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Criteria for Assessing the Relevance of Including Microorganisms in Wastewater Surveillance

GUIDANCE AND RECOMMANDATIONS MARCH 2025

REFERENCE FRAMEWORK

Québec

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FIREWORD

The Institut national de santé publique du Québec (INSPQ) is Québec's public health expertise and reference centre. Its mission is to support the Ministre de la Santé et des Services sociaux, Québec's Minister of Health and Social Services, in carrying out their public health responsibilities. The Institute's mission also includes, to the extent determined by its mandate from the Ministre, supporting Santé Québec, the Nunavik Regional Board of Health and Social Services, the Cree Board of Health and Social Services of James Bay, and other institutions in the exercise of their public health mission.

The Guidance and Recommendations series brings together various scientific works under a single banner; these works highlight the best available scientific knowledge and add a contextual analysis that uses different criteria and deliberations to make recommendations.

This reference framework presents the criteria for assessing the relevance of including specific microorganisms in wastewater surveillance, and is intended to guide the development of this surveillance.

The framework was developed as a joint initiative by the Direction des risques biologiques (DRB), the Direction de la santé environnementale, au travail et de la toxicologie (DSETT), and the Laboratoire de santé publique du Québec (LSPQ) under the INSPQ's COVID-19 surveillance mandate.

This document is intended for professionals and managers involved in guiding infectious disease surveillance or wastewater surveillance programs, as well as the scientific community working in the field.

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LIST OF ACRONYMS AND ABBREVIATIONS

CDC	Centers for Disease Control and Prevention
CEAEQ	Centre d'expertise en analyses environnementales du Québec
COVID-19	Coronavirus disease 2019
INSPQ	Institut national de santé publique du Québec
LSPQ	Laboratoire de santé publique du Québec
MSSS	Ministère de la Santé et des Services sociaux
NASEM	National Academies of Sciences, Engineering, and Medicine
NAAT	Nucleic acid amplification test
CPHLN	Canadian Public Health Laboratory Network
WWTP	Wastewater treatment plant

KEY MESSAGES

- A wide range of pathogens can be detected in wastewater. Wastewater surveillance can therefore be a valuable tool for public health.
- Various initiatives have been launched in several countries to prioritize and assess the inclusion of pathogens in wastewater surveillance, led by both public health organizations and academic researchers.
- This reference framework aims to define guiding principles and evaluation criteria for including infectious agents in the development of wastewater surveillance programs. It draws on expertise gained since the launch of Québec's wastewater surveillance program in March 2021, as well as insights from similar initiatives.
- The decision to include a microorganism must be based on the specific surveillance objectives to ensure that the information collected effectively supports public health decision-making.
- The identified evaluation criteria take into account:
  - Whether the microorganism should be included in the exercise, according to:
    - Its detectability in wastewater;
    - Its public health significance according to the epidemiological situation;
  - Feasibility and the capacity to obtain quality data from wastewater samples;
  - The usefulness and added value of wastewater surveillance data in decision-making;
  - Ease of implementation and supportive contextual factors.
- The weight assigned to each evaluation criterion can be adjusted according to its relevance to the objectives and the available level of evidence.
- The reference framework can be used in different contexts, according to the desired scope of the approach and available resources, i.e.:
  - An ad-hoc need concerning a specific pathogen;
  - Regular evaluation of the targets included in a program;
  - Short-term prioritization of a limited number of infectious microorganisms for which surveillance can be easily deployed (e.g., pathogens monitored by other jurisdictions);
  - Long-term planning in program development.

SUMMARY

The COVID-19 pandemic accelerated the development of wastewater surveillance. This type of surveillance has significant potential as it captures data from the entire population served, irrespective of healthcare use or diagnostic testing. In addition, it can be used to detect a wide range of pathogens. Wastewater surveillance programs worldwide are increasingly diversifying their surveillance targets to take full advantage of the infrastructures used to collect, analyze, process, and disseminate surveillance data. This reference framework aims to define the guiding principles and evaluation criteria for including infectious agents in the development of wastewater surveillance programs. While wastewater surveillance can inform various other types of analysis (drugs and medications, biomarkers, contaminants, etc.), they are not directly covered in this document.

The framework was developed based on the expertise and experience acquired following the Québec research project and deployment of the Québec wastewater surveillance program in March 2021. The process also included a summary exploration of the scientific and grey literature, including the review of several international initiatives. There appears to be a consensus on three main categories of criteria in the documents consulted: public health significance, analytical feasibility, and added value for decision-making.

Guiding principles

The following general principles were applied to guide reflection on the criteria to adopt for the reference framework:

- The choice of pathogens must meet a surveillance need of public health authorities and be useful in decision-making by providing relevant information for assessing the epidemiological situation.
- The chosen perspective is that of protecting human health, although animal and environmental reservoirs may also be taken into account as part of a "One Health" approach.
- Wastewater surveillance is part of an integrated approach to infectious disease surveillance. It can provide a different perspective or redundancy in information sources, thereby increasing the number of indicators and decision-makers' confidence regarding the situation observed.
- The analytical feasibility for a specific pathogen may need to be addressed through further work and methodological development, if justified by public health needs. In such cases, it is important to weigh the effort such development would require against the expected public health benefits.

- Although equity is not a standalone evaluation criterion, it is incorporated into the assessment of certain criteria, namely to enhance the representativeness of populations that are otherwise less well covered. In addition, risks of stigmatization must be taken into account when deploying surveillance.
- The results of the evaluation are specific to the geographical and temporal context in which it is conducted. Re-evaluation may be necessary over time, particularly in light of changes in the epidemiological situation and methodological advances.

Evaluation criteria

The proposed evaluation criteria are grouped into four blocks:

1. **Inclusion of pathogens:** The purpose of this stage is to determine whether a pathogen should be retained for further assessment before proceeding to the other three evaluation blocks. It is divided into two components: detectability in wastewater and public health significance in terms of the microorganism's epidemiology. If the microorganism is retained following this first stage, the surveillance objective(s) are defined to guide evaluation in the other blocks.
2. **Analytical quality of data:** This second block examines the sensitivity and specificity of analytical methods when using the wastewater matrix for microorganism detection and quantification.
3. **Usefulness and added value**: These criteria aim to compare wastewater surveillance with other existing modalities, and to identify whether it will benefit public health decision-making.
4. **Ease of implementation and supportive context**: This block considers the ease of deploying surveillance for a specific pathogen, as well as supportive contextual factors, such as the buy-in of stakeholders and decision-makers.

The evaluation grid proposes factors to consider for each criterion in order to help determine the most suitable response to the situation. The evaluation should also take into account the importance of each criterion to the surveillance objective(s) determined in Block 1, as well as the level of evidence available to evaluate the criterion.

Context for application of the evaluation criteria

The reference framework can be used in different contexts, according to the intended scope of the approach and available resources, i.e.:

- Assessing the ad-hoc need to add a pathogen;
- Regular re-evaluation of the microorganisms monitored in a program;
- Short-term prioritization of a limited number of microbial targets for which surveillance can be easily deployed (e.g., pathogens monitored in wastewater by other jurisdictions);

- More comprehensive, formal prioritization of a large number of pathogens for long-term planning in program development.

The proposed framework provides a systematic evaluation approach, drawing on the findings of scientific work and of the documents consulted, which converge on the key aspects to be evaluated. However, as it is difficult to formulate generic criteria that apply to all pathogens and epidemiological contexts, and as the framework has been tested on a limited number of these, it may need to be adapted in certain contexts. It would be valuable to validate the reference framework to ensure the effectiveness of this proposed tool in different contexts.

