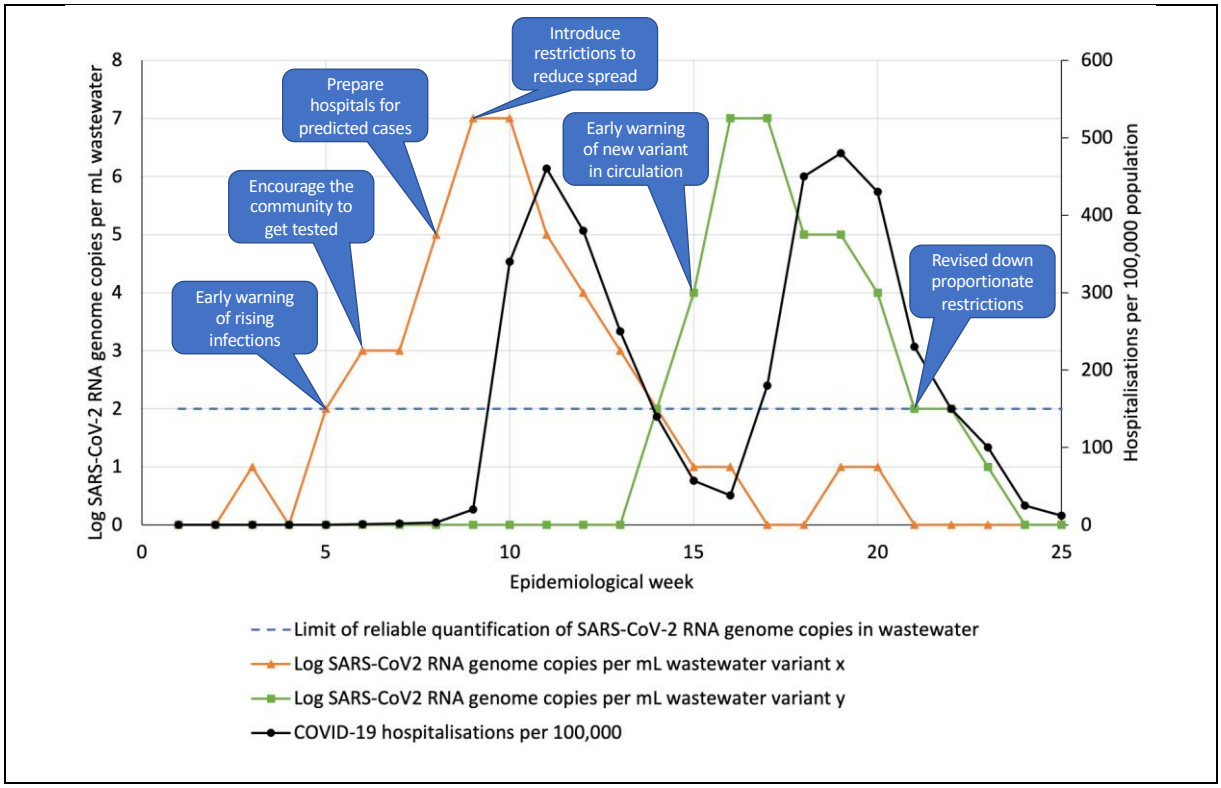
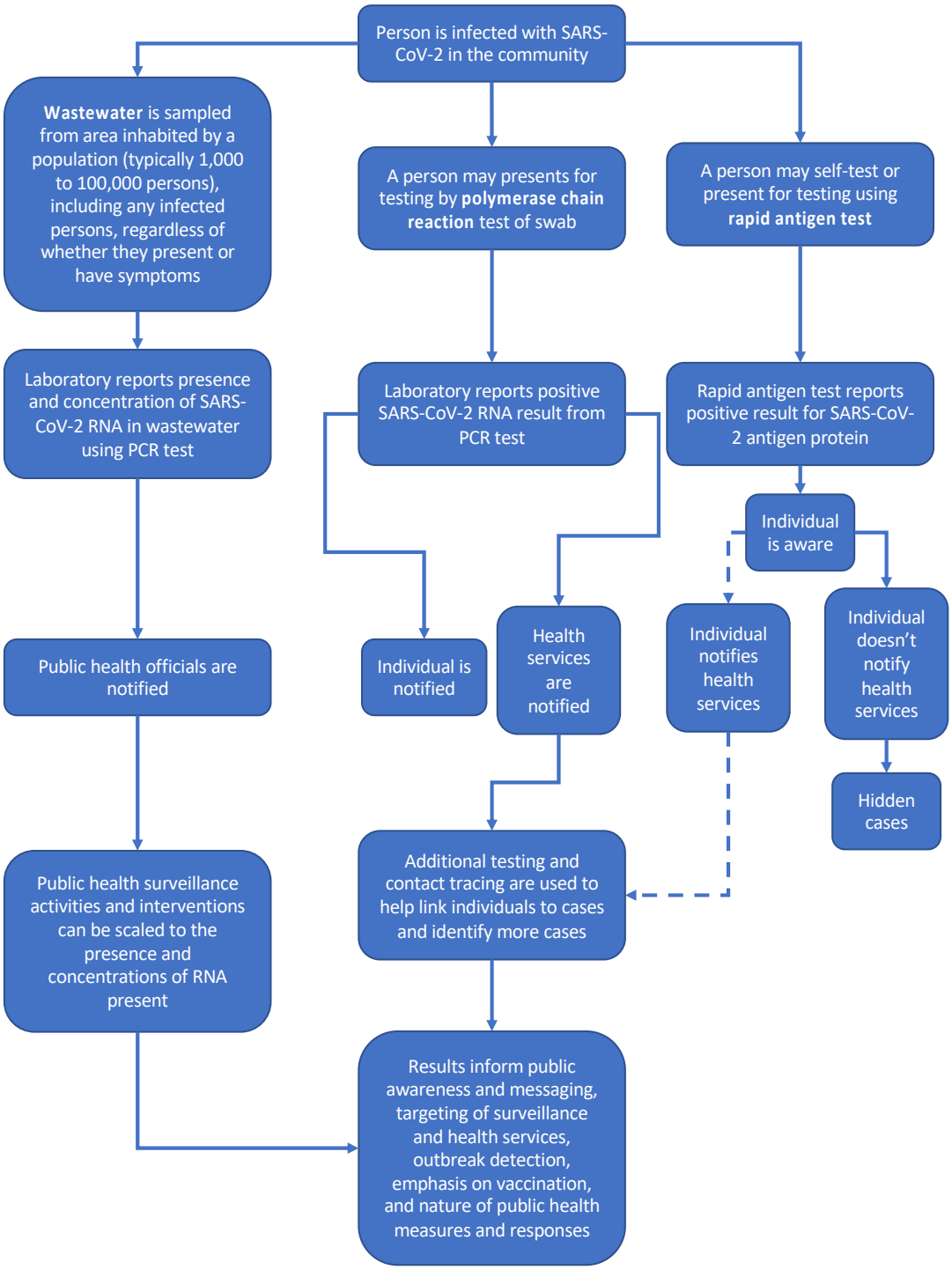


Fig. 2. Illustration ES data compared to hospitalization data and potential use cases for public communication, public health decision-making and targeting restrictions.



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 diagnostic tests and approximately 0.5 to 2 days for both diagnostic 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 that shed the virus but that might not have presented for diagnostic 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 rapid antigen testing, nasopharyngeal testing and wastewater testing from the perspective of a public health agency.

3. Applications of environmental surveillance for COVID-19

Public health leadership

Leadership by the agencies responsible for public health, and with overall responsibility for COVID-19 management 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 operators and managers, and laboratories.

However, the health sector is the end user of the information and therefore needs to take the lead in designing surveillance programmes, merging and linking the SARS-COV-2 ES data with other surveillance platforms, and coordinating interpretation and communication of the findings. 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 fund the SARS-COV-2 ES program since it is not a water and sanitation sector function – it is about accessing the information encoded in wastewater to provide an unbiased indicator of COVID-19 incidence.

Uses of SARS-COV-2 ES to support public health surveillance

Before initiating a SARS-COV-2 ES programme, it is important to consider how SARS-COV-2 ES is anticipated to add value to health sector decision-making for the COVID-19 response (Table 1).

