Wastewater and environmental surveillance for one or more pathogens

Guidance on prioritization, implementation and integration

Pilot version

6 Dec 2024

Disclaimer

This document is currently a working draft open to public comment and pilot testing in regions and countries during 2025. The content, findings and conclusions of this document are subject to ongoing update. The document will pass though final clearance from WHO and CDC once public and pilot feedback has been incorporated and agreement from the WES expert group and relevant disease focal points has been reached.

The findings and conclusions of this report are those of the authors and do not represent the official position of the World Health Organization, the United States Centers for Disease Control and Prevention (CDC) or other institutions.

Contents

List of boxes, tables and figures

Acknowledgements

WHO steering group and disease focal points

Steering
Kate Medlicott – Water, sanitation, hygiene and health (WSH), Acya Acme - Emerging diseases and zoonoses (EZD), Jane Cunningham – Emerging diseases and zoonoses (EZD), Emilie Peron – Surveillance systems (SRV), Josefina Campos – Collaborative intelligence (COL)

Disease focal points
Mpox: Ana Hoxha – Acute events epidemiology (AEE), Rosamund Lewis – Emerging diseases and zoonoses (EZD), Lorenzo Subissi – Emerging diseases and zoonoses (EZD), Olivier Le Polain - Acute Events Analytics (ANA)
Polio: Ousmane Diop - Polio Surveillance and laboratory (PSL), Mufti Zubair Wadood - Surveillance, Labs & Data (SLD)
Cholera: Laurant Sax - High Impact Epidemics (EHI)
Influenza: Melissa Rolfes Global Influenza Programme (GIP)
COVID-19: Jane Cunningham – Emerging diseases and zoonoses (EZD, Acya Acme - Emerging diseases and zoonoses (EZD)
Typhoid: Anna Minta - Immunization Analysis & Insights (IAI), Musa Hindiyeh - Immunization Analysis & Insights (IAI)

Regional advisors

(to be added after regional pilot phase)

US-CDC steering group and disease focal points

Steering
David Berendes - Global WASH, Division of Foodborne, Waterborne, and Environmental Diseases (DFWED); Jennifer Murphy - Global WASH, DFWED; Heather Reese – National Wastewater Surveillance System (NWSS), Division of Infectious Disease Readiness and Innovation (DIDRI);

Wastewater and environmental surveillance focal points
Megan Gerdes – NWSS, DIDRI; Alexandra Kossik - Global WASH, DFWED; Maya Ramaswamy - Global WASH, DFWED;Megan Bias – NWSS, DIDRI

Disease focal points
Mpox: Christina Hutson – Division of High-Consequence Pathogens and Pathology
Polio: Amanda Wilkinson – Global Immunization Division; Nancy Gerloff – Division of Viral Diseases
Cholera: David Shih, Joan Brunkard, Javier Gallard, Maryann Turnsek, Ash Wadhwa, Xin Wang – DFWED
Influenza: Alicia Budd – Influenza Division
COVID-19: Cria Gregory, Clinton Paden, Coronavirus and Other Respiratory Viruses Division
Typhoid: David Shih – DFWED

Lead consultants

Monica Nolan, WHO consultant medical epidemiologist, Australia
Daniel Deere, WHO consultant environmental microbiologist, Australia

Expert contributors

Jean-Martin Brault, World Bank Group, USA; Juliana Calabria de Araujo - Universidade Federal de Minas Gerais, Brazil; Angus Dawson, Long Yoo Yin School of Medicine, Singapore; Ana Maria de Roda Husman, National Institute for Public Health and the Environment (RIVM), The Netherlands; Nishita d'Souza, Michigan State University, USA; Steen Ethelberg, Statens Serum Institut, Denmark; Bernd Gawlik, European Commission – Joint research centre, Italy; Nicholas Grassly, Imperial College London, UK; Fatma Guerfali, Institute Pasteur, Tunisia; Rashidul Haque, icddr,b, Bangladesh; Joanne Hewitt, Institute of Environmental Science and Research , New Zealand; Nalia Ismail - Instituto Nacional De Saude – Mozambique; Tim Julian – Swiss Federal Institute of Aquatic Science and Technology (eawag), Switzerland; Amy Kirby, US Centers for Disease Control and Prevention, USA; Bilge Alpaslan Kocamemi, Turkish Water Institute (SUEN), Turkiye; Supriya Kumar (Observer), Bill and Melinda Gates Foundation, USA ; Sophie Magnet , Independent consultant, Switzerland; Douglas Manuel, Ottawa Hospital, Canada; Christine Moe, Emory University, USA; Placide Mbala - Institut National d'Hygiène Publique, Democratic Republic of Congo; Kerrigan McCarthy - National Institute for Communicable Diseases, South Africa; Gertjan Medema - KWR Water Research Institute – The Netherlands; Scott Meschke - University of Washington, USA; Imran Nisar, Aga Khan University, Pakistan; Titik Nuryastuti, Universitas Gadjah Mada, Indonesia; Lusungo Nyirenda, Lusaka Sanitation Programme, Zambia; Michael Owusu - Kwame Nkrumah University of Science and Technology, Ghana; Samuel O. Oyola - International Livestock Research Institute, Kenya; Tarja Pitkänen - Helsinki One Health, Finland; Maximillian, Riess, ECDC, Sweden; Renee Street - South African Medical Research Council -South Africa; Mami, Taniuchi, University of Virginia, USA; Eunice Ubomba Jaswa, Water Research Commission, South Africa; Márta, Vargha, National Center for Public Health and Pharmacy, Hungary; Henry Willis, RAND, USA; Judith, Wong, National Environment Agency (NEA), Singapore; Mukhlid Yousef - National Institute for Communicable Diseases, South Africa.