1 CONTEXT

Wastewater-based epidemiology is a branch of epidemiology involving the analysis of pathogens, chemicals, and biomarkers present in various forms of human excreta to study the health of populations. The COVID-19 pandemic accelerated its development for the purpose of health surveillance. Beyond COVID-19, wastewater surveillance can be used to detect and monitor different microbiological and chemical targets. Many recent initiatives and publications (1-3) highlight the growing interest in fully exploring the potential of this surveillance approach. Wastewater surveillance programs worldwide are increasingly diversifying the targets they monitor to optimize the use of wastewater surveillance infrastructures, much of which was developed during the pandemic.

In Québec, the SARS-CoV-2 wastewater surveillance program has established an operational structure for data collection, analysis, processing, and dissemination. The INSPQ supports the ministère de la Santé et des Services sociaux (MSSS) in this program, in collaboration with the Centre d'expertise en analyse environnementale du Québec (CEAEQ) of the ministère de l'Environnement, de la Lutte aux changements climatiques, de la Faune et des Parcs (MELCCFP).

Evaluation of Québec's wastewater surveillance program (4) demonstrated that, with SARS-CoV-2, this approach provides complementary surveillance information that makes it possible to characterize virus transmission using a different tool from those used in conventional surveillance (e.g., clinical indicators). The users consulted reported good acceptance of the program and of its indicators in their surveillance practices. It was also noted that wastewater surveillance is useful for increasing confidence in decision-making.

Literature reviews produced by the INSPQ (5) and by the Public Health Agency of Canada (PHAC) (6) on the detection of pathogens other than SARS-CoV-2 in wastewater has demonstrated the potential for extending wastewater surveillance to other microbial targets of interest to public health, while highlighting the importance of assessing their relevance based on clear objectives.

Given the large number of pathogens that can be detected in wastewater, and the limited resources available for surveillance, it seems important to develop a reference framework that supports a systematic approach to assessing potential microbial targets for development. The defined evaluation criteria can also be used to support the evaluation of ad-hoc requests for wastewater surveillance in specific epidemiological contexts.

2 INTRODUCTION TO THE REFERENCE FRAMEWORK

2.1 Objective

This reference framework aims to define the guiding principles and evaluation criteria for assessing pathogen relevance for wastewater surveillance and to propose different contexts for the use of this approach.

2.2 Framework development method

This reference framework was primarily developed based on the expertise and experience acquired by the authors since the deployment of the Québec wastewater surveillance program. The authors are members of a multidisciplinary working group in the fields of infectious disease surveillance, environmental health, and molecular biology. The process was enriched by a summary exploration of the scientific and grey literature, carried out in May 2024. Table 1 presents the main parameters used in this literature review. The keywords used are intended to represent four concepts: surveillance, wastewater, microorganisms, and prioritization. These keywords were combined with Boolean operators. The search was conducted until no further results were added.

NOTE: This literature exploration does not constitute a systematic literature review. The search strategy was developed to provide an overview of the existing literature and to identify key elements related to pathogen surveillance in wastewater. The documents selected were retained as part of a targeted exercise and by no means represent an exhaustive review of publications on the subject. They do, however, help identify the key evaluation criteria relevant to this context.

Reference frameworks from other recognized public health organizations were also consulted. The various reference frameworks found in the literature (section 4) were analyzed to extract the relevant evaluation criteria.

Table 1 Main parameters of the search strategy for the scientific and grey literature

DATABASES	KEYWORDS		DATES
PubMed, Google	• *wastewater based surveillance* • *wastewater based epidemiology* • *wastewater* • *pathogens* • *surveillance strategy*	• *prioritization criteria* • *prioritization* • *selection criteria* • ranking • *infectious disease*	2020 – May 2024

2.3 Scope

Although wastewater surveillance can be used for many types of analysis (drugs and medications, biomarkers, contaminants, etc.), this reference framework applies specifically to infectious microorganisms, including antimicrobial-resistant microorganisms. The framework applies to all conceivable wastewater collection points, from individual facilities (airport, hospital, seniors' residence, etc.) to wastewater treatment plants (WWTPs). It is also primarily designed from a *vigie* (see note) perspective, as this aligns with the current context of the wastewater-based program. However, it could be applied to other surveillance objectives, if the data collected and expertise developed through the program support the use of wastewater for these purposes.

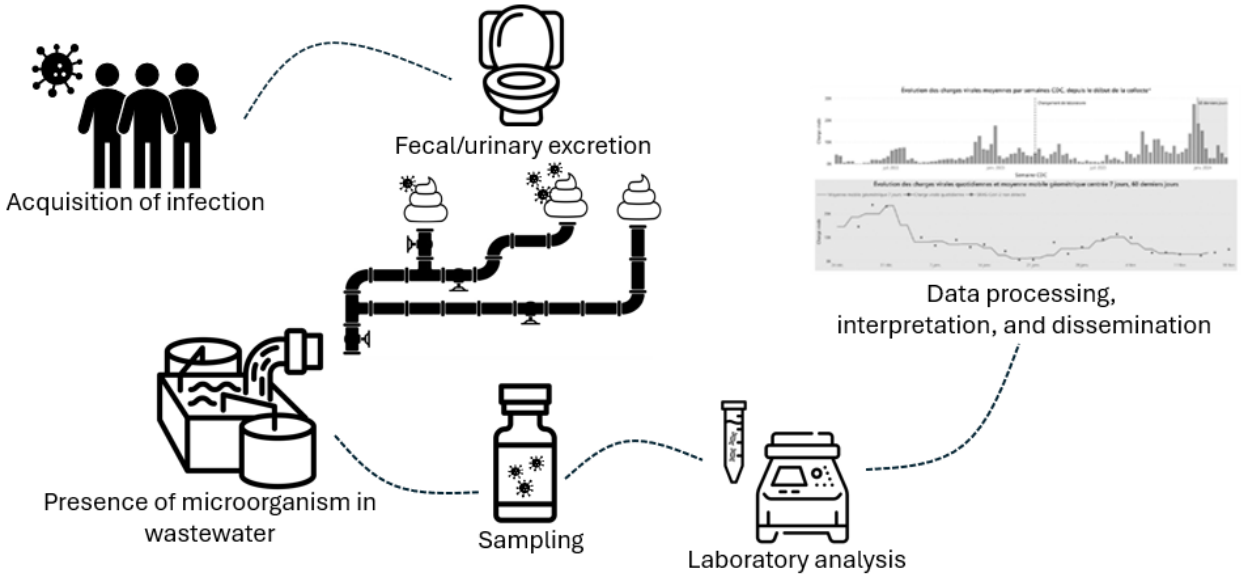
NOTE: In the Québec context, a distinction is made between the terms vigie and surveillance. Vigie serves to identify health threats and deploy interventions to control them. Surveillance, on the other hand, refers to tracking the population's health status and its determinants to identify trends that can guide service planning, especially for prevention (7). As this distinction does not exist in English, the word surveillance is used in this translation to encompass both terms.