All ES applications provide a population-level indicator for COVID-19, covering relatively large populations for each sample collected (Figs. 3). SARS-COV-2 ES data is independent of healthcare-seeking behaviours and access to and use of clinical testing. The benefits of SARS-COV-2 ES vary according to factors such as phase of the pandemic, the method used to collect wastewater 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 limits of detection of the testing methods used at the level of the community. This is particularly relevant in no or low prevalence settings, to confirm absence of virus circulation or warn about (re)emergence of the virus (like for the polio ES).
- Most programmes in high prevalence settings involve quantification of results to identify increasing or decreasing trends, or plateaus, in community COVID-19. SARS-CoV-2 concentrations in wastewater do not accurately translate the number of COVID-19 infections in the community *(58)* due to three confounders that prevent precise correlation of numbers of excretors who contributed to the viral load in wastewater: variability in rates and patterns of virus shedding; concentration of human excreta in wastewater given water use patterns (e.g., flush volumes and variability in greywater and blackwater separation; and fluctuation in flow rates in wastewater systems (e.g. due to rainfall or industrial and commercial discharges). Studies examining the relative contributions from faeces, sputum, urine and saliva to the wastewater signal illustrate some of these complexities *(59)*. However, these

confounders are accounted for to some extent in some programmes using normalization methods (see section 5).
- In the most advanced cases, SARS-COV-2 ES programmes monitor variants, including both known new variants of interest or concern, and in some cases searching for new and emerging variants.

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

- More frequent sampling with more rapid turnaround of results provides better early warning.
- Finer spatial sampling scales (smaller wastewater catchments) allows better targeting of mitigation responses to those areas.
- Safeguarding high risk settings such as age-care facilities, dormitories, and prisons.

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

- ES programmes are technically relatively simple in areas with a high proportion of the population connected to sewers, allowing sampling points to capture most of the population resident in the sewered area. Most programmes cover such applications.
- ES programmes are more challenging in areas with high proportions of individual on-site sanitation systems (i.e., septic tanks and pit toilets). However, successful applications have been developed using samples from open drains following lessons from ES for polio eradication*, (8,9)*, or septic tanks of public toilets *(60)*, and making use of passive samplers *(61)*.

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

- Information from SARS-COV-2 ES will help to fill information gaps in situations of limited or inconsistent levels of diagnostic testing.
- SARS-COV-2 ES can play a valuable role in remote areas, where access to diagnostic testing is limited, particularly if methods for areas not connected to sewerage systems can be implemented and integrated with the broader public health surveillance system.
- ES is particularly valuable during periods of low or high community COVID-19 prevalence. During periods of high prevalence diagnostic testing resources can become overwhelmed or persons may see little value in getting tested. During periods of low prevalence diagnostic testing will mostly return negative results making it relatively expensive compared to ES.

A summary of example use cases for SARS-COV-2 ES that have been demonstrated successfully and consistently in multiple contexts is provided in Table 1. The use cases are supported by short case studies in Annex 1.

Note that most of the case studies illustrate multiple use cases because 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 serve a single purpose.

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 (Legend: +++ = primary benefit, ++ = secondary benefit, + = adjunct benefit)							Setting or level where application has greatest benefit, and comments on benefits	Case study (Annex 1)
		Provides early warning	Encourages diagnostic testing	Informs decisions on control interventions	Encourages compliance with control interventions	Informs decisions on hospital care capacity	Informs decisions on targeted clinical testing	Improves vaccine uptake		
Tracking increasing and decreasing trends at community level to help ***target COVID-19 responses and interventions***	Observing increasing and decreasing trends at community level to, once confirmed, provide an early indication (4–7 days) of changes in incidence and levels of virus circulation assists 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 diagnostic testing or failing reporting system or increase in self-testing Larger population sizes	1
Finding outbreaks in places thought to be COVID-19-free	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 COVID-19 is thought to have been eliminated or locations where COVID-19 cases have not been identified	6
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 diagnostic testing, and reduces complacency about control interventions (e.g. masking, distancing, vaccination).	+	+++	+	++			+ +	Low to moderate prevalence	2, 3, 6
Cost-effective ***targeting of public health surveillance*** (diagnostic testing resources)	Allows deployment of scarce diagnostic testing resources in hotspot areas with higher SARS-COV-2 ES signals.	+	++	++			+++		Spatially differentiated, low to moderate prevalence Larger population sizes	3
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	4

Use case	Description	Benefits for COVID-19 response strategy (Legend: +++ = primary benefit, ++ = secondary benefit, + = adjunct benefit)							Setting or level where application has greatest benefit, and comments on benefits	Case study (Annex 1)
		Provides early warning	Encourages diagnostic testing	Informs decisions on control interventions	Encourages compliance with control interventions	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 COVID-19 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 bubbles or groups to be contained. Augment data in areas with low uptake of diagnostic testing.	4, 7, 8
- transport vessels	- sullage tanks of ships and aircraft arriving at borders								Permit transport vessels to be tested before disembarkation	
- 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	5
Detecting emergence of ***novel variants*** (albeit challenging in sewage samples)	Involves whole-genome sequencing to identify novel variants circulating in the environment.	+++							Moderate to high prevalence	1
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	-

4. 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, analyse and interpretation of the data, and using the data to inform decision-making and risk communication *(62)*. 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.
- 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 and how they relate to residential locations of populations and to public health districts.
- Laboratories that do the testing, report the results, and undertake quality management, and that need expertise in handling wastewater samples and molecular biology.
- Information technology and communications personnel that undertake spatial mapping and data interpretation, prepare reports and maintain dashboards on behalf of all parties.

A successful programme requires health sector leadership and multisector coordination. Dedicated, specialized resources need to be committed to meet the organizational, technical, and financial requirements to implement a 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 program, the costs could be hundreds of thousands of US dollars per year for a smaller jurisdiction (e.g., a city) and millions of dollars per year for a larger jurisdiction (e.g., a region). However, the benefits will outweigh the costs from savings made by reducing costs for other forms of public health surveillance and in 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 programmes (e.g., for polio, which is using a large network of ES sampling sites, but also to a lesser extent typhoid, AMR and routine wastewater testing).

Leadership and coordination should be clear and would ideally be provided by the public health surveillance agency. The objective of the SARS-COV-2 ES programme is to inform decision-making processes for COVID-19 monitoring and management as part of the broader COVID-19 response strategy. This requires linking the SARS-COV-2 ES programme with the broader public health COVID-19 response. 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, permit 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 lack sanitation services.

Box 1 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 1. 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 and regional levels. Assess which actors are already engaged. Understand the receptivity and interest of the necessary actors to participate. They include the primacy public health agency, the COVID-19 incident management and control agency, the wastewater management agency, and actors undertaking wastewater sampling, processing of samples and molecular genetic testing. Ideally, normative bodies that provide laboratory standards, review and accreditation as part of quality assurance.
- ☐ **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 incident 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 levels, providing early warning of the emergence of COVID-19 cases, indications of changes in COVID-19 incidence and incursion and spread of variants. 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 incident 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 diagnostic testing and hospitalization surveillance to the extent possible. Set up data dictionaries, data management systems and reporting systems and dashboards for coordination and data sharing.
- ☐ **Identify opportunities to build on existing capacities to ensure time and cost efficiencies.** Align sampling with existing sampling programmes. 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. If possible, make use of other wastewater surveillance programmes (e.g., for polio, typhoid, antimicrobial resistance genes, 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 analyses of samples will be conducted at a single centre or multiple centres. In the latter case, interlaboratory comparison is essential. 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 samples. 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 a different agency or entity to 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, 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 only RNA 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 incident 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.

5. Key considerations for data collection, analysis and interpretation

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 programs, along with many published protocols *(64–67)*. Sections below summarize 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. 4.

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

- Type of environmental sample – municipal or institutional sewage, biosolids/faecal sludge, open drains, or surface water
- Sample type and volume (grab, composite, passive *(61)*;
- Virus concentration approach (membrane filtration, centrifugation, protein precipitation and purification); and
- RNA method -amplification and reverse transcription-polymerase chain reaction quantitation using analysis – e.g., gene targets, primers and probes.

Methods and approaches need to be fit-for-purpose for particular 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 variables *(68)*.

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 organisational capacity. Some limited guidance is available for unsewered and lower-resource settings (8, 9), particularly where SARS-COV-2 ES programmes have been able to leverage existing capacity for polio ES. Where possible, the guidance below notes considerations for settings with low sewerage coverage and low financial resources and laboratory and organisational capacity and provides examples of non-commercial methods that can be developed locally. For all settings, it is important to ensure that planning, coordination and capacity requirements (Section 4) are in place before a SARS-COV-2 ES programme is initiated.

Design of sampling sites

SARS-COV-2 ES programmes should be optimised 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.

In general, SARS-COV-2 ES programmes are multi-tiered. 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. Spatially more 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 planes 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 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 COVID 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 would relate 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, time- or flow-integrated sampling methods become more important which means that sampling sites may need to consider more sophisticated sampling devices or passive samplers rather than just grab samples. 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 *(50)*, 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. Therefore, samples need to be collected from places such as wastewater collection vessels, pipes and inflows to treatment plants.