WHO gratefully acknowledges funding support from the Bill and Melinda Gates Foundation (BMGF), the European Commission Health emergency preparedness and response (EC-HERA) and the Institute of Philanthropy (IoP). WHO also gratefully acknowledges in-kind support and partnership with the United States Centres for Disease Control and prevention (CDC) and partial support to CDC from Interagency Agreement with the US Agency for International Development (USAID).

Acronyms

Acronym	***Meaning***
AFP	Acute flaccid paralysis
AMR	Antimicrobial resistance
APHL	Association of Public Health Laboratories
ARG	Antimicrobial resistance gene
BMGF	Bill & Melinda Gates Foundation
BSC	Biological safety cabinets
BSL	Biosafety Level
CDC	United States Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
DOI	Declaration of interest
dPCR	Digital polymerase chain reaction
E. coli	*Escherichia coli*
ESBL	Extended beta-lactamase-producing
ERG	External Review Group
FAO	Food and Agriculture Organization of the United Nations
Flu	Influenza
GDG	Guideline Development Group
GEPI	Global Polio Eradication Initiative
GI	Gastrointestinal
GLOWACON	Global Consortium for Wastewater and Environmental Surveillance for Public Health
GTFCC	Global Task Force on Cholera Control
GWASH	Global Water Sanitation and Hygiene [of the CDC]
ICC-PCR	Integrated cell culture-polymerase chain reaction
IVA/IVB	Influenza A virus / Influenza B virus
MPXV	Monkeypox virus
MS2	MS2 is a F-RNA bacteriophage. It can be used as an internal control in PCR analysis
NADH	Nicotinamide adenine dinucleotide

NGO	Non-governmental organization
NGS	Next generation sequencing
NWSS	National Wastewater Surveillance System [of the CDC]
OHS	Occupational Health and Safety
PCR	Polymerase chain reaction
PHSM	Public health and social measures
PMMoV	Pepper mild mottle virus
PPE	Personal protective equipment
PV	Poliovirus
QMS	Quality Management System
qPCR	Quantitative polymerase chain reaction
QSE	Quality System Essentials
rDNA	Ribosomal ribonucleic acid
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcription-polymerase chain reaction
RT-qPCR	Reverse transcription- quantitative polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
STI	Sexually transmitted infections
TAT	Turn around time
UNEP	United Nations Environment Programme
VOC/VOI/VUM	Variants of concern, variants of interest, variants under monitoring.
WASH	Water, sanitation and hygiene
WBE	Wastewater Based Epidemiology
WES	Wastewater and Environmental Surveillance
WGS	Whole genome sequencing
WHO	World Health Organization
WOAH	World Organisation for Animal Health
WWTP	Wastewater treatment plant

Glossary

Terms	***Meaning or use in this document***
Antimicrobial resistance	Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi and parasites have antimicrobial resistance genes (ARG) and are no longer susceptible to antimicrobial products. AMR is increasing due to the use and misuse of antimicrobials and a complex ecology involving humans, animals and the environment. AMR is a significant threat to global human, animal and plant health.
Collaborative Surveillance	Collaborative surveillance [1] is intended to reinforce frameworks and strategies for strengthening surveillance, risk assessment, and response to emergencies. Collaborative surveillance is the systematic strengthening of capacity and collaboration among diverse stakeholders, both within and beyond the health sector, with the ultimate goal of enhancing public health intelligence and improving evidence for decision-making. The collaborative surveillance concept builds upon robust routine public health surveillance, health systems monitoring, and laboratory surveillance, while drawing insights from other data sources and applying advanced data and analytical approaches to enable the generation of contextualized insights on hazards, threats and risks, populations affected, and their contexts. WES supports the operationalisation of collaborative surveillance as it is relevant across diseases and sectors (environmental, human and animal), supports routine monitoring and emergency surveillance objectives throughout the cycle of prevention, preparedness, response, and recovery, and is be used at different geographical levels. WES must be viewed as a component of broader national surveillance capabilities, with findings triangulated with other surveillance approaches to generate robust intelligence and inform public health decisions and actions.
Environmental monitoring	Environmental matrix (e.g., water) sampled and tested with the goal of identifying locations as risk factors for exposure to the substance of interest (e.g., sampling of a drinking water or food crop irrigation water source for a waterborne pathogen of interest).
Genomic Characterization	A laboratory method that uses a sample of wastewater or environmental water to seek information on relevant genetic information present, and to utilise that information to inform action.
Multimodal surveillance	Simultaneous application of multiple coordinated modes of surveillance. Typically including laboratory confirmed clinical surveillance and often syndromic surveillance for infectious diseases. Potentially integrated with one or more other forms of surveillance, such as serosurveillance, and the subject of this text: wastewater and environmental surveillance.
Multitarget WES	Simultaneous testing for multiple targets in wastewater and/or human-impacted environmental samples. This may include multiple targets for one pathogen or multiple pathogens as well as other genetic or chemical information.
Next generation sequencing	High speed parallel sequencing to determine the order of nucleotides in entire genomes or targeted regions of DNA or RNA.
Non-sewered	Lacking a functional connection to the sanitary sewer system.