3 INFECTIOUS DISEASE SURVEILLANCE IN WASTEWATER

3.1 General principles

Wastewater-based epidemiology operates on the principle that pathogens of interest can be detected in wastewater. They are primarily present due to their excretion in stool or urine by infected individuals, who may be symptomatic or asymptomatic. Moreover, greywater (from personal hygiene use, such as showers or hand washing) can also contribute to the presence of these pathogens in wastewater when they have been directly deposited on the skin. Pathogens excreted in domestic, industrial, or commercial wastewater then travel through the municipal sewer system to a WWTP. Their analysis can be based on sampling at various points in the network: within a building, in a neighbourhood, or at the end of the network at the WWTP. The sampling location and method will determine the population and time period represented. To analyze the contents of the sample, nucleic acid amplification testing (NAAT) of a specific target gene is generally used to detect the presence of the microorganism or to quantify it. Genomic analysis can also be used to identify variants present in wastewater. Indicators are constructed from the laboratory results to inform public health authorities of the population's health status and inform decision-making (figure 1).

Figure 1 How wastewater-based epidemiology works



Wastewater-based epidemiology is a surveillance tool that complements traditional clinical data (cases, hospitalizations, deaths, proportion of positive clinical tests, etc.). Compared with these data, wastewater data have the advantage of being independent of healthcare use and access to clinical tests. Wastewater data are therefore less influenced by testing policies, access to healthcare, and individuals' behaviours and beliefs. In addition, since asymptomatic individuals can excrete pathogens in stool or urine, wastewater surveillance can detect the presence of the pathogen independently of the presence of symptoms. Moreover, a single wastewater sample represents a proportion of a population, making it more cost-effective than clinical surveillance, which requires one sample per individual.

This approach nonetheless has certain limitations. Wastewater data are volatile, as they vary according to environmental factors (e.g., rain, NAAT inhibitors), which can affect detection. Considering that these variables inherent to the method generate noise in the dataset, a longer time series of data is required to obtain an accurate understanding of variability and detection levels in order to properly identify trends. Moreover, these data only provide an indirect estimate of the actual burden, without indicating the exact number of cases or the severity of the infections. The choice of sampling sites greatly influences population representativeness, which can lead to inequalities in the surveillance of certain subgroups. Densely populated urban areas with extensive sewage connections are frequently selected to cover a high percentage of the population. However, rural populations not connected to sewer systems may also be important and valuable for wastewater surveillance, depending on the objectives pursued. Where appropriate, these populations should also be considered in the sampling strategy, despite the logistical and technical challenges associated with them. Furthermore, the disproportionate contribution of certain sectors (e.g., industry, healthcare facilities) can skew geographical representativeness; the selection of sampling sites must also take this parameter into account.

3.2 Wastewater surveillance objectives and modalities

Pathogen surveillance in wastewater helps to meet various surveillance objectives aimed at informing public health authorities. The modalities and deployment are adapted to the needs of the particular objective. In assessing the relevance of different infectious agents for wastewater surveillance, it is essential to consider its objectives to ensure that the information obtained meets these objectives and, ultimately, effectively informs public health stakeholders.

Wastewater surveillance can help achieve a number of objectives:

- Detect the emergence or re-emergence of a microorganism (e.g., poliovirus);
- Detect a change in the circulation levels of an endemic microorganism (e.g., SARS-CoV-2);
- Detect the start of circulation of a cyclical or seasonal microorganism (e.g., influenza);
- Help detect an outbreak and support its investigation (e.g., norovirus in care facilities);
- Monitor the characteristics and genomic evolution of circulating microorganisms (e.g., antibiotic resistance).

By meeting some of these objectives, wastewater surveillance can help to anticipate and document the burden of microorganisms on the population or their impact on the healthcare network, or to monitor the effectiveness of public health measures.

The objectives of wastewater surveillance define its modalities:

- **Qualitative surveillance**

  Qualitative surveillance involves providing information on the absence or presence of the pathogen, without providing information on the concentrations detected. It mainly applies to targets that are not already circulating in the community. It is used to detect the emergence (or re-emergence) of new cases.

- **Quantitative surveillance**

  Quantitative surveillance makes it possible to track circulating microbial load levels. This objective applies mainly to endemic pathogens. It can also be used to characterize the trend in pathogen transmission (increase, decrease, stability) within the community. Microbial load levels can provide information on the intensity of community transmission over different periods.

- **Genomic characterization**

  Genomic characterization involves analyzing the genome of infectious agents to identify the variants circulating within a community. It makes it possible to detect and track target-specific mutations, as well as to identify the presence of specific strains. Genome sequencing is therefore essential for gaining a better understanding of the evolution and spread of pathogens in the population.

Regardless of the objectives and the surveillance method used, the information obtained by wastewater surveillance can be used to monitor the epidemiological situation on an ongoing basis, or to anticipate epidemiological changes.

3.3 Possible deployments

The objectives, pathogen, and surveillance modalities all inform the choice of wastewater surveillance deployment plan. The different possible sampling locations, types, and frequencies influence the results and how they are interpreted.

From a temporal perspective, surveillance can be continuous, used for longitudinal tracking, or conducted on an ad-hoc basis to respond to suspected or confirmed threats. The frequency of sampling will depend on the objectives. A high sampling frequency (e.g., daily) maximizes early detection and mitigates the volatility associated with quantification. A lower sampling frequency (e.g., weekly) makes it possible to monitor less rapidly evolving situations, while reducing costs.

From a geographical perspective, sampling can be deployed at different collection points. The selected collection point will determine the population covered by the surveillance. Sampling at a WWTP at the end of a sewage network can cover all or a significant part of a municipality. It is also possible to sample a specific neighbourhood or facility. The number of collection points and their distribution across the area influence the representativeness of the results obtained.

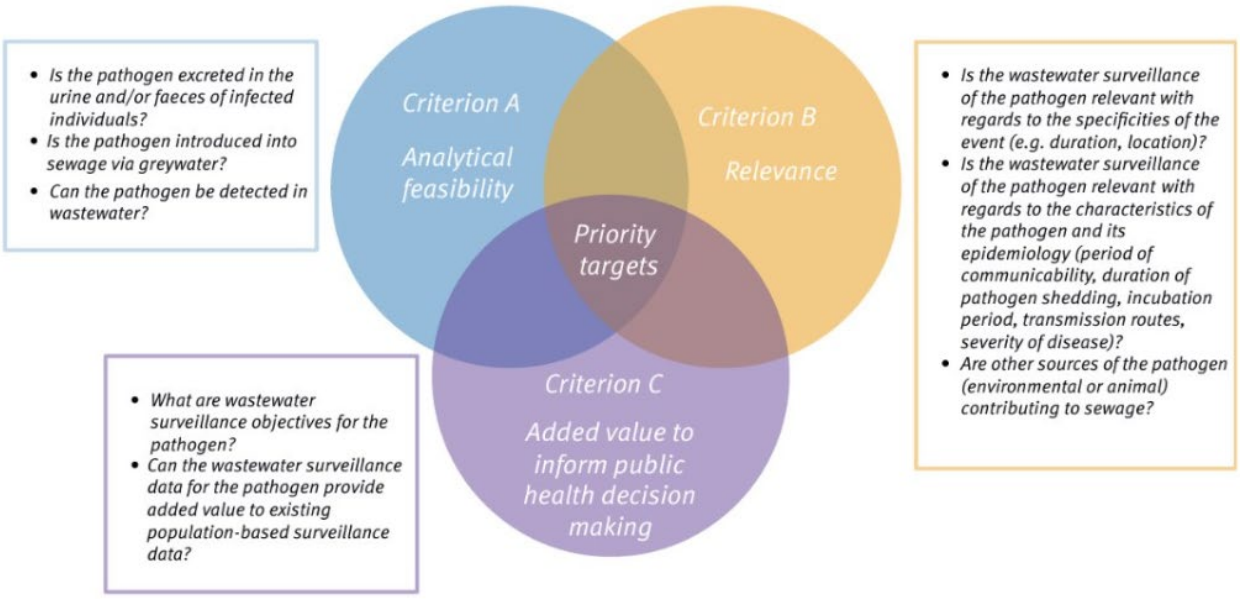
4 OVERVIEW OF SIMILAR INITIATIVES

During the COVID-19 pandemic, a number of wastewater surveillance systems were rapidly deployed around the world. This section presents an exploration of initiatives around the world that have established structured frameworks for evaluating the inclusion of pathogens in these systems.