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

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 after primary screening, and 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 buildings.

In low resource settings, programmes have monitored septage from specific locations not connected to sewers including drainage network confluence points, as recommended by the polio ES program, or where on-site systems such as septic tanks are used, or sullage tanks on boats or aircrafts. Some programmes have successfully demonstrated the use of SARS-COV-2 ES in environmental waters *(69, 70, 71)*.

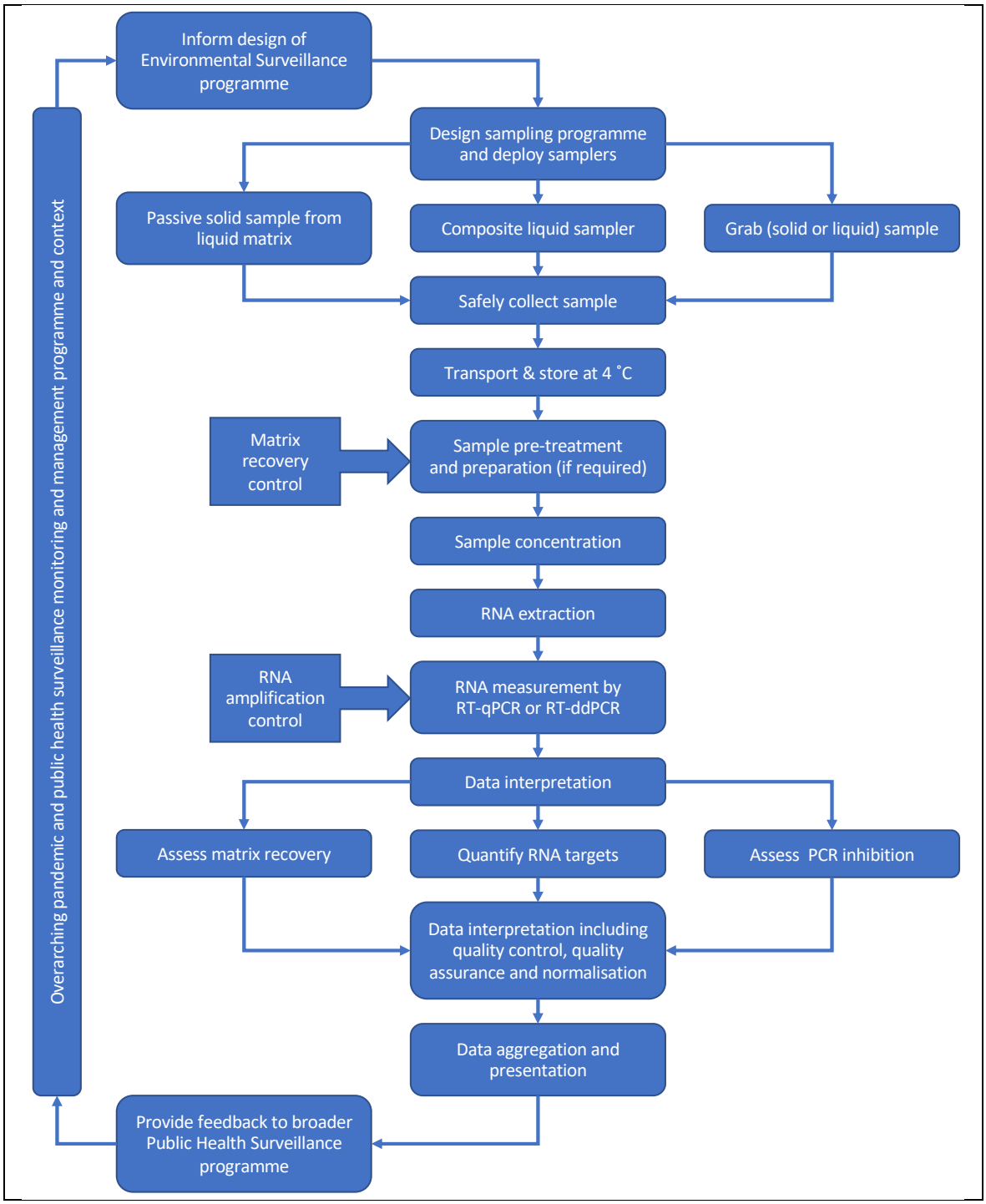


Fig. 4. Typical workflow for SARS-COV-2 ES programmes

Protection of sampler safety is critical when sampling from wastewater – regardless of COVID-19 *(72)*. 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. Finally, handling untreated wastewater presents risks due to a wide range of faecal–oral and respiratory pathogens, and sometimes chemicals.

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 in good time.

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.

Sampling methods

Sampling equipment and volume

Sampling equipment and volume depend on the use case and context *(73)*. To date most SARS-COV-2 ES sampling has taken place on liquid wastewater with increasing use of passive samplers in some areas.

- 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 sometimes power for 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.
- Grab sampling methods involve collecting samples of 100–250 mL, similar to bacteriological testing of wastewater. 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 can be collected at an optimal time of day – albeit it is not clear when that is. Most programmes target during peak morning sewage flow for instance, partly because sampling occurs in the morning to enable 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. If available, *Escherichia coli* or other more specific biological indicator measurements might assist with identifying any elevated non-sewage inputs but doing so requires specialist interpretation.
- Passive sampling places a medium in the wastewater to capture viruses and their RNA *(61)*. 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.

Collecting samples of large volume is of limited value since inhibitors from wastewater need to be kept at concentrations that will allow detection of viral RNA using the PCR. Hence, a common sample size is about 100 mL of wastewater or 1 g of settled sludge.

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 preliminary data on variants of concern (VoCs), requires a different design and implementation. For instance, studies in Italy have reported routine monthly or bi-monthly surveys for such variants *(74)*. Typical SARS-COV-2 ES sampling frequencies are shown in Table 2.

Table 2. 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 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 or release	*Aims to detect presence of SARS-CoV-2 RNA or variant of concern at point of entry of transport vessel or holding point. The result may be required before clearance of the transport vessel or its passengers and crew, or people held in quarantine or be used for rapid tracing. Evidence of SARS-CoV-2 RNA persistence would assist in better understanding the suitable sampling frequency in such contexts.*	*Once – at the time of arrival or before release from holding*

Sample transport

- Samples need to be stored at refrigeration temperatures (not frozen) wherever practicable, at least seeking to prevent samples becoming warm. Freeze–thaw processes significantly reduce the concentration of detectable RNA.

Sample storage

- Samples should be stored in a refrigerator 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 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 spike is added to samples before storage, if they are to be stored for longer than about 24 hours (see Matrix recovery spike, below).

Laboratory analysis

Protection of laboratory worker safety is critical to prevent exposure to a wide range of enteric and respiratory pathogens, and sometimes chemicals. 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.

Choice of methods

- The choice of analytical methods used will be influenced by the availability of testing methods 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.
- Specific commercial kits and reagents are available for such testing *(75)*. Credible, independent, third-party evidence and/or local trials to match the method to the context are recommended before committing to any one method *(76)*.

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 to be 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 for lower-resource settings.

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 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) or flocculation with skim milk *(77)*.
- The choice of concentration method depends on factors such as preferences 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 *(78)* 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.

RNA extraction

- RNA is typically extracted using commercially developed 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.

RNA detection and quantification

- RNA detection and quantification methods are largely the same as those used for clinical testing and are typically provided as commercial kits. They include reverse transcription quantitative polymerase chain reaction (RT-qPCR) with fluorescent probes, and reverse transcription digital polymerase chain reaction (RT-dPCR). 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. Laboratories are increasingly developing methods for sequencing viral RNA (section 6).

Quantification

- 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, 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, 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 be tested to assess inhibition.
- 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.
- Variants of interest can be detected in wastewater using genetic targets specific for those variants *(79, 80)*. SARS-CoV-2 variants with targeted single nucleotide polymorphisms (SNPs) (single nucleotide differences unique to specific variants) can be detected using real-time PCR (RT-PCR) assays *(22)*. Identification of novel variants and SNPs can be achieved using sequencing, either whole genome sequencing or of target regions, such as the spike protein *(23)* (section 6).
- 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 proven 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 proving 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.

Data interpretation

The sensitivity of SARS-COV-2 ES methods to detect the presence of infected people in the water catchment varies depending on factors such as:

- the variant-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 relative to the time window represented by the sampling;
- the extent of dilution and degradation of viral RNA in the water matrix due to inflow and infiltration into the sewer (rainwater and runoff, groundwater, 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 diagnostic 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 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 polio 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.