One Health	One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems.
Polymerase chain reaction	Polymerase chain reaction (PCR) is used to amplify a specific targeted segment of DNA. The result can be quantitative using quantitative PCR (qPCR) or digital PCR. The addition of a reverse transcription (RT) step allows detection and quantitation of RNA targets.
Sewage surveillance	Sewage surveillance, wastewater surveillance, wastewater based epidemiology, and wastewater and environmental surveillance (WES) are often used interchangeably. See WES definition below.
Sewered	Having a functional connection to the sanitary sewer system.
Wastewater, fecal sludge and environmental samples	For the purposes of WES, the sample of sewage, faecal sludge, or other human-impacted environmental waters. Environmental waters are of most relevance in locations with low coverage or dysfunctional sewered systems or from non-sewered settings. The sample is selected to identify if a pathogen or other target of interest is present within the individuals contributing to the upstream catchment of the wastewater, faecal sludge or environmental water sample relevant to the sampling period. Note that WES is distinct from environmental monitoring (see separate definition) but WES for waterborne pathogens, such as cholera and typhoid, may overlap to some extent with environmental monitoring.
Wastewater Based Epidemiology	Wastewater Based Epidemiology (WBE). Wastewater based epidemiology, wastewater surveillance, sewage surveillance, and wastewater and environmental surveillance (WES) are often used interchangeably. See WES definition below.
Wastewater and Environmental Surveillance	**Wastewater and Environmental Surveillance (WES). Surveillance using samples from sewage, or other human- impacted environmental waters. Environmental waters are of most relevance in locations with low coverage of sewered systems. Within this document the term is reduced in scope from environmental surveillance more broadly (e.g. it does not cover air, soil or other environmental samples) and is broader in scope than sewage surveillance that entails sampling from sewered systems only. In this document, WES refers to the combined:** • **Purposive collection of samples from sewage, wastewater, or environmental water from sampling points representing defined catchments** • **With known input from human sanitation and hygiene activities, primarily faecal excreta, but also vomitus, urine, sputum, respiratory and other secreta, blood, skin and fomites.** • **Which may have input from non-human zoonotic sources** • **That are analyzed for target pathogens and/or nucleic acids for the explicit and exclusive purpose of public health surveillance** • **Where such practice is consistent with the ethical principles of public health surveillance**
Whole-genome sequencing	A method for analyzing entire genomes, e.g. all the detectable genetic information that can be sequenced in a wastewater sample.

Summary

Introduction

Wastewater and Environmental Surveillance (WES) is surveillance using population samples from sewage where sewers are present, or other human-impacted environmental waters in locations without sewer networks (see full definition in Glossary).

This document provides an overview framework for prioritization, implementation and integration of WES as part of multi-modal public health surveillance. It is aimed at health ministries and disease-specific prevention and control programmes as well as other WES stakeholders such as operators of sewage and sanitation systems, the water and environmental sectors, and researchers all operating at national and subnational levels. Its purpose is to guide the dynamic development, prioritization and integration of WES programs for one or multiple targets from the multitude of potential targets considering both current and future threats. Although WES can be applied to a wide variety of substances, this document is limited to WES applications for human infectious diseases.

This pathogen agnostic guidance framework is part of a package of documents and other relevant resources such as collaborative surveillance, pandemic preparedness and One Health surveillance and response. This packages includes;

- Wastewater and environmental surveillance for one or more pathogens: Guidance on prioritization, implementation and integration
- A long list of potential pathogens for WES application (Annex 1)
- Decision support tools (Annex 2 -5)
- Detailed WES summaries for specific pathogens (separate sheets)
  Currently these are; poliovirus, SARS-CoV-2; influenza A and B viruses, monkeypox virus, *Vibrio cholerae,* and Typhi and Paratyphi with more to be completed.

Potential added value of WES

WES has been shown to provide information of public health relevance on the presence (above limits of detection), spatial and temporal trends, and/or genomic characterisation of various pathogenic biomarkers at the population level defined by geography. WES may provide additional value to existing surveillance modalities, addressing critical surveillance gaps and strengthening overall disease surveillance and response. Sometimes WES can serve as the principal community surveillance tool, but to-date there are few examples of this. In principle, since many human pathogens have been detected in wastewater, these WES applications apply to many other pathogenic and other targets.

WES does not rely on clinical symptoms, health seeking behaviors or access, cost and quality of health services. WES can have advantages of relative timeliness, coverage, cost and acceptability compared to event-based surveillance. WES may be used to adaptively respond to changing surveillance needs because of its flexibility in sampling, population coverage, representativeness, and timeliness. However, WES cannot connect affected individuals directly with care.

Situating WES within cross-cutting and disease specific initiatives

WES needs to be linked to and coordinated with relevant disease -specific surveillance and response activities as well as overall surveillance systems and relevant cross-cutting initiatives at local, regional and global levels as described in Section 2. This includes integration into items such as; global disease

control strategies, national plans on communicable disease surveillance and response and plans for health security, international and cross-border WES. To ensure fit for purpose WES implementation, a national coordination structure encompassing all relevant actors should be established and led by public health actors such as the Ministry of health or national public health or environmental agency. Figure 2.2 illustrates sectoral roles.