4.1 In public health organizations

While preparing for the Paris 2024 Olympic and Paralympic Games, Santé publique France explored the possibility of extending wastewater surveillance, already in place in France for SARS-CoV-2, to other targets to monitor the population's health during the events (8). The French authorities compiled a list of 60 infectious agents of potential interest. Each pathogen on the list was evaluated according to: 1) analytical feasibility; 2) epidemiological relevance, given the characteristics of the microorganism and specificities of the event; and 3) the value added to existing surveillance for public health decision-making (figure 2). Their study involved a literature search and consultation with experts using the Delphi method (9). Through this process, they identified six priority targets: poliovirus, influenza A virus, influenza B virus, mpox virus, SARS-CoV-2, and measles virus.

Figure 2 Framework for identifying priority pathogen targets for wastewater surveillance during the Paris 2024 Olympic and Paralympic Games, France



Note : Reproduced from "Pathogen prioritisation for wastewater surveillance ahead of the Paris 2024 Olympic and Paralympic Games, France", by Toro, L., 2024, Euro Surveillance, 29 (28), p.2400231.

In the United States, wastewater surveillance is conducted by the Centers for Disease Control and Prevention (CDC). To better understand how wastewater surveillance can help detect and respond to health threats, an initiative has been set up to evaluate new targets for inclusion. This four-part evaluation considers whether there are existing public health actions, whether the pathogenic microorganism is present in wastewater, and if existing clinical assays can be adapted for its specific detection in wastewater, as well as the geographical distribution of cases (10).

In 2023, the National Academies of Sciences, Engineering, and Medicine (NASEM) published a first report on wastewater surveillance for public health action (11). The report presents a framework for identifying candidate pathogens for wastewater surveillance along with several examples of its application (e.g., influenza, enterovirus, antimicrobial resistance). The framework sets out three key areas of assessment. First, public health significance considers the current or potential severity and distribution of the disease in the population. Second, analytical feasibility assesses whether the microorganism can be detected in wastewater, whether cost-effective methods for its analysis exist, and if the results can be calibrated to public health outcomes. Third, usefulness for action explores the availability of non-wastewater sources of data, the advantages, if any, of wastewater data over alternative data sources, complementarity with other surveillance systems, and the ability of the data to provide early warnings to inform public health decision-making.

In Canada, the Canadian Public Health Laboratory Network (CPHLN) led an initiative to evaluate infectious microorganisms to monitor in wastewater in order to guide approaches on the provincial and national levels. A list of priority criteria in five categories (surveillance objective, relevance, analytical feasibility, added value, equity) was developed by expert consensus.

4.2 In the scientific literature

In addition to these efforts by public health organizations, researchers have undertaken initiatives to guide target prioritization for wastewater surveillance. The following publications define different frameworks for selecting and prioritizing relevant targets for wastewater surveillance.

For the WastPan project in Finland, Tiwari et al. (12) aim to develop wastewater surveillance schemes to track several pathogens simultaneously. Their criteria for selecting new pathogens include:

- Public health significance: Priority is given to targets that pose significant risks to public health.
- Feasibility of detection: The target must be detectable in wastewater using available methods.

- Epidemiological importance: Priority is given to targets with a known epidemiological impact and transmission potential.
- Regulatory and surveillance requirements: This criterion supports the inclusion of pathogens that must be monitored under regulatory requirements or are critical to ongoing public health surveillance.

Gentry et al. (13) propose a ranking system for selecting communicable diseases to include in wastewater surveillance in the Detroit area. Their criteria for the selection of new pathogens include:

- The epidemiological data: Local epidemiological data is used to identify prevalent and emerging microorganisms.
- Health impact: Potential impact on the community.
- Detection capacity: The capacity of assays to reliably detect the target in wastewater samples.
- Availability of resources: Availability of resources and infrastructure to support the surveillance.

Eaton et al. (14) present a framework for evaluating health determinants for wastewater-based epidemiology. Their criteria for selecting new targets include:

- Public health need: The geographic scale must be defined in order to understand the determinant of health and its impact.
- Technical feasibility: There must be existing technology to detect the targets in wastewater using reliable and validated methods.
- Data interpretation: The pathogenic microorganisms must be specific to the targeted determinant of health, and have a stable and consistent relationship over time with this determinant of health.

Overall, this overview of the scientific literature and various international initiatives leads us to consider three key elements, which are found in the majority of documents consulted: public health significance, analytical feasibility, and added value for decision-making. These three attributes guided the development of the present reference framework.

5 REFERENCE FRAMEWORK TO ASSESS THE RELEVANCE OF INCLUDING MICROORGANISMS IN WASTEWATER SURVEILLANCE

Based on this overview of the literature, the working group developed the present framework to assess the relevance of microorganisms for wastewater surveillance. The framework has been built around guiding principles, which informed the development of a criteria grid. These criteria can be used to assess the relevance of microorganisms for wastewater surveillance in different contexts.

5.1 Guiding principles

The following general principles were adopted to guide reflection on the criteria used to evaluate pathogens in the context of this reference framework.

Needs of public health authorities

The selection of infectious targets must meet a surveillance need of the public health authorities and ultimately be useful for action. It should be noted that surveillance alone is itself also a public health measure. In this regard, it is possible that merely having information on the presence, level of circulation, or trend of an infectious agent is useful to public health stakeholders for assessing its epidemiological situation (situational awareness). Moreover, preparing for the next pandemic is an essential priority for public health authorities, and requires building and maintaining surveillance infrastructure that can respond quickly to needs in times of crisis. The experience gained from the wastewater surveillance of certain targets can be transposed and applied to other threats, including those posed by pathogens with high pandemic potential (15).

Human health perspective within a "One Health" context

The chosen perspective focuses on human health. The objects of surveillance are therefore primarily intended to represent the epidemiology of infections in humans. In this context, the presence of non-human reservoirs, which may contribute to the microbial load found in the wastewater, may reduce the precision of the measurement and limit its use. However, from a "One Health" perspective (16), the microorganism's presence in the environment or in an animal reservoir may also indicate an increased risk to humans. In such cases, it may be desirable to detect its presence or monitor its level of circulation in animals (wildlife and livestock) and the environment. The presence of a non-human source of microorganisms in wastewater is therefore not automatically an exclusion criterion for the pathogen; depending on the objectives pursued, it provides information whose impact on the interpretation of results can affect the usefulness of the approach.