The precise number of infected people in a wastewater catchment cannot be accurately estimated based on SARS-COV-2 ES results. However, this is not a major limitation since the purpose of SARS-COV-2 ES is to understand the spatial extent of COVID-19 and trends in its levels. 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. Assays for other viruses more routinely shed by humans can provide a normalization control. Such viral targets include phages (crAssphage, *Bacteroides* HF183 and *Lachnospiraceae* Lachno3 genetic markers) and viruses routinely present in human faeces (pepper mild mottle virus). Conventional and widely used bacterial faecal indicator organisms, such as *E. coli*, can be used as a low-cost and widely available normalizing marker. Likewise, ammonia conductivity and other chemical parameters, can provide some normalisation indicators and can cost less. Industrial water, stormwater, snowmelt, greywater and groundwater might contain some background concentrations of these indicators that need to be taken into account.

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 (e.g., between home and work; for shopping, tourism and recreation);
- members of the population using on-site sanitation (e.g., septic tanks, pits) will not be captured in sewer-based sampling programmes;
- wastewater catchment 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, and the willingness and ability of potentially infected people to get tested, such as:

- availability and recommendations of use of specific tests with different sensitivity, specificity and predictive values such as nasopharanygeal or saliva specimens analysed with PCR tests, rapid antigen tests or other;
- availability of testing stations and personal tests within a reasonable distance;
- cost of tests – both at testing stations and for personal tests;
- wait times in queues for testing;
- opening hours of testing stations;
- concerns about the potential implications of a positive test result for freedom of movement;
- cultural and behavioural factors encouraging or discouraging testing;
- policies encouraging, requiring or discouraging testing; and
- capacity of testing and reporting systems.

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

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.

There can be challenges in comparing different methods between laboratories and work groups. Therefore, there are benefits in standardizing methods, 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).

Dashboards can be used to present data at local and national levels paired with public health advice. Examples of such dashboards include; South Africa, Hungary, the Netherlands, Switzerland, the United Kingdom, Victoria (Australia) and USA. 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 results by public health agencies should include testing response decision-support process flow diagrams or algorithms.
- Formulation and communication of public health advice should; help to focus diagnostic 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 dashboards useful to public health agencies and the public, includes:

- physical location of sample collection and catchment (represented spatially and by name);
- population monitored as represented by each sample;
- historical results from the same location;
- current and historical results from nearby and comparable locations;
- reported COVID-19 cases from the same location for the same period as sample collection;
- trends (rising, falling or steady); and
- implications of high, medium or low levels relative to a benchmark (e.g., using traffic light indicators).

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

- gene target;
- assay detection limits; and
- quality assurance and quality control process and performance on method sensitivity and specificity.

6. Emerging research

A range of research projects and innovations are in progress to improve ES for SARS-CoV-2 and other microorganisms *(81)*. 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 trials of genome sequencing and next-generation sequencing for variant detection. This requires molecular biology, computational *(82)* and bioinformatics capability that is not readily available in many lower-resource contexts. ES has the potential to detect novel variants that emerge, as well as to increase understanding of the ecology and potential zoonotic potential of SARS-CoV-2 that is not being identified in human clinical samples *(83, 84)*. Potentially, ES could be used to monitor wastewater or other water sources from animal rearing operations and transport hubs to support global pandemic intelligence.

Research needs for SARS-COV-2 ES are being coordinated and promoted via the EU *(85, 86)* and Global Water Research Coalition *(87)* to optimize the benefits of data sharing and coordinated research among SARS-COV-2 ES programme managers, researchers and funding partners.

7. Details of guidance development

Search strategy

Multiple lines of evidence were used to inform this guidance.

- Precedence was given to evidence sourced from a systematic 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 3,000 sites in 58 counties covering all 6 WHO regions.
- 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;
    - 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 qualitative expert interviews with participating members of the Global Water Research Coalition.

Evidence review and quality appraisal

Data was extracted from the individual papers and grey literature according to the three scoping questions described in section 1. Unlike other areas such as rapids 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 in programmes at scale. As such the document summarises evidence from published and grey literature that meets these three 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. *Use cases*	• *Published and grey literature included* • *Scale of application* • *Extent to which ES supports public health decision making*
	2. *Capacity, planning and coordination needs*	• *Published and grey literature included* • *Degree to which ES supports public health decision making*
	3. *Methods for sampling, analysis, data interpretation*	• *Method described in published literature including description of methods or protocol* • *Method is commonly used* • *Method has been used in an at scale programme*

Evidence to decision-making process

Evidence was synthesized into guidance text based on quality assessment and evidence to decision criteria and presented to GDG 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 by ERG members and feedback compiled into the final document.

Plans for updates

WHO continues to monitor the situation closely for any changes that may affect this interim guidance. Should any factors change, WHO will issue a further update. Otherwise, this interim guidance will expire one year after the date of publication.

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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.

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 analysed.
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-dPCR	Reverse transcription digital polymerase chain reaction
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
Sewage	Wastewater that has been or used 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
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

Annex: Illustrative case studies

Case study 1: Observing increasing and decreasing trends at community level, aiding in tracking emergence of novel variants

Summary	**Early detection of fourth epidemic wave of COVID-19 in Gauteng Province, South Africa, together with later confirmation of the presence of Omicron variant, and efforts to include wastewater indicators for preparedness and alert systems.**
Date	**Mid-November to December 2021**
Location	**Gauteng Province, South Africa.** Most urbanized province of South Africa, total population 15.8 million (26% of South African population), 680 people/km^2.
Details	• In Gauteng province, South Africa, the third wave of COVID-19 (predominantly due to the Delta variant) ended in epidemiological week 34. The incidence of laboratory-confirmed cases remained below 30 cases/week until week 47. Levels of SARS-CoV-2 in wastewater were undetectable or under 1.5 log genome copies/mL from week 37 until week 42. • In weeks 43–45, a first increase in SARS-CoV-2 levels in wastewater from various treatment plants across the province was observed. The first increase in laboratory-confirmed clinical cases was observed in epidemiological week 45 by which time 1–3 successive increases in levels in wastewater had been observed. The fourth wave officially started in week 47. • S-gene target failure was detected in clinical samples of laboratory-confirmed SARS-CoV-2 patients in week 46 leading to the discovery and characterization of Omicron variant in week 48. • SARS-CoV-2 levels in wastewater were presented to the Technical Working Group (TWG) of the COVID-19 Ministerial Advisory Committee on 17 November 2021, just before the discovery of Omicron. At that stage, members of the TWG, including the Centre for Epidemiological Modelling and Analysis, agreed that wastewater-based surveillance provided useful early warning and committed to work with the National Institute for Communicable Diseases (NICD) to support development of more robust wastewater indicators. Subsequently, members of the TWG reported that wastewater-based epidemiology had predicted the fourth wave. • Sequencing and variant analysis of RNA amplified from wastewater samples successfully detected evidence of Omicron in weeks 47 and 48.
Benefit use case	Early warning; Health system preparedness, Indicator-based surveillance, Good correlation with clinical genomics and lineages.
Pandemic context	Wastewater surveillance between epidemic waves in a previously high-prevalence area with low vaccine uptake.
Governance	NICD conducts and funds some testing, and co-ordinates testing by partner laboratories, and disbursement of funds from agencies such as the Water Research Commission, to testing partners.
Stakeholders involved	• Partner laboratories – co-ordinate sample collection and testing, and provide testing results to NICD. • NICD – national public health institute responsible for epidemiological monitoring of communicable disease including SARS_CoV-2, and also the co-ordinator of the ES network • National Department of Health – convenors of the COVID-19 incident management team, responsible for advising Cabinet on SARS-CoV-2 levels and appropriate public health interventions • Cabinet – Ministers of various government portfolios responsible for regulations to limit SARS-CoV-2 transmission • Provincial health departments –health care providers and responsible for preventive, administration of diagnostic testing, curative and palliative health care services.
Incidence settings	Transition from *clusters of cases* to *community transmission* (as per WHO 2022 surveillance guidance). Incidence rate in Gauteng province was 2–6 cases/100 000 during weeks 40–44.
Implications for other incidence settings	Suitability in areas where only imported cases or clusters of cases are detected: • Sampling of influent at large wastewater treatment plants may yield negative results when only imported or sporadic cases are present, due to dilution and environmental degradation of RNA. • Wastewater-based detection of SARS-CoV-2 at large wastewater treatment plants may identify the transition from *imported cases* or *clusters of cases* to *community transmission*. This transition is often precipitated by super-spreader events, or by the emergence of a new variant that can escape pre-existing immunity.
Capacity needs	Capability to: • sample influent wastewater from multiple large wastewater treatment plants weekly (or more frequently) during periods of low incidence and transport refrigerated samples within a day; • reliably undertake molecular biological analysis in a laboratory; and