WES programme elements

WES programs consists of three inter-related activities set out in Section 3:

- **Routine WES at strategic sentinel sites** with ongoing systematic collection, analysis, interpretation, and dissemination to monitor spatial and temporal trends and patterns of pathogen circulation, and which may provide early warning.
- **Time-limited agile WES** is a new WES activity or a change from routine WES sampling, targets, strategies, analytic methods and/or reporting. Triggers include evidence of changing or emerging threats and emergencies, including from routine WES. Other time-limited WES activities may be to establish local burden and epidemiology to fill knowledge gaps or to help evaluate impact of public health measures (e.g., introduction of a vaccine).
- **Supportive activities to plan, prepare and improve WES –** do not involve implementation of WES directly, but support readiness for future WES addition, expansion and improvement (e.g., identifying sites, targets, approaches and methods)

Selection and prioritization of pathogen targets

The following key criteria are proposed to prioritize pathogens for inclusion in WES programs with consideration of the local context as detailed in Section 4.

- **Public health significance -** current or future threat posed by the pathogen or public health target and the potential value that WES could provide in early detection and mitigation relative to existing surveillance and response options
- **Technical feasibility -**sensitivity, specificity and predictive value of WES relative to public health action needs, factoring in pathogen shedding, host range, target degradation in waters, sampling and analytic methods and interpretation of results
- **Operational feasibility -** suitability of and access to sampling points, and capacity to finance, organize, undertake, utilize and sustain a WES program with favorable cost-benefit and appropriate governance
- **Acceptability -** consultation with key stakeholders on legal, ethical and social license of WES for the pathogen, surveillance objective and populations of interest with acceptable mitigation strategies
- **Integration potential** of WES as part of disease specific surveillance and response, as well as into any existing WES programs with multi-target WES

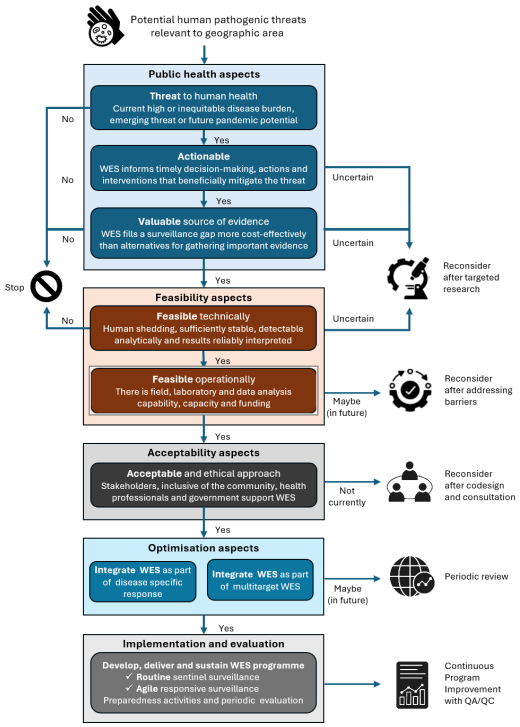


Figure 4.2 Prioritization process for selecting targets for WES implementation

Prioritizing WES using these criteria can enable implementation that adds value by enabling public health responses to achieve improved outcomes efficiently for a wide range of disease outbreak scenarios.

Cross cutting aspects

Cross-cutting aspects are pathogen agnostic and encompasses programme planning considerations, site selection and sampling strategies, sampling capacity needs, laboratory capacity needs and quality management, method selection considerations, and data to action pathways. Pathogen specific aspect are included in the six targets sheet which should be read together with cross-cutting aspects set out in Section 5.

A target-agnostic WES workflow is given (Figure 5.1) to assist evaluating whether one or more pathogens can be efficiently combined. Sample site selection, sampling strategies and interpretation are a key element of WES as the relationship of sampling catchment area and human and other contributions is dynamic and complex in both sewered and non-sewered settings. Context specific considerations for sampling approaches, frequencies, capacity needs and potential to align for multiple targets is set out (Table 5.2 - 5.4). High-quality and reliable laboratory data are a core component of a successful WES program. Building or strengthening environmental microbiology laboratory capacity is one of the first steps in WES program development.

Research needs and future updates

Section 6 sets out priority research needs drawn from the GLOWACON technical working group. Research needs cover eight main areas:

1. Identify priority pathogens for WES
2. Develop improved cost-effective, robust tools and techniques for the sampling, detection and analysis of priority targets
3. Develop improved cost-effective, robust tools and techniques for the interpretation of WES data
4. Promote integration of WES results as part of collaborative surveillance into mainstream public health decision-making and public communications
5. Promote ethical practice for WES for public health purposes
6. Enhance the use of WES in non-sewered settings
7. Strengthen WES capability and capacity including in human resources
8. Identify other potential use cases for WES for public health and One Health purposes to inform future program development priorities

WHO, with CDC, will periodically update this guidance package following pilot implementation in 2025 and as rapidly evolving new scientific and applied research comes to light.

Methods

WHO, with CDC, developed this guidance package following best practice method set out in WHO procedure for norms and standards development and as set out in Section 7. Contributing experts were selected seeking a balance of academic, implementation and disease specific surveillance experience, as well as gender and regional representation. All members of the expert group were screen for any conflicts of interest. Disease leads contributed to the six pathogen specific target sheets.