Integrated infectious disease surveillance

Wastewater surveillance is part of an integrated infectious disease surveillance approach. It is intended to complement other surveillance approaches. As such, it can be used to obtain different information by, for example, providing insights on the circulation of a disease when many people are asymptomatic (e.g., polio). It is therefore expected that the relationship with the usual surveillance indicators (e.g., cases, deaths, hospitalizations) will not be perfect. This is why usefulness must be evaluated based on the expected level of community transmission, for which there is often no gold standard. Wastewater data can also be used to confirm information obtained from another surveillance source, increasing decision-makers' confidence in the situation observed and actions to be taken. Such redundancy between indicators also ensures continuity if an event disrupts an indicator (e.g., change in test indications). The existence of other sources of surveillance data for the pathogen is therefore not an exclusion criterion. In fact, a variety of complementary indicators allows for a more accurate and contextualized interpretation of the epidemiology, particularly in a pandemic context (11,17).

Efforts required for development

Analytical feasibility, while essential, may need to be addressed through further work and methodological development, if justified by a significant public health need. Therefore, the absence of any well-documented method in the literature or developed by other organizations should not prevent the selection of a pathogen if the evaluation supports its relevance. This criterion nonetheless allows us to gauge the effort that may be required to deploy wastewater surveillance for an infectious agent. This effort should be justified by the expected public health benefits.

Equity and transparency

There is no specific criterion for equity issues related to wastewater surveillance. They are, however, considered in the criteria used to evaluate population representativeness. One of the potential benefits of wastewater surveillance is that it improves surveillance in remote areas where little surveillance is carried out. However, the risks of stigmatizing the sub-populations captured by surveillance activities need to be considered in their deployment. Mitigating these risks requires maintaining transparency with the target populations. Communicating the results and including the population in the decision-making on wastewater surveillance data helps to create a favourable climate of trust.

Context-specific evaluation

While the proposed evaluation criteria (and the principles put forward) are intended to be independent of any epidemiological context, the evaluation results for each infectious microorganism are specific to their context and time of application, mainly due to the potential for developments in the epidemiological situation and methodological advances. In the event of an epidemiological change for an infectious microorganism, a re-evaluation may be necessary to

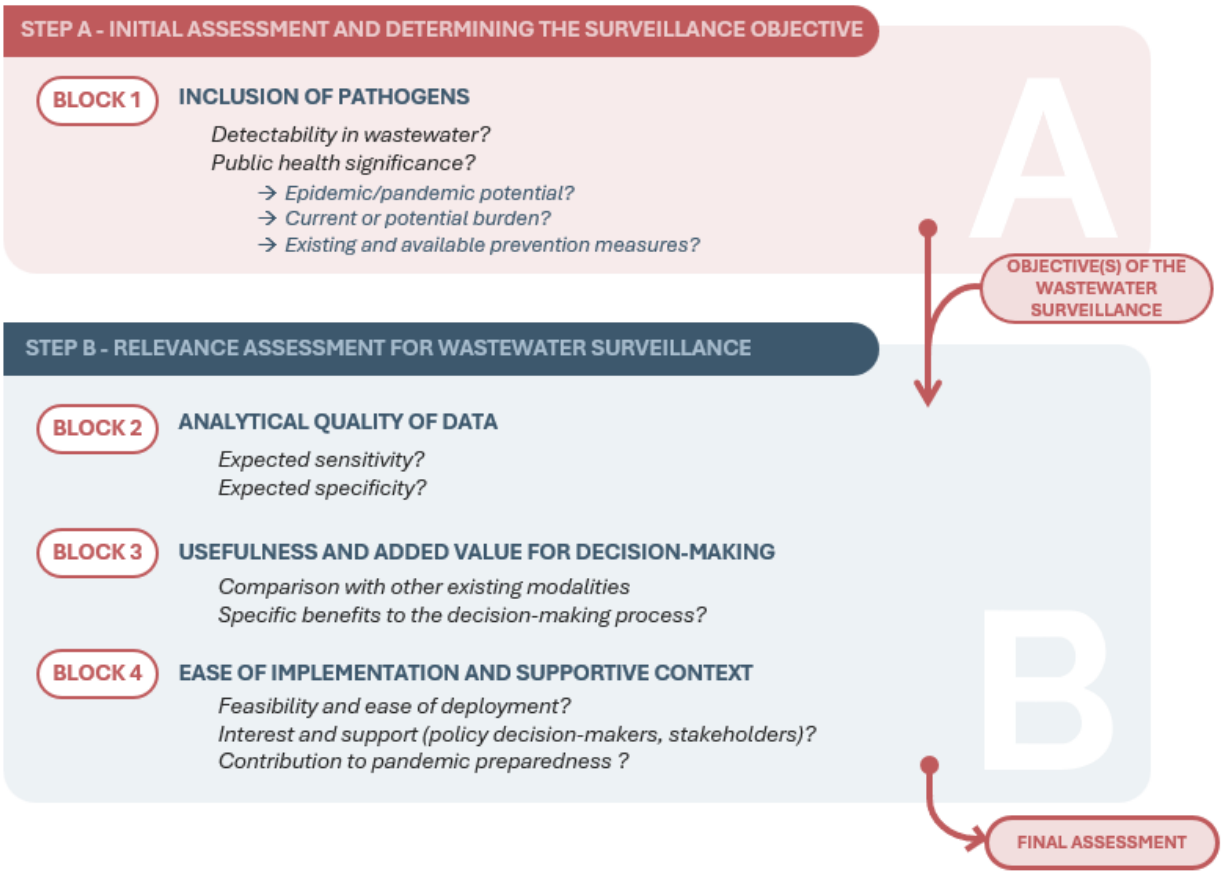
take the new context into account. The evaluation is also specific to the geographical context in which it was carried out. For example, findings from urban settings on a province-wide scale are not necessarily transposable to a small, remote community, and vice versa.

5.2 Evaluation criteria

A grid of 18 evaluation criteria was developed to structure the approach and ensure that all key factors are considered when evaluating the target pathogen. The criteria grid is presented in appendix 1.

- These criteria have been grouped into two main steps and four separate blocks (figure 3).
- Each block evaluates a specific aspect of the pathogen being considered for inclusion in wastewater surveillance.
- Each criterion is presented in the form of a multiple-choice question (appendix 1).
- Each criterion is also accompanied by sub-questions (appendix 1), which represent factors to consider that may influence the answer to the main question. They are provided to help guide the evaluation process. These factors are neither exhaustive nor prescriptive. New sub-questions can be added when using the questionnaire to consider angles not initially anticipated. Similarly, certain factors that do not need to be systematically considered can be ignored.
- Four criteria are considered essential for wastewater surveillance, and an unfavourable response to any one of these criteria means that the microorganism is rejected for inclusion and the evaluation process is terminated.
- This approach is mainly qualitative and relies on the judgement of the experts consulted. It provides an overview of the strengths and weaknesses of wastewater surveillance for a specific pathogen.
- Given the diverse objectives of wastewater surveillance, the importance of each criterion to the established surveillance objective should affect its weight in the evaluation. Depending on the surveillance objective, a criterion may be irrelevant and not taken into account in the evaluation. At the other extreme, a specific positive response may be necessary to guarantee the success of an objective.
- As this field of expertise is still developing, it is important to qualify the evaluation according to the level of evidence available for each criterion.