	• access provincial and national policymakers to present interpreted results or indicators, so that these may be used together with other signals to inform preparedness activities.
Resource settings	Medium-resource setting: • Grab samples are easily collected. • Concentration, PCR detection and quantification are relatively easily performed. A variety of inexpensive concentration methods may be used. It is essential to apply a selected method consistently to make longitudinal comparisons meaningful.
Implications for other settings	Suitability in low-resource settings: • Highly suitable where clinical testing has limited accessibility or high cost. • Useful in sewered communities but can be applied to runoff water in unsewered communities.
Comment on cost–benefit	Cheaper than case-based surveillance. Allows surveillance of large population groups at minimal cost using accessible samples, and with limited ethical implications regarding intrusion on privacy.
Sampling method	Grab samples, or passive in-line samplers.
Test method(s) used	https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-reports/wastewater-based-epidemiology-for-sars-cov-2-in-south-africa/
URL	https://www.nicd.ac.za/diseases-a-z-index/disease-index-covid-19/surveillance-reports/weekly-reports/wastewater-based-epidemiology-for-sars-cov-2-in-south-africa/

Case study 2: Risk communication

Summary	**National sewage surveillance system in Hungary used for early warning and to alert the public in increasingly affected areas.**
Date	**Continuous, starting from June 2020**
Location	**Hungary**
Spatial context	22 sampling points representing the entire geographical area of the country.
Details	• Weekly samples are collected from the three wastewater treatment plants (WWTPs) in Budapest and 19 treatment plants in major cities representing 40% of the population. • Results including viral RNA concentration (low, moderate, elevated, high) and trend (decreasing, stagnant, increasing, sharply increasing) are communicated weekly to the public. Press release receives wide media attention, covered by almost all major online newspapers. • Results are also communicated to the chief medical officer, the county public health offices and the operative board responsible for outbreak management.
Benefit use case	Alerting the public to the pandemic situation encouraged increased use of diagnostic testing and uptake of vaccinations, and reinforced compliance with masking, distancing advice. Data are also used in decision-making by the operative board on capacity planning and hygiene measures.
Pandemic context	Awareness raising was especially important in the increasing phase, or when case numbers are high for an extended period, resulting in people becoming accustomed to the situation and relaxing personal hygiene behaviour.
Governance and design	Surveillance system is coordinated by the National Public Health Centre, building on existing systems of sampling, transport and analysis. Funding is provided from national and EU COVID response funds. Wastewater surveillance is used as a complementary data source for informing public health decisions of the operative board.
Stakeholders involved	Samples are collected by WWTP staff and transported to the National Public Health Centre by the local public health authorities. SARS-CoV-2 analysis is performed on national level in the National Public Health Centre in collaboration between environmental and clinical laboratories.
Incidence settings	Community transmission – level 2–4. (as per WHO 2022 surveillance guidance)
Implications for other settings	Only useful when the increase in viral RNA concentration is detectable at an urban WWTP level.
Capacity needs	Capability to: • carry out nationally representative sampling; • provide samples to a laboratory in a timely manner (within a day, kept cold); • reliably undertake molecular biological analysis in a laboratory; and • interpret, communicate and use the results to inform COVID-19 control response in a timely manner.

Resource settings	High-resource setting. Requires experienced water samplers and analysts.
Implications for other resource settings	Suitability in low-resource settings - In-house flat membrane ultrafiltration method was developed for concentration of sewage samples using membranes originally developed for wastewater treatment *(88)*. Membranes were available from the national manufacturer. This solution was more cost-effective than commercially available concentration units and not susceptible to supply shortage.
Comment on cost–benefit	Costs of environmental sampling are relatively low compared with representative clinical testing of a similarly large population (4 million people).
Sampling method	Automated sampling, where available, or grab samples in the morning peak period: https://pubmed.ncbi.nlm.nih.gov/33971598/
Test method(s) used	• Flat-sheet ultrafiltration for concentration of the liquid phase: https://pubmed.ncbi.nlm.nih.gov/33971598/ • QIAamp Viral RNA Mini Kit: https://www.qiagen.com/us/products/diagnostics-and-clinical-research/sample-processing/qiaamp-viral-rna-kits/ • LightCycler 480 Instrument II platform, LightCycler Multiplex RNA Virus Master kit. Target: N1 gene
URL	• Reports: https://www.nnk.gov.hu/index.php/koronavirus/szennyvizvizsgalatok • Data: https://sphere.waterpathogens.org/dataset/403decc4-5c94-49ef-9998-7440e809f14d • Examples of news coverage: https://telex.hu/koronavirus/2022/01/25/koronavirus-orokitoanyag-szennyviz-minta-nnk; https://index.hu/belfold/2022/01/25/szennyviz-koronavirus-orokitoanyag-emelkedes/

Case study 3: Cost-effective targeting of diagnostic testing resources

Summary	**Comparing wastewater concentrations to test data in different city areas indicated undertesting in one city area.**
Date	**September 2020 to February 2021.**
Location	**Rotterdam, The Netherlands**
Spatial context	Rotterdam is the second largest city in the Netherlands and Europe's largest seaport. Its population is approximately 650 000 with a high proportion of people of non-Dutch origin. Density is 3000 people/km^2.
Details	• Case data and wastewater data in different city areas were matched by zip-code • Trends in COVID-19 incidence (reported cases per 100 000) matched trends in SARS-CoV-2 concentration in wastewater in these city areas (population 6 500 – 128 000) • Comparing incidence to wastewater concentrations indicated a consistently high wastewater-to-incidence ratio in one city area, suggesting more undertesting in this city area • The municipal health service directed mobile test facilities to this city area and targeted an information campaign to promote testing.
Benefit use case	Provided additional, objective information about virus circulation in the population of city areas, independent of testing behaviour and availability.
Pandemic context	Second wave, high prevalence
Governance	Close collaboration between Municipal Health Service (GGD Rotterdam-Rijnmond), Erasmus Medical Center and the wastewater monitoring organizations (KWR, P4UW), with weekly joint comparison of wastewater data and reported cases from each city area.
Stakeholders involved	• Municipal Health Service: surveillance, public health response • Erasmus Medical Center: collection of General Practitioner and Hospital data, virus sequencing • KWR: wastewater monitoring and coordination • P4UW: wastewater normalization, data analysis • IMD: installing and maintaining autosamplers • AQUON: sampling • Water authorities and city: access to sites
Incidence settings	Overall high incidence, between 10 and 100 per 100 000. All wastewater samples positive for SARS-CoV-2.
Implications for other incidence settings	The objective nature of wastewater surveillance also applies to low prevalence settings, and is valuable in settings where case testing is low (due to testing aversion or limited availability). For smaller populations, the variability in virus shedding by infected persons may cause too much variability in the wastewater concentration to be able to reliably discriminate undertesting.
Capacity needs	Capability to: • safely sample regularly (3x per week) from wastewater points that are representative for city areas (sewer mains, pumping stations) with 24h composite autosampler;

	• provide samples to a laboratory in a timely manner (within a day, kept cold); • reliably undertake molecular biological analysis in a laboratory; • interpret and communicate the results to Municipal Health Service, and • compare wastewater data against case data for well-matched populations • mobilize testing facilities to city areas, launch information campaign.
Resource settings	High-resource setting: Requires pre-defined selection of city areas and installation of autosamplers Requires experienced water samplers, rapid transport and analysts. Requires combination of case data and wastewater data at same resolution
Implications for other resource settings	Suitability in low-resource settings: Highly suitable where clinical testing has limited accessibility or high cost. Could work with in-line passive samplers (cheap, simple, safe), but these will give higher variability so require more data for sufficient certainty. Useful in sewered communities. Could be applicable to small rivers/streams in unsewered communities.
Comment on cost–benefit	Relatively cheap addition to case-based surveillance, providing insight in virus circulation that is not seen by case-based surveillance. In this case study, the precise costs and benefits were not quantified.
Sampling method	Autosamplers collecting 24h (flow) composite samples. Could work with passive in-line samplers provided the samples are normalized for their 'fecal strength'.
Test method(s) used	• Concentration: Centricon® Plus-70 30kDa Centrifugal Filter Units https://pubs.acs.org/doi/10.1021/acs.estlett.0c00357 • Extraction: Nuclisense Viral RNA Kit https://pubmed.ncbi.nlm.nih.gov/34371414/ • RT-qPCR: N2 and E gene • Flow normalization
URL	• WSPHERE description of use case Dutch sewage surveillance use case (arcgis.com) • News report (in Dutch) • Signaalfunctie coronatest rioolwater leidt tot groot bevolkingsonderzoek in Rotterdam - Waterforum • Municipal Health Service report (in Dutch) • 20210401-Rapportage-RGT-Lansingerland-en-Charlois-V1.1.pdf (ggdrotterdamrijnmond.nl)