1 Introduction

1.1 Background

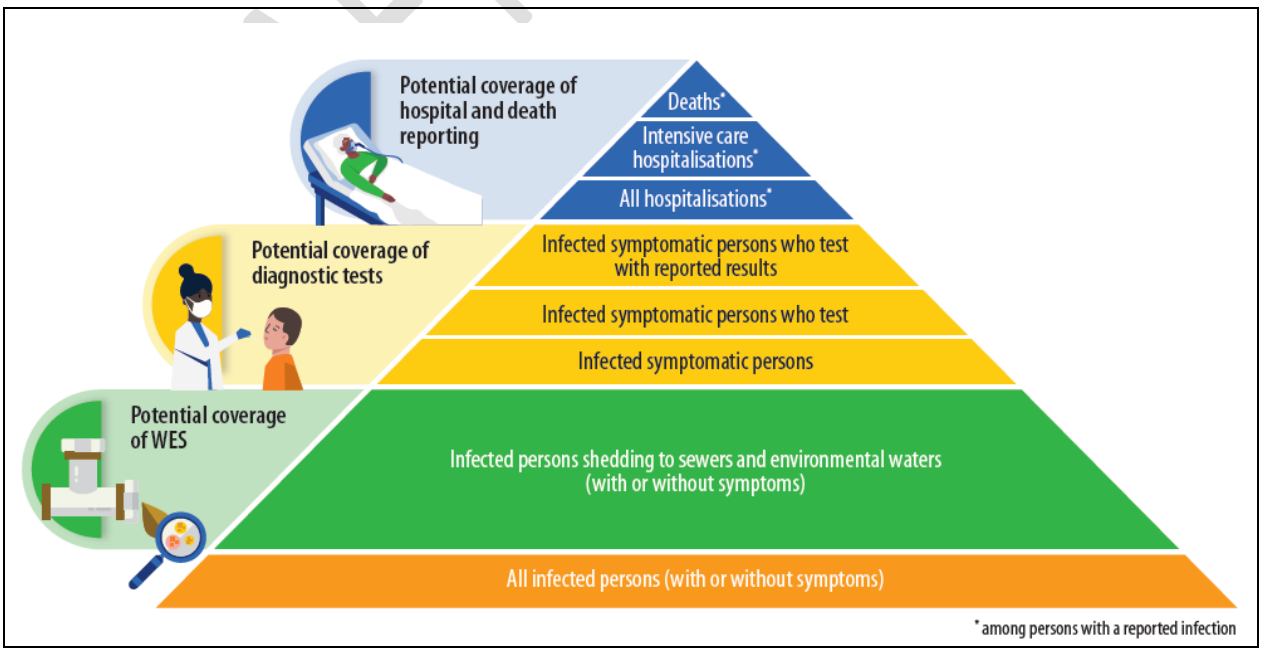
Wastewater and Environmental Surveillance (WES) is surveillance using population level samples from sewage where sewers are present, or other human-wastewater impacted environmental waters in locations without sewer networks (see full definition in Glossary).

WES has been shown to provide population-level information on the presence above detection limits, spatial and temporal trends, and/or genomic characterisation of various pathogens of public health relevance. The inclusion of WES can strengthen overall multimodal surveillance by cost-effectively complementing and filling gaps in other forms of surveillance to enable timely public health decisions. Well established uses of WES include poliovirus as part of polio eradication efforts and SARS-CoV-2 in the COVID-19 pandemic response (Box 1.2).

WES and case-based surveillance have different relative strengths and provide complementary information. Unlike case-based surveillance, WES often provides early warning ahead of reported cases because it can detect asymptomatic cases and is not dependent on healthcare-seeking behaviour or access to health services and diagnostic testing. WES provides population-level information with a single test. It can promote greater health equity by providing information about populations who are under-represented in case-based surveillance.

Rapid technological and operational advances and empirical evidence from WES activities are greatly expanding the potential utility of WES as a cost-effective and flexible population-level surveillance tool. These have relevance for a wide range of other infectious diseases and related targets of public health significance (Table 4.4). Figure 1.1 illustrates of the potential coverage of WES in relation to all infected persons and those identified through various event-based surveillance approaches.

Figure 1.1. The relationship between all infected person, potential coverage of WES and various levels of individual event based surveillance (Source: Adapted from Havelaar et al. 2007).



These documents set out evidence and use cases for WES of SARS-CoV-2 and key considerations for implementation. The Scientific Brief provided early high level information with the Interim Guidance and Guidance providing more in-depth guidance on if, how, and in what circumstances application of routine and agile WES could be deployed to complement clinical and other surveillance. These include documentation of varied at-scale applications including early warning of cases or surges in community infection trends as well as the emergence and spread of emerging variants through peer reviewed publications with original research and reviews.

WHO recognises the need for support in designing, implementing and sustaining integrated multi-source and multi-sectoral surveillance systems that include WES. Together these systems provide evidence to support local, practical, and context-aware public health decision-making. This is a complex and dynamic task. Known current and emerging epidemic and pandemic threats continue to evolve, sometimes very rapidly; as illustrated by recent Public Health Emergencies of International Concern (PHEIC) such as COVID-19 and mpox. Climate change, extreme weather events, armed conflict and other factors are impacting vector and pathogen distribution, water and sanitation infrastructure, agricultural and animal husbandry practices, population movement, vaccination uptake and more. All have consequences for changed endemic and epidemic disease epidemiology. Diverse global settings have different local epidemiology, public health system capacity and resources.