Figure 3 **Summary of pathogen evaluation criteria for wastewater surveillance development**



STEP A — INITIAL ASSESSMENT AND DETERMINING THE SURVEILLANCE OBJECTIVE

BLOCK 1: INCLUSION OF PATHOGENS

This first block assesses whether a pathogen and its epidemiological situation present characteristics that warrant further assessment. It has two components:

- The pathogen's detectability in wastewater
  - **Question 1** **(ESSENTIAL) Is the microorganism detectable in wastewater?**

- Public health significance, which determines whether the pathogen's epidemiological situation poses a potential or significant threat to the population, and whether effective interventions are possible.
  - **Question 2** **(ESSENTIAL) Does the microorganism pose the risk of an outbreak or have epidemic/pandemic potential in the area in question?**
  - **Question 3** **Does the microorganism currently represent a burden on the community and healthcare system?**
  - **Question 4** **Would the microorganism represent a burden on the community and healthcare system in the event of an epidemic (pandemic preparedness)?**
  - **Question 5** **Will prevention or control measures be available and deployable if a signal is detected?**

SURVEILLANCE OBJECTIVE(S)

If the pathogen is deemed relevant for inclusion in this initial assessment, the surveillance objective(s) are to be defined before proceeding to the criteria in the next block. This step is essential, since in the following blocks these objectives will guide evaluation of the criteria, the importance of which will be adjusted based on their relevance to the objective. These objectives are based on those defined in section 3.2. Several objectives can be pursued for the same pathogen.

STEP B — RELEVANCE ASSESSMENT FOR WASTEWATER SURVEILLANCE

BLOCK 2: ANALYTICAL QUALITY OF DATA

The second block addresses the analytical quality of the data obtained from wastewater. It examines two key aspects: the expected sensitivity and specificity of the detection methods in the wastewater matrix. Both laboratory methods and the significance of information from wastewater for the pathogen are evaluated.

- **Question 6** **(ESSENTIAL) Does the population at risk contribute to the wastewater?**
- **Question 7** **(ESSENTIAL) Are the quantities expected at the selected sampling site sufficient for detection or quantification (depending on the objective)?**

- **Question 8 Are analytical methods suitable for achieving the objectives?**
- **Question 9 Are there any strains non-pathogenic to humans that could be detected in wastewater?**
- **Question 10 Is there a relationship between the wastewater indicator and the microorganism's level of circulation?**
- **Question 11 Are there any animal, environmental, or industrial reservoirs that may contribute to the microorganism's presence in wastewater?**

BLOCK 3: USEFULNESS AND ADDED VALUE FOR DECISION-MAKING

The third block assesses the usefulness and added value of wastewater surveillance for public health decision-making. It compares wastewater surveillance to other existing modalities, and examines the specific benefits that this approach can bring to the public health decision-making process.

- **Question 12 Is the planned spatial resolution useful to the intervention?**
- **Question 13 Does wastewater surveillance capture a population that complements those reached by other surveillance modalities?**
- **Question 14 Is it possible to obtain a temporal resolution that is useful for decision-making?**
- **Question 15 Do the wastewater data represent an additional data source to other surveillance systems?**

BLOCK 4: EASE OF IMPLEMENTATION AND SUPPORTIVE CONTEXT

The fourth and final block addresses the context, as well as the ease of deploying wastewater surveillance for the evaluated microorganism. The interest and support of policy decision-makers and stakeholders is taken into account, as is the contribution to pandemic preparedness.

- **Question 16 Do the existing infrastructure and processes support the deployment of surveillance of this microorganism in a way that aligns with the objectives?**
- **Question 17 Do the community and/or decision-makers support the surveillance of this microorganism?**
- **Question 18 Does deploying wastewater surveillance for this microorganism contribute to pandemic preparedness?**

FINAL EVALUATION

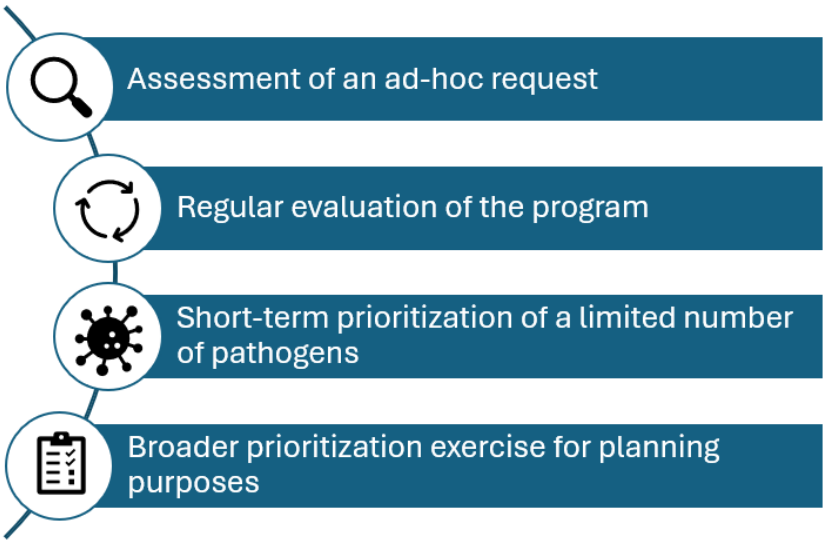
The answers to each of these criteria will inform the final evaluation of the pathogen, and its resulting inclusion or prioritization for inclusion in wastewater surveillance. To consolidate the results of this evaluation, the method and template provided in appendix 2 can be used to highlight key findings from the data collected. This synthesis provides an executive summary of the assessment of the pathogen's relevance for wastewater surveillance, based on each of the proposed evaluation blocks.

NOTE: The results of the evaluation are specific to the geographical and temporal context in which it is carried out. Re-evaluation may be necessary over time, particularly in light of changes in the epidemiological situation and methodological advances.

5.3 Contexts for application of the evaluation criteria

The proposed criteria grid provides a systematic approach for evaluating the various factors that support or oppose the inclusion of an infectious agent in a wastewater surveillance program. This can be done in different contexts (figure 4), which will affect the extent of the evaluation required and, consequently, the resources needed.

Figure 4 Contexts for applying the evaluation criteria to assess the relevance of including microorganisms in wastewater surveillance



First of all, the criteria can guide the response to an ad-hoc request to include an infectious microorganism in the wastewater surveillance program, often in response to a specific epidemiological situation (e.g., the measles outbreak in Québec in spring 2024).

Similarly, the grid can be used to periodically reassess the elements of a program to determine whether the monitored targets remain relevant in light of their epidemiological situation. Use of the criteria helps standardize the response in situations where the relevance of including a pathogen in wastewater surveillance must be assessed or reassessed quickly.

Likewise, the criteria grid can support the short-term prioritization of pathogens for inclusion in wastewater surveillance. Evaluating a limited number of microorganisms—particularly those for which deployment is facilitated by established methodologies, for example pathogens already monitored in wastewater by other public health jurisdictions—is well suited to this context. A small team of experts from relevant fields (laboratory science, wastewater management, environmental health, infectious diseases) could conduct a comparative evaluation of target pathogens using the various criteria, drawing on insights from a quick examination of the literature.