Case study 4: Informing early and localized restrictions in pockets of re-emergence and *targeted surveillance for early warning of circulation*

Summary	**Early warning of COVID-19 emergence among a public housing community in a high-rise building in the urban city of Melbourne, Australia.**
Date	**Mid-August 2021**
Location	**Melbourne, Victoria, Australia**
Spatial context	Single building – urban public housing, high-rise, > 500 residential apartments.
Details	• In the context of an expanding Delta variant wave with increasing cases and unexpected wastewater detections in urban Melbourne catchments, localised surveillance was initiated at all urban high-rise social housing estates using passive samplers. • This was because there was both a high risk of amplification and high vulnerability to poor health and social outcomes as had occurred in these settings in Melbourne's first wave. • After a short period of surveillance, an unexpected wastewater result with a high quantitative level was returned in the absence of any known cases among residents. • More frequent wastewater sampling was initiated and a further positive result was returned. • On the basis of these results, public health action was taken : community engagement including but not limited to phone text messages encouraged targeted clinical testing and this resulted in uptake of testing, identification of cases directly and through subsequent contact tracing. • Cases were offered alternative accommodation and ongoing wastewater sampling returned negative results providing reassurance that the outbreak was contained. • The early warning from wastewater coupled with the prompt response and culturally competent community engagement helped reduce the spread and contain the cluster and avoid additional restrictions which would likely have been required if the cluster had spread.
Benefit use case	Early warning in localised setting in a community which is characterised by high-amplification risk and vulnerability to health and social harms due to COVID disease or COVID related restrictions

Pandemic context	Early phase of Delta variant wave in the State of Victoria, Australia (www.coronavirus.vic.gov.au/victorian-coronavirus-covid-19-data).
Incidence settings	Clusters of cases: (as per WHO 2022 surveillance guidance) Most clinical and wastewater samples outside quarantine facilities had been non-detects in weeks before in Victoria, while neighbouring state of NSW had a rapidly expanding Delta wave. Recent sewage samples in nearby central Melbourne areas were showing detections and variant detection of Delta had been found in recent Melbourne cases and visitors from NSW.
Implications for other incidence settings	Suitability in high-prevalence settings: Of value in localised settings where there is high amplification and high vulnerability when the incidence is low such as large aged care and corrections facilities and there is specific public health actions which would result including response from the community themselves to increase testing and/or vaccine uptake – this was an early unexpected detection use case.
Capacity needs	Capability to effect rapid end to end turn around from sample to results including: safely and feasibly identify sampling points and sample from building wastewater connection points; provide samples to a laboratory in a timely manner (within a day, kept cold); reliably undertake molecular biological analysis in a laboratory; and interpret, communicate and use the results to inform COVID-19 control response in a timely manner.
Resource settings	High-resource setting: Requires experienced water samplers and analysts. High cost anticipated if pandemic spread, so high value of early detection and containment required and is used adaptively linked to risk of incursion and perceived value (as noted in comment on cost–benefit ratio, below).
Implications for other resource settings	Suitability in low-resource settings: Provided the method is functional, the use case is of equal value in low-resource settings. However, with most samples under such a programme likely to test negative, such a programme might be considered costly and would not be used routinely but may be considered adaptively.
Comment on cost–benefit	A high health and economic cost of uncontained pandemic spread, so a high value was placed on early detection and containment – the cost per test was relatively small relative to the wider cost. In this case study, the precise costs and benefits were not quantified.
Sampling method	Passive sampler on the sewer line: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8291133/
Test method(s) used	Electronegative membrane in passive sampler: www.ncbi.nlm.nih.gov/pmc/articles/PMC8291133/ www.monash.edu/engineering/davidmccarthy Qiagen Extraction Kit: www.qiagen.com/au/products/discovery-and-translational-research/dna-rna-purification/rna-purification/microbial-rna/rneasy-powerwater-kit/ Perkin Elmer China CDC SARS-CoV-2: https://perkinelmer-appliedgenomics.com/home/products/sars-cov-2-real-time-rt-pcr-assay-ce-ivd/
URL	www.abc.net.au/news/2021-08-15/victoria-records-25-new-covid-19-cases/100378244 Investigation into the detention and treatment of public housing residents arising from a COVID-19 'hard lockdown' in July 2020 | Victorian Ombudsman

Case study 5: Identifying known variants

Summary	**Evidence of transition from SARS-CoV-2 Beta to Delta variant of concern in wastewater samples, during the second and third waves of infection in South Africa.**
Date	**Mid-April to mid-August 2021**
Location	**Mangaung, Free State, South Africa**
Spatial context	Mangaung (formerly Bloemfontein) is the judicial capital of South Africa. It is an urban area with a population of approximately 800 000 and a density of 120 people/km^2.
Details	• Case-based genomic surveillance is routinely carried out by the Network for Genomic Surveillance in South Africa (NGS-SA) where specimens from selected diagnostic laboratories are sent for sequencing. • the second wave of COVID-19 was dominated by the Beta variant starting in week 45, 2020, and ended in week 5, 2021. The third wave of infection was dominated by the Delta variant, which started in week 18 in 2021 and ended in week 35 in 2021. • Using next-generation sequencing with a protocol adapted from the ARTIC protocol (https://artic.network/ncov-2019), wastewater samples from plants in Mangaung, Free State Province, provided evidence of the transition from the Beta variant (weeks 16–25) to the Delta variant (weeks 26–33) during the third wave of infection. • The proportion of fragments containing mutations specific to the Beta variant decreased in week 25 (Beta mutations were at 93% in week 24, declining to 20% in week 25), while the

	proportion of fragments containing mutations specific to the Delta variant increased over the same period (Delta mutations were at 100% in week 26). • The transition from Beta to Delta was also demonstrated in sequencing data generated by NGS-SA from clinical specimens obtained from Mangaung patients: the Beta variant was dominant in weeks 16–23, whereas the Delta variant was dominant in weeks 26–33.
Benefit use case	Provides additional evidence to policymakers when correlation of lineage information is high between sequences obtained from wastewater samples and clinical genomics.
Pandemic context	Wastewater genomic surveillance
Governance	Led by the National Institute for Communicable disease (NICD) and funded by the Water Research Council (WRC), South Africa, and Bill and Melinda Gates foundation.
Incidence settings	Introduction and transmission of known variant of concern during a transition from *clusters of cases* to *community transmission* in a new epidemic wave (as per WHO 2022 surveillance guidance).
Implications for other incidence settings	Genomic sequencing of SARS-CoV-2 RNA fragments from wastewater may have value when and where testing rates are low, in low-prevalence settings, or when the sequencing capabilities from clinical samples are limited.
Capacity needs	Capability to: • safely sample influent wastewater from multiple large wastewater treatment plants weekly (or more frequently) during periods of low incidence; • transport samples to a laboratory in a timely manner (within a day, kept cold); and • reliably undertake laboratory methods to identify mutations specific to Beta and Delta variants.
Resource settings	Medium-resource setting: • Grab samples are easily collected. • Concentration, PCR detection and quantification are relatively easily performed. • Sequencing methods are expensive but may be conducted through local and international partners or agencies.
Implications for other resource settings	Suitability in low-resource settings: • Highly suitable where clinical testing has limited accessibility or high cost. • Useful in sewered communities but can be applied to runoff water in unsewered communities.
Comment on cost–benefit	Cheaper than case-based surveillance. Allows surveillance of large population groups at minimal cost using accessible samples, and with limited ethical implications regarding intrusion on privacy.
Sampling method	Grab samples, or passive in-line samplers
Test method(s) used	• Concentration : Centricon® Plus-70 Centrifugal Filter Units • Extraction: QIAamp Viral RNA Kits • Sequencing: ARTIC protocol
URL	• www.merckmillipore.com/ZA/en/product/Centricon-Plus-70-Centrifugal-Filter-Units,MM_NF-C3043 • www.qiagen.com/us/products/diagnostics-and-clinical-research/sample-processing/qiaamp-viral-rna-kits/ • https://artic.network/ncov-2019

Case study 6: Finding outbreaks in places thought to be COVID-19-free

Summary	**Detection of unrecognized community cases of COVID-19 in using wastewater-based surveillance for SARS-CoV-2**
Date	**July to November 2021**
Location	**Stratford, New Zealand**
Spatial context	Stratford is a town in Taranaki, New Zealand with a population of 6,100 (10,100 in the wider Stratford district). The wastewater system services 2,200 properties (97% of the urban Stratford area), with all other dwellings serviced by septic tanks.
Details	New Zealand implemented national wastewater-based surveillance for SARS-CoV-2 in 2021 following a trial late in 2020. Wastewater was collected from the town of Stratford from 28 July 2021. SARS-CoV-2 RNA was first detected in a wastewater sample collected on 2 November 2021, arrived at the laboratory on 3 November, and was reported on 4 November. At the time were no known cases of COVID-19 in the Stratford district. Six clinical cases were subsequently identified who did not transmit the virus to anyone outside of their household, at least in part because of the heightened awareness that wastewater testing provided.