WES may be considered in a One Health context noting many zoonotic pathogens co-circulate among humans or represent a potential epidemic or pandemic threat and that WES also has applications directly and exclusively to animal health [2,3]. There is also potential use of WES to contribute to the pressing global problem of antimicrobial resistance (AMR) that needs to be considered as part of the larger and more complex AMR integrated One-Health surveillance approach (ref forthcoming). Finally, WES can be applied to a wide variety of biological and chemical substances many with public health significance (e.g., pharmaceuticals, illicit drugs, various biomarkers).

1.2 Purpose

This guidance package builds on previous pathogen specific WES guidance [4,5]. It aims to assist local decision makers with a practical framework to consider the potential utility of WES programs for one or more targets as part of cost-effective, multimodal surveillance systems.

This framework includes both technical and practical contextual considerations, centered on local public health needs, specific surveillance objectives and the actions enabled by surveillance information. It describes the potential value, strengths and limitations of WES, including where WES has been conceptually and methodologically proven. Key knowledge gaps research priorities are identified to improve the application and utility of WES.

This overview document is accompanied by detailed WES summaries for six specific pathogen groups, which will be expanded as additional priority pathogens are identified (Section 1.5). Together, they provide an evidence-informed practical framework to assess inclusion of WES for one or more pathogens within the users' context.

1.3 Target audience

The target audience of this guidance package is entities considering establishment, modification and sustainability of WES as part of their collaborative surveillance systems. This includes consideration of WES as for preparedness for emerging and future human health threats. Entities include:

- Health ministries (national, regional) and disease-specific prevention and control programmes
- WES and surveillance related stakeholders, inclusive of public health, environmental and other One Health related agencies
- Policy makers, researchers and professionals with diverse expertise including those operating at national and subnational levels
- Other key stakeholders are those from the water and sanitation sector inclusive of operators of sewage and sanitation systems

1.4 Scope

This guidance package focuses on WES applications for human infectious diseases. Key questions addressed in this document are:

- In what circumstances can WES fill gaps and strengthen multimodal surveillance systems by providing actionable intelligence for public health decisions?
- What are the key use cases for routine ongoing use as well as for time-limited agile WES?
- What is the evidence for given use cases for WES and their strengths and limitations?
- How can local and contextual factors inform which pathogens to prioritise in a new, modified or sustained WES program?
- How are aspects such as; the public health significance of a pathogen, technical and operational feasibility, acceptability and integration into surveillance and response considered?
- What are the key ethical and legal considerations unique to WES for infectious diseases?
- What are the governance, planning and other activities needed to prepare to implement routine and/or agile)WES?
- What are the minimum capacity needs to implement WES considering aspects such as sampling, transport, laboratory, data collection and bioinformatics analysis, interpretation, integration and public health response?
- How can individual or multi-target WES programs be most cost-efficient and effective; are trade-offs needed (e.g., between sensitivity and optimal resource use)?

The questions and the guidance package will require periodic revision given the rapid development in WES applications, publications and technological innovation, this package will need regular updates.

This package does not cover WES for exclusively animal pathogens other biological and chemical substances such as pharmaceuticals and, illicit drugs which may have relevance for public health surveillance and potential synergies.

1.5 Pathogen specific WES summaries

Detailed pathogen specific WES summaries are included in this guidance package for globally significant diseases and their causative pathogens. Thus far the following six have been completed with more to follow. They are;

- Cholera *(Vibrio cholerae)*
- Influenza *(Influenza viruses A and B)*
- Mpox *(Monkeypox virus)*
- Polio (*Poliovirus*)
- COVID-19 (*SARS-CoV-2*)

- Enteric fever of typhoid and paratyphoid (S*almonella entiritidis* serotypes Typhi and Paratyphi*)*

A limited number of new specific WES summaries will be added as pathogens and related targets are prioritized.

These pathogen summaries synthesize the published evidence, including: the demonstrated and potential WES use cases to support public health decision making; relevant background; technical and operational feasibility; additional WES methodological considerations in relation to sampling, laboratory methods, analysis, interpretation and acceptability; integration into disease specific and overall surveillance and response, as well as multitarget WES. Limitations, key knowledge gaps and applied research priorities are also described.

Additional specific WES summaries will be completed and are likely to include one or more arboviruses (e.g. Zika virus), antimicrobial resistance, vaccine preventable diseases (e.g measles) as well as other pathogens or targets which are prioritized through regional consultations.

Box 1.2. Examples of established uses of WES

Poliovirus

WES is integrated as an important source of evidence to inform polio eradication complementing case-based surveillance of acute flaccid paralysis (AFP) including identification of silent circulation, the type of poliovirus and its likely source. The *Global Polio Eradication Initiative* (GPEI)[6], inclusive of WHO, issued its most recent *Field Guidance for the Implementation of Environmental Surveillance for Poliovirus* in 2023[5]. These compliment and build on the prior *Guidelines for environmental surveillance of poliovirus circulation* (2003)[7] and the draft *Guidelines on environmental surveillance for detection of poliovirus* and expansion plan (2015)[8].
The quality and number of WES sites continues to improve and expand; as of Dec 2023 there were 900 routine WES sites in 86 countries including wild poliovirus endemic countries, countries prone to circulating vaccine derived poliovirus outbreaks and other (GPEI 2024). Poliovirus WES also includes an agile outbreak response triggered by a clinical AFP case, WES detection or other heightened risk. Further details and examples of routine sentinel and agile WES are provided in the poliovirus WES summary (available here).