Finally, the criteria grid is also useful for long-term planning exercises requiring a more exhaustive and formal prioritization of a large number of pathogenic microorganisms. This systematic exercise would make it possible to consider pathogen targets that would require methodological development or different strategies for sampling deployment. This exercise could be based on established criteria, using prioritization methodologies like the Delphi method or multi-criteria analysis (18,19). In such cases, a method for calculating a score for quantitative evaluation could be developed and validated. As this approach requires greater resources and the involvement of multiple experts, it must be justified by a willingness to invest in the development of this surveillance tool, or to develop medium- or long-term guidance. This prioritization exercise should draw on a range of expertise to address all the criteria, sometimes in contexts where limited scientific evidence is available to support the evaluation. It is also possible to group pathogens according to, for example, their mode of transmission, thus limiting the number of resources involved and the time devoted to the exercise.

6 STRENGTHS AND LIMITATIONS OF THE REFERENCE FRAMEWORK

This framework has been designed based on a review of key publications on the subject, and enriched by the multidisciplinary expertise of the editorial team. The authors come from diverse fields of expertise, including infectious diseases, molecular biology, environmental health, health surveillance, and wastewater-based epidemiology. Together, they have drawn on their knowledge and skills to cover all relevant angles. The findings of all the scientific work and documents consulted converge on the main aspects to be evaluated, increasing confidence in the proposed tool. Moreover, by enabling a systematic analysis of the relevance of microorganisms for wastewater surveillance, this reference framework ensures a transparent decision-making process in the development of this surveillance approach. One of the major strengths of the evaluation grid is its flexibility and the range of contexts in which it can be used. However, given the wide variety of possible contexts, this framework does not prescribe a methodology for consultation (e.g., Delphi method) in the evaluation of pathogens, as this will have to be tailored to the purpose and scope of the intended prioritization exercise.

This framework has so far been tested on a limited number of pathogens, and may require some adjustments when applied. It is, after all, difficult to propose generic responses to the various criteria that apply to all pathogens and epidemiological situations. Some criteria may therefore need to be modified or adapted when using the framework in certain contexts to ensure it remains a relevant and effective tool. To ensure a good level of consensus between multiple assessors, interrater reliability may need to be validated to confirm the consistency in evaluation results across several observers. Finally, the framework may need to be updated to reflect new knowledge and developments in the use of wastewater for epidemiological surveillance, even though its general structure should ensure its longevity as a tool.

7 CONCLUSION

The scientific literature lists over 600 pathogens that are potentially detectable in wastewater (6), illustrating the great potential of this source of information. The large number of possibilities, combined with the limitations of available resources, justify the use of a systematic approach when evaluating candidate pathogens for inclusion in wastewater surveillance. The reference framework proposed in this document provides an analysis structure and precise criteria to facilitate a rigorous and transparent evaluation of potential targets.

These criteria cover essential factors such as public health significance, feasibility and analytical quality, and usefulness and added value for decision-making. In addition, they consider logistical aspects and the context in which the program is implemented, offering a comprehensive evaluation tool that can be adapted to the different needs of stakeholders. This reference framework can be used in a variety of contexts, from ad-hoc analysis of specific pathogens to strategic planning of surveillance programs.

The detailed analysis that will result from the use of the criteria grid will provide a clearer and more structured vision of the benefits and challenges of wastewater surveillance for each pathogen, thus promoting informed decisions and more effective surveillance planning.

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APPENDIX 1 CRITERIA GRID

Block 1: Inclusion of pathogens			
Detectability in wastewater			
1. *ESSENTIAL: Is the microorganism detectable in wastewater? Factors to consider: - Is the microorganism excreted in either stool or urine? - Do hygiene measures contribute to the presence of the microorganism in grey water? - Has the microorganism ever been detected in wastewater?	The microorganism is not detectable in wastewater	The microorganism is detectable in wastewater	
Public health significance			
2. *ESSENTIAL: Does the microorganism pose the risk of an outbreak or have epidemic/pandemic potential in the area in question? Factors to consider: - How is the microorganism transmitted? - What is its basic reproduction number (R0)? - What's the duration of contagiousness? - Is it transmissible in the presymptomatic and asymptomatic periods? - What is the level of immunity in the population? - Are the reservoir or vectors present in the area? - Does the pathogen have a high risk of genetic mutation? - Does the microorganism have a special surveillance status (e.g., extreme surveillance)?	The microorganism has no epidemic or pandemic potential	The microorganism has epidemic potential, which may be limited or theoretical	The microorganism has high or proven epidemic or pandemic potential
3. Does the microorganism <u>currently</u> represent a burden on the community and healthcare system? Factors to consider: - What are the prevalence, morbidity, and mortality associated with the microorganism within the context? - How many potential years of life lost (PYLL) are associated with infection by this pathogen? - What costs does this pathogen entail for the healthcare system and society?	The microorganism does not represent a significant burden for the community and healthcare system	The microorganism imposes a moderate burden on the community or healthcare system (e.g., local outbreak)	The microorganism imposes a heavy burden on the community (e.g., Strep A) or the healthcare system (e.g., influenza), with significant impacts

Block 1: Inclusion of pathogens			
Public health significance			
4. Would the microorganism represent a burden on the community and healthcare system in the event of an epidemic (pandemic preparedness)? Factors to consider: - What is the extent of transmission and anticipated severity? - How many potential years of life lost (PYLL) could be associated with an epidemic caused by this pathogen? - What costs would an epidemic caused by this pathogen entail for the healthcare system and society?	The microorganism does not represent a significant burden for the community or healthcare system	The microorganism presents a risk of a large number of expected cases with low to moderate severity, or a risk of a small to moderate number of expected cases with high severity	The microorganism presents a risk of a large number of expected cases with high severity
5. Will prevention or control measures be available and deployable if a signal is detected? Factors to consider: - Are the following measures possible? (Non-exhaustive list): o Education, awareness, and communication campaigns o Investigation and/or tracing of cases and contacts o Increased clinical screening o Vaccination campaigns o Non-pharmacological mitigation measures like isolation, quarantine, masking, etc.	No prevention or control measure available or deployable	Some prevention or control measures available, but their deployment is limited or has never been carried out	Effective prevention or control measures are available and deployable if a signal is detected

Block 2: Analytical quality of data			
Sensitivity			
6. *ESSENTIAL: Does the population at risk contribute to the wastewater? Factors to consider: - What is the population at risk of excreting the microorganism? - In what geographic area is the microorganism circulating or could the microorganism circulate? - Are the at-risk populations and communities connected to a wastewater collection system from which wastewater samples can be taken?	The population at risk does not significantly contribute to the wastewater	The population at risk contributes to the wastewater	
7. *ESSENTIAL: Are the quantities expected at the selected sampling site sufficient for detection or quantification (depending on the objective)? Factors to consider: - What is the proportion of excretors among infected individuals? - What is the quantity excreted by infected individuals? - What is the prevalence of infection in the target population? - Can the microorganism be found at the planned sampling site (persistence in wastewater)?	The expected quantities in wastewater are too small to be detectable or quantifiable	Detection/quantification is only possible when there is high community circulation of the microorganism	The expected quantities are large enough for detection/quantification, regardless of the microorganism's level of circulation in the community
8. Are analytical methods suitable for achieving the objectives? Factors to consider: - Is there any evidence of the microorganism's detectability in wastewater? - What analytical methods are suitable for the pathogen, and how advanced are they? o Sampling o Extraction of genetic material o NAAT detection/quantification o Variant detection	Analytical method non-existent or difficult to develop	Analytical method requires some development	Existing, deployable analytical method