	Following the reporting of the positive wastewater sample, there was extensive messaging from national, regional, iwi and community-based health providers, alerting the public to a potential case(s) of COVID-19 in community, and encouraging observance of public health measures, vigilance regarding symptoms, and for symptomatic individuals to get tested. From 5 November, additional testing and vaccination clinics were established, resulting in 1,145 COVID-19 tests being undertaken over the following 10 days, compared with 67 for the 10 days prior. Increased vaccination rates were also observed, and several community events were cancelled to minimize potential exposure. Following the positive detection, wastewater sampling increased to daily samples. SARS-CoV-2 RNA was detected in wastewater samples collected on 6, 7, 9, 10, and 13-16 November 2021. These results strongly suggested that the positive detection was due to a least one case(s) was resident in Stratford (rather than being a visitor). Wastewater testing in nearby towns suggested the case(s) were confined to Stratford. On 11 November, six clinical cases were identified in the Stratford community, all from the same household (3 adults, 3 children). The first case experienced symptoms from 28 October following travel to Auckland where there was an active Delta outbreak. No transmission of COVID-19 occurred outside of this household, demonstrating the actions of the family and the community were sufficient to eliminate the virus. No further detection in the wastewater, or community cases occurred until 3 months later supporting elimination of the virus from this community. Heightened awareness provided by the results of wastewater testing and the partnership by stakeholders, including the District Health Board and iwi healthcare providers, contributed to this outcome.
Benefit use case	Early detection of cases. Rapid isolation and elimination of COVID-19 in the area. Extensive messaging across a range of platforms alerting the whole country to the potential presence of cases and reinforced uptake of public health measures (mask-wearing, social distancing, hand hygiene, location tracking/"scanning in") and rapid increase in vaccination.
Pandemic context	Extended period of no cases in the country. Detection in Stratford wastewater happened during the Delta outbreak thought to be confined to Auckland city.
Governance and Stakeholders involved	• New Zealand Ministry of Health • Taranaki District Health Board • Ngāruahine, Taranaki Regional Council • Ngāti Ruanui, local iwi (Maori tribe) • Tui Ora - Community-based health and social service provider • Institute of Environmental Science and Research - Undertakes wastewater testing for SARS-CoV-2 and surveillance activities • Stratford District Council - Collection of wastewater samples for testing
Incidence settings	Low incidence setting
Implications for other settings	Less relevant in high incident settings
Capacity needs	Capability to: • safely sample influent wastewater from multiple large wastewater treatment plants weekly (or more frequently) during periods of low incidence; • transport samples to a laboratory in a timely manner (within 24hours, kept cold); and • rapid communication and action of results in coordination with national and local authorities and local stakeholder.
Resource settings	• Medium to high resource settings
Comment on cost–benefit	Early case detection, isolation and contract training for a small outbreak is highly cost effective compared to a larger scale effort with more cases had the outbreak not been contained. Success enabled by: ready buy-in from wastewater utilities in supporting sample collection; and the ability to utilize autosamplers to collect composite samples; quick turnaround time for reporting results (2 days) utilizing overnight couriers; strong collaboration between national, regional, iwi and community health authorities and providers in supporting the response through messaging, testing and vaccination initiatives, buy in from the community, clear and constant messaging to public from the media.
Sampling method	• Initially weekly sampling undertaken at the wastewater treatment facility. Sampling frequency increased to daily following the detection and confirmation of a SARS-CoV-2 positive wastewater sample. • Composite samples collected at the inlet to the treatment plant using an autosampler from typically 10am to 10am.

Test method(s) used	• Viruses were concentrated from 0.25L wastewater to 1.25 mL using PEG precipitation. RNA was extracted using the High Pure Viral Nucleic Acid Extraction Kit (Roche Molecular Biochemicals Ltd), SARS-CoV-2 RNA was detected using a two-step RT-qPCR using Chinese CDC N gene primers and probes. • Method described in Hewitt J, Trowsdale S, Armstrong BA, Chapman JR, Carter KM, Croucher DM, Trent CR, Sim RE, Gilpin BJ. Sensitivity of wastewater-based epidemiology for detection of SARS-CoV-2 RNA in a low prevalence setting. Water Res. 2022 Mar 1;211:118032. doi: (https://doi.org/10.1016/j.watres.2021.118032)
URL	• Hewitt J, Trowsdale S, Armstrong BA, Chapman JR, Carter KM, Croucher DM, Trent CR, Sim RE, Gilpin BJ. Sensitivity of wastewater-based epidemiology for detection of SARS-CoV-2 RNA in a low prevalence setting. Water Res. 2022 Mar 1;211:118032. doi: (https://doi.org/10.1016/j.watres.2021.118032) • www.tdhb.org.nz/news/documents/media_release_2021_11_05.shtml • www.stratford.govt.nz/our-council/news?item=id:2fyyj1r5j17q9sj7kq21 • www.tdhb.org.nz/news/documents/media_release_2021_11_10.shtml • www.health.govt.nz/news-media/media-releases/six-cases-covid-19-stratford

Case study 7: Establishing a National Non-Sewered Surveillance Programme

Summary	**Establishing a non-sewered sanitation environmental surveillance programme to complement the wastewater treatment works surveillance programme.** **The non-sewered sites selected for investigation include high-density informal settlements where social distancing is challenging, shared use of ablution facilities is common. Non-sewered areas have numerous sampling points: faecal sludge from different sanitation systems, faecal collection systems and greywater / blackwater run-off. The challenge was to develop a cost-effective and practical approach for incorporating non-sewered areas with the sewered approach as part of city-wide surveillance and provide community-level data.**
Date	**Different at sites: beginning around mid-Jan 2021 with large-scale sampling from mid-March to Feb 2022.**
Location	**Gauteng Province, Limpopo Province, Western Cape Province, KwaZulu-Natal Province South Africa**
Spatial context	Within peri-urban settlements across 21 different test sites across 4 provinces. Most sites are located in proximity to major urban settlements
Details	• Many developing countries have a mix of sewered and non-sewered coverage. Around 40% of the country relies on non-sewered sanitation systems • The river and run-off samples have been proven to be a useful resource to be able to detect SARS-CoV-2 RNA and thereby detect community infection. The data showed that as the cases rise, the Ct values drop accordingly, indicative of a higher viral load. In all provinces, there is some evidence of a correlation for the second wave in January 2021 and again in July 2021 for the third wave. • In Gauteng province this trend was more predominate. Sites tend to be very densely populated and the run-off water highly polluted with similar characteristics to untreated domestic wastewater). Site testing occurred much earlier than other provinces and the second wave was captured in trend analysis of water samples. Another peak in COVID-19 detection in the run-off is noted in March 2021 although clinical case data for the province did not follow the same trend. This may be due to a level of infection within the community which is unreported and untested due to financial constraints. • Composite samples taken from Urine Diversion Dehydrating Toilets (UDDTs) in the KwaZulu-Natal province did not yield positive results (in terms of detection of SARS-CoV-2 RNA). Further, this sampling method was proven to be costly, labour intensive and unproductive. This sample collection method was abandoned in favour or more community-wide surveillance methods. • Passive sampling devices *(52)* have been adapted for use in non-sewered contexts in South Africa. This method was proven to be practical and cost-effective method for sampling non-sewered areas. This method can be equally useful in other non-sewered contexts in other developing countries.
Benefit use case	Application countries who do not have extensive sewerage infrastructure, Early warning; Health system preparedness, Indicator-based surveillance, Good correlation with clinical data at a provincial level.
Pandemic context	ES between waves in a previously high-prevalence area with low vaccine uptake. The areas targeted have social distancing challenges and use of shared sanitation facilities with varying levels of cleaning and disinfection protocols undertaken. The areas were targeted for this specific reason as the risk for disease transmission is higher due to the lack of individual household sanitation facilities.