SARS-CoV-2

In August 2020 WHO published a Scientific Brief on the *Status of Environmental Surveillance for SARS-CoV-2 virus*[9] on the potential use of WES to provide evidence to inform management of COVID-19. WHO's Interim Guidance on *Environmental surveillance for SARS-CoV-2 to complement public health surveillance* followed in April 2022[10] and was further updated in Guidance on environmental surveillance for SARS-CoV-2 to complement other public health surveillance in September 2023[11].

These documents set out evidence and use cases for WES of SARS-CoV-2 and key considerations for implementation. The Scientific Brief provided early high level information with the Interim Guidance and Guidance providing more in-depth guidance on if, how, and in what circumstances application of routine and agile WES could be deployed to complement clinical and other surveillance. These include documentation of varied at-scale applications including early warning of cases or surges in community infection trends as well as the emergence and spread of emerging variants through peer reviewed publications with original research and reviews.

Further details and examples of routine sentinel and agile WES are provided in the SARS-CoV-2 WES summary (available here).

2 Situating WES within cross-cutting and disease-specific initiatives

2.1 Linking national, regional and global levels

WES needs to be linked to and coordinated with relevant disease -specific surveillance and response activities as well as overall surveillance systems and relevant cross-cutting initiatives at local, regional and global levels. As such there are a wide range of individuals and institutions (including from the health, environment, water and sanitation sectors) who may need to be consulted and have a role in WES program design, implementation or as end users of WES information. The various cross-cutting initiatives and programs may have relevance with global, regional, country and subnational dimensions and will depend on the WES pathogen and use case application. Collaboration, coordination and institutionalisation is vital for success and also challenging.

2.1.1 Global

There are multiple global and related regional and national programs and initiatives where WES has a current or likely contributory role. These include:

- Collaborative surveillance for fit for purpose, multimodal, multisectoral surveillance [1,12]
- Health emergency prevention, preparedness, response and resilience (HEPPRR) including strengthened infectious disease surveillance in emergency and conflict settings [14]
- Epidemic and pandemic preparedness including for pathogens (and viral families) that pose a high threat including unknown pathogen X[13]
- Global genomic surveillance including for epidemic and pandemic threats [15,16]
- Global biosecurity initiatives[17,18]
- Antimicrobial resistance inclusive of integrated surveillance[13,19]
- One Health approaches to human and zoonotic diseases which recognize the interrelationship between human, animal and environmental health [2,3];

There are also numerous disease and syndrome specific programs where WES has a current or potential role which are multidisciplinary and typically multisectoral. Key illustrative examples (which are not exhaustive), are:

- The Global Polio Eradication Initiative[6]
- The expanded Global Influenza Surveillance and Response System[20] inclusive of influenza and SARS-CoV-2
- Various collaborative laboratory networks such as CoViNet[21], the coronavirus laboratory network inclusive of environmental surveillance
- Global Arbovirus Initiative[22]
- Global Typhoid response including the Take on Typhoid Coalition[23]
- Global Task Force on Cholera Control[24]
- Mpox response as part of the Public Health Emergency of International Concern response

There are many other depending on the pathogen/disease targeted; such as meningitis, vaccine preventable diseases including measles and hepatitis, various sexually transmitted infections, acute hemorrhagic fevers, neglected tropical diseases.

2.1.2 National

At the national level, WES strategy should be included within broader frameworks of relevant national policies and strategic frameworks which are country-specific. Examples include the National

Action Plan for Communicable Disease Surveillance and Response and the National Action Plan for Health Security as well as other cross-cutting or disease specific national plans. These will likely be embedded within one or more of the broader regional and global initiatives outlined above.
This level of WES integration is possible once WES moves from pilot and research projects to at scale implementation.

2.1.3 International / cross-border

In addition to country level WES applications, there are also international applications which may be relevant to cross-border, multi-country, regional and global geographies. Human pathogens are rapidly transported across borders directly by humans, or by animals and trade via aircraft, boats and overland transport as well as environmental and wild zoonotic carriage. Emerging pathogens found to be circulating in one location can be transported rapidly to other locations including across international borders. With these considerations, WES international applications may be categorised as:

- **Indirect:** Country-specific WES results may provide early warning for other countries (i.e., Indicating heightened risk of incursion)
- **Multi-country cross border or regional:** WES program activities specifically planned to respond to a regional cross border event (e,g., multi-country outbreak or other)
- **Global:** WES program designed principally for global benefit such as global sentinel surveillance of aviation hubs (+/- maritime hubs) with country partners benefitting global surveillance for early detection of emergence and spread [25]

2.2 Integration with disease specific surveillance and response

The goal of the integrated, multimodal surveillance systems, including WES, is to provide cost-effective systems that help reduce disease burden and socioeconomic harms. Use of WES should provide relevant local information to identify, monitor and mitigate substantial human health threats in a way that is not available or more costly with other surveillance types.