Block 2: Analytical quality of data

Specify

9. Are there any strains non-pathogenic to humans that could be detected in wastewater? Factors to consider: - What is the impact of the presence of vaccine strains? - Is there a high degree of similarity between the target pathogen and microorganisms naturally found in the environment?	Non-pathogenic strains may interfere with detection in wastewater		No non-pathogenic strains can be detected in wastewater
10. Is there a relationship between the wastewater indicator and the microorganism's level of circulation? Factors to consider: - Is the duration of excretion limited to the contagious period? - Are there chronic carriers who continue to excrete without being contagious? - Is the microorganism capable of multiplying in the wastewater environment?	No stable relationship exists	There is some expected variability in the relationship or the relationship is weak	There is a stable relationship between wastewater indicators and clinical indicators
11. Are there any animal, environmental, or industrial reservoirs that may contribute to the microorganism's presence in wastewater? Factors to consider: - What is the geographical distribution of reservoirs and vectors? - Is the wastewater content subject to environmental contamination? - Are there any agricultural/industrial activities in the area covered that could contribute to the presence of the microorganism in the wastewater?	The presence of significant reservoirs interferes with the surveillance objectives	A few reservoirs may exist, but their contribution is limited or does not interfere with the surveillance objectives	No significant reservoirs exist or, if they do, they support the achievement of the surveillance objectives

Block 3: Usefulness and added value for decision-making			
12. Is the planned spatial resolution useful to the intervention? Factors to consider: - Are population-based interventions relevant at this scale? - Where do the risks of infection and transmission occur that could be targeted by prevention and control measures?	The spatial resolution of the wastewater data is insufficient for the intervention	The spatial resolution of the wastewater data is useful to the intervention	
13. Does wastewater surveillance capture a population that complements those reached by other surveillance modalities? Factors to consider: - What proportion of infected individuals are asymptomatic? - What proportion of symptomatic cases are detected by the existing surveillance systems? - Can asymptomatic individuals transmit the infection?	Other surveillance modalities already capture the majority of cases	Wastewater surveillance complements the population captured by other surveillance modalities	Wastewater surveillance provides a way to capture a population that is difficult/impossible to capture through other surveillance modalities
14. Is it possible to obtain a temporal resolution that is useful for decision-making? Factors to consider: - How long is the time period from infection to excretion? - How long is the time period before symptom onset? - How quickly can a wastewater surveillance logistics chain be deployed?	Wastewater surveillance provides a signal too late to support intervention	Wastewater surveillance provides a synchronous or timely signal for intervention	Wastewater surveillance can provide an early warning (ahead of other indicators) for intervention
15. Do the wastewater data represent an additional data source to other surveillance systems? Factors to consider: - What are the other sources of data? - What is the quality and reliability of the existing data?	There are already several sources of high-quality data for the object of surveillance	There are already some data sources of varying quality, but wastewater data can be used to confirm or supplement them	Wastewater is the only source of data for this microorganism, or it provides a different kind of information for this microorganism

Block 4: Ease of implementation and supportive context			

16. Do the existing infrastructure and processes support the deployment of surveillance for this microorganism in a way that aligns with the objectives?
Factors to consider:
- Can existing processes be used or adapted for:
  o The sampling plan (frequency/site)?
  o Data processing (indicators)?
  o Data dissemination (reporting)?
	Surveillance of this microorganism cannot be deployed using the infrastructure and processes in place	Surveillance of this microorganism can be deployed with minor adjustments to the infrastructure and processes in place	Surveillance of this microorganism can be deployed quickly and easily using the infrastructure and processes in place

17. Do the community and/or decision-makers support the surveillance of this microorganism? Factors to consider: - Is there media interest? - Is it consistent with policy or regulatory frameworks? - Is there public engagement and/or concern? - Is funding available?	The community and/or decision-makers do not support surveillance of this microorganism	There is community and/or decision-maker support for surveillance of this microorganism	The community and/or decision-makers strongly support the surveillance of this microorganism
18. Does deploying wastewater surveillance for this microorganism contribute to pandemic preparedness? Factors to consider: - Will new methodologies be developed as a result of the wastewater surveillance? - Will the wastewater surveillance lead to new partnerships? - Will wastewater surveillance prompt the development of new channels of communication and dissemination?	Deployment would contribute little to future pandemic preparedness efforts	Deployment would theoretically contribute to future pandemic preparedness efforts	Deployment would contribute directly and concretely to future pandemic preparedness efforts for a high-risk pathogen

APPENDIX 2 QUALITATIVE ASSESSMENT METHOD

The final evaluation summarizes the strengths and weaknesses of wastewater surveillance for a given pathogen, systematically considering all factors in the criteria grid. This can be achieved using the following template to compile the key findings from the evaluation data collected. This synthesis provides an executive summary of the relevance of including a pathogen in wastewater surveillance, based on each of the criteria blocks.

Procedure

Step 1: Complete the Block 1 evaluation.

- Document all information relevant to the evaluation of each criterion (Column A). The list of factors to consider (appendix 1) should guide the evaluation.
- Use the responses (Column B) selected from the options (appendix 1) to make a critical judgement on the criterion.
  - If the answer to an essential question (*) is unfavourable (i.e., the leftmost option in the grid), the evaluation can be terminated.

Step 2: Define the surveillance objective.

- Possible wastewater surveillance objectives are included in section 3.2 of the reference framework.

Step 3: Complete the evaluation in blocks 2, 3, and 4.

- Document all information relevant to the evaluation of each criterion (Column A). The list of factors to consider should guide the evaluation.
  - The blocks can be documented sequentially or simultaneously.
- Use the responses (Column B) selected from the options (appendix 1) to make a critical judgement on the criterion.
  - If the answer to an essential question (*) is unfavourable (i.e., the leftmost option in the grid), the evaluation can be terminated.

Step 4: Compile the key findings (Column C) to make a qualitative assessment.

The relative weight assigned to each evaluation criterion can be adjusted according to its relevance to the surveillance objectives and the available level of evidence (see section 5.2 of the main document).

Fictional example:

Microorganism XYZ				
	Criteria	Column A Relevant information	Column B Applicable response selected	Column C Highlights Important¿This.is.a.fictitious.example.using.broad.generalizations¡
Block 1	1*	Use this column to document all relevant information (facts, findings, knowledge, limitations, etc.) to guide how each criterion is assessed.	Use this column to document the response selected from the criteria grid (appendix 1).	High.pandemic.potential?with.risk.of.mutation.generating.new.circulating.variants.for.which.herd.immunity.is.low¡.Potentially.large.burden.on.the.healthcare.system¡
	2*			
	3			
	4			
	5			
Surveillance objective				Document.the.wastewater.surveillance.objective
Block 2	6*			There.are.quantification.challenges.when.levels.are.low¡.The.variability.in.environmental.samples.makes.it.difficult.to.interpret.trends¡
	7*			
	8			
	9			
	10			
	11			
Block 3	12			Although.monitored.through.several.clinical.indicators?the.majority.of.these.are.subject.to.biases.influencing.their.quality¡.Wastewater.provides.a.complementary.angle.that.captures.the.entire.population.regardless.of.access.to.testing?as.well.as.asymptomatic.patients¡.Evidence.of.the.potential.for.early.detection.is.still.limited¡.
	13			
	14			
	15			
Block 4	16			Wastewater.surveillance.is.much.less.costly.than.clinical.surveillance?and.offers.very.high.coverage.rates¡.The.infrastructure.and.processes.already.in.place.are.transferable?¡e¡?surveillance.for.this.pathogen.can.be.quickly.deployed¡
	17			
	18			

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