Governance	Research programme developed by the Water Research Commission (WRC). The team included partners involved in the national sewerage surveillance programme (SACCESS) network led by the NICD- namely, the University of Pretoria and Waterlab PTY Ltd. WRC provided funding to evaluate the business case for future uptake.
Stakeholders involved	• WRC – national water research hub • Waterlab PTY LTD – project co-ordination of partnerships, sampling and testing. • University of Pretoria – laboratory testing of samples • Local partnerships (communities, NGOs, municipalities) for sampling were critical to extend the technical capacity for routine sampling and transport of samples to laboratories.
Incidence settings	Transition from second to third wave
Implications for other incidence settings	Suitability in areas where only imported cases or clusters of cases are detected: • Sampling of influent at large wastewater treatment plants may yield negative results when only imported or sporadic cases are present, because of dilution and environmental degradation of RNA. • Wastewater-based detection of SARS-CoV-2 at large wastewater treatment plants may identify the transition from "imported cases" or "clusters of cases" to "community transmission". This transition is often precipitated by super-spreader events, or by the emergence of a new variant that can escape pre-existing immunity following vaccination or prior infection.
Capacity needs	Capability to: • Local sampling partnerships to be established. This requires a co-ordinated effort and different approvals specific to each site (community leaders, municipality, NGOs). • Safely sample run-off, stream and rivers weekly; • Transport samples to a laboratory in a timely manner (within a day, kept cold); • Reliably undertake molecular biological analysis in a laboratory; and • Clinical data sets to complement data (if possible).
Resource settings	Medium-resource setting: • Passive samples can be left at site and easily collected the next day. • Concentration, PCR detection and quantification are relatively easily performed. The research team have used skimmed milk for viral concentration which has proven to efficient and cost-effective.
Implications for other resource settings	Suitability in low-resource settings: • Highly suitable where clinical testing has limited accessibility or high cost. • Useful in non-sewered communities in which residents may limited financial capacity for individual testing.
Comment on cost–benefit	Cheaper than case-based surveillance. Allows surveillance at community-level in non-sewered environments at minimal cost (compared to individual testing) using accessible samples. It offers the benefit on surveillance that was traditionally not available to countries with low sewerage infrastructure. It thus opens possibilities for the surveillance of other pathogens of concern e.g., Cholera, Polio, typhoid.
Sampling method	Grab samples, or passive in-line samplers.
Test method(s) used	• Pocock, G., Coetzee, L., Mans, J., Taylor, M., & Genthe, B. (2020). Proof of Concept Study: Application of Wastewater-Based Surveillance to Monitor SARS-CoV-2 Prevalence in South African Communities. Pretoria: WRC. http://wrcwebsite.azurewebsites.net/wp-content/uploads/mdocs/TT%20832-20%20final%20web.pdf • Pocock, G.; Coetzee, L., Mans, J. & Genthe, B.. ((in prep)). Development of a framework for water quality based COVID 19 Epidemiology surveillance Framework for Non-Sewered Communities. WRC Research Project 2020-2021-00686. Pretoria: WRC.

Case study 8: Non-sewered surveillance filling gaps in clinical surveillance

Summary	**Environmental surveillance in non-sewered areas for multiple enteric pathogens and antimicrobial resistance genes**
Date	**From June 2019**
Location	**Dhaka, Bangladesh**
Spatial context	12 sites in Mirpur wards of Dhaka were already established for surveillance of poliovirus, antimicrobial resistance, and other enteric pathogens in June 2019. Expanded in the second quarter of 2020 to 33 ES sites covering low-, mid-, and high-income Dhaka North City Corporation areas of Dhaka

	(https://es.world/country/BGD) to track SARS-CoV-2. 30 additional sites are being established in the Dhaka South City Corporation areas to represent the whole city better in 2022.
Details	• The sewage system in Dhaka, a city of 21 million, is made up of mostly informal and some formal sewage networks. Only 20% of the sewage ends up in a wastewater plant. In this setting, the environmental surveillance activity started with blue line tracing all the informal sewage system to completely map the sewage network. Using the shapefiles of the blue lines and WorldPop data, Novel-T, a mapping company, developed interactive maps of our study area (https://es.world/country/BGD). • Determined the catchment population and area for site selection using these interactive maps. • Established 33 sites throughout the Dhaka North City Coporation areas of Dhaka that represents low-, middle-, and high-income areas. • Measured weekly physiochemical properties of the wastewater using Aquaread probe (pH, total dissolved solids, GIS point, temperature, etc) and collected weekly 6L grab samples using the Bag Mediated Filtration System from all 33 sites • Samples were process on the day of collection and nucleic acid extraction and RT-qPCR for N1 and N2 gene (CDC assays) the following day. • A2i, a Bangladesh government agency on digital information, shares weekly case data for our study area with our team. • Developed a dashboard to display the ES data from our study and the case data from the government (https://dhakacovidtracker.research.virginia.edu/) to make the ES data more digestible for the public health stakeholders. • Results are shared weekly to the national COVID task force, comprised of public health stakeholders and researchers, via a weekly summary report and an interactive dashboard for mitigation efforts. • Good correlation between the ES data and the clincal case data. • The strongest correlation is around 5 days where the ES data precedes the rise or fall in the clinical data. • Developed a panel of VOC RT-qPCR assays to detect VOCs in wastewater. Using these assays, the most prevalent VOC in circulation was Beta in April to May 2021, follow by Delta from June to December 2021, and Omicron from January 2022 to recent. • Currently, NGS sequencing the wastewater to detect VOCs using the Illumina's COVIDSeq Kits. • Expansion into the Dhaka South City Corporation is underway with 30 additional ES sites.
Benefit use case	ES serves as complementary surveillance to track COVID transmission on a community level, especially useful when clinical surveillance is incomplete or lacking.
Pandemic context	SARS-CoV-2 was first detected in the ES on March 23, 2022, before the rise of cases in Dhaka
Governance and stakeholders involved	Research investigators from icddr,b, the University of Virginia, and Imperial College London in collaboration with the Institute of Epidemiology Disease Control and Research (IEDCR), the leading government institute for COVID-19 research and response, and the Directorate General of Health Services. Results are shared weekly with the national COVID task force, comprised of public health stakeholders and researchers, via a weekly summary report and an interactive dashboard (https://dhakacovidtracker.research.virginia.edu/) for mitigation effort
Incidence settings	Overall high incidence, especially during the delta and omicron waves. For the most part, all wastewater samples positive for SARS-CoV-2 during the pandemic.
Implications for other incidence settings	Suitability in low to high incidence settings: • Highly sensitive method to detect low to high burden on virus in wastewater. • Highly suitable when the transmission is low to detect re-emergence • Good correlation with case data to track the ebbs and flows of the pandemic
Capacity needs	Capability to: • Local government support for ES activity (access to ES sites, approval to sample at those sites) • Team to collect and process the samples within 6 hours of collection maintaining cold chain • Laboratory capable of processing, nucleic acid extraction, PCR, and NGS sequencing • Access to clinical data of the catchment population
Resource settings	Medium-resource setting: • Ability to collect and process samples within 6 hours • No problem with cold chain transportation of samples • Laboratory team is capable of performing sample concentration, nucleic acid extraction, PCR, NGS sequencing

Implications for other resource settings	Suitability in low-resource settings: • Highly suitable where clinical testing is limited. • Highly suitable when clinical surveillance is incomplete or lacking altogether • Useful in areas where there is a converging informal and or formal sewage network.
Comment on cost–benefit	USD $100 per ES sample which is much more cost-effective than testing individuals to understand community level transmission of COVID-19.
Sampling method	Weekly six-litre grab samples are collected at all 33 sites using the Bag Mediated Filtration Kit and processed following protocols described in Philo SE, Ong AQW, Keim EK, Swanstrom R, Kossik AL, Zhou NA, Beck NK, Meschke JS. *Development and Validation of the Skimmed Milk Pellet Extraction Protocol for SARS-CoV-2 Wastewater Surveillance*. Food Environ Virol. 2022 Feb 10:1–9. www.ncbi.nlm.nih.gov/pmc/articles/PMC8830996/. Following the secondary concentration, total nucleic acid is extracted using the QIAamp Mini Stook Kit (Qiagen).
Test method(s) used	RT-qPCR for N1 and N2 gene (CDC assays) on the BioRad CFX96 platform. TaqMan Array Card for 60-plus other enteric pathogens (ie: poliovirus, cholera, typhoid, etc) on the ViiA7 platfrom (Life Technologies). NGS for VOCs using COVIDSeq Kits (Illumina) on the MiSeq and NextSeq platforms (Illumina).

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