Figure 2.1 shows a schematic of integrated disease surveillance including WES illustrating:

- The **collaborative involvement of multiple sectors**, such as stakeholders from the human health, animal health and environment sectors inclusive of water and sanitation.
- **Integration with other surveillance types** at key points including the design stage and end stage for integrated analysis and decision-making, i.e.,
  - design and refinement of surveillance systems and their governance to meet context specific disease surveillance and response needs; and
  - integration of surveillance information from multiple sources to provide timely combined intelligence to inform public health decisions and actions.
- **Implementation of WES and other surveillance in parallel**
  - includes site selection, sampling and transport, laboratory analysis, interpretation and reporting of WES specific results.
  - In parallel to other contributing event-based surveillance activities (human +/- zoonotic or vector) and other surveillance and information gathering.
  - The relative importance of different surveillance and information streams may vary.

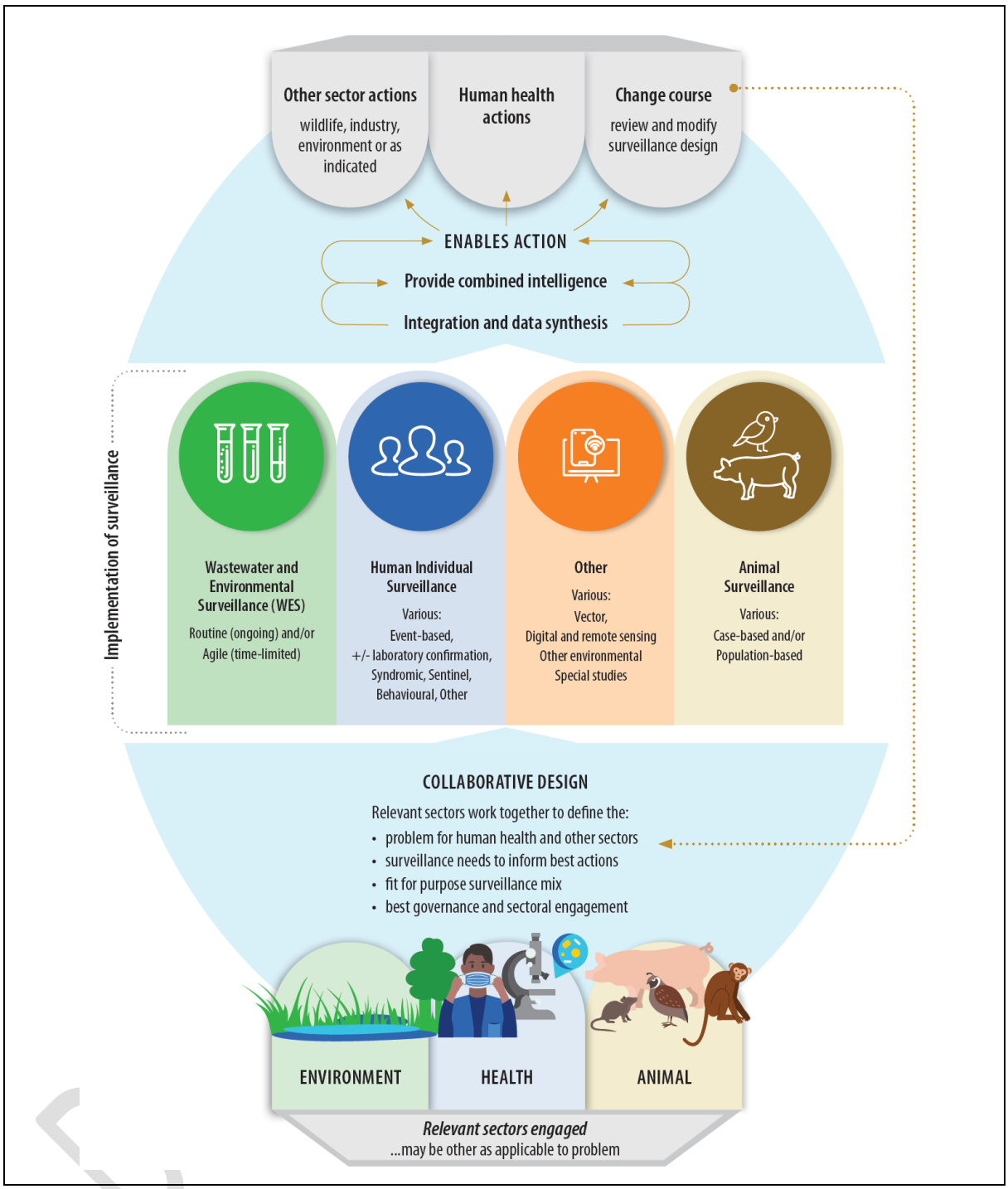


Figure 2.1. Illustration of effective integrated disease surveillance including WES.

2.3 Key actors and roles for design and implementation

Human health surveillance always requires a multidisciplinary team with expertise relevant to the phases of program design, implementation, monitoring and evaluation. For routine implementation, expertise in public health (such as infectious disease epidemiology, surveillance, data management, bioinformatics, communications and others) as well as specialized laboratory expertise (if a laboratory component) is needed. Input from community representatives is also important to gain insights into community perspectives particularly if the surveillance and/or disease affect marginalized or vulnerable individuals. Specifically, WES requires expertise in:

- sanitation systems, sampling and environmental laboratory analysis,
- design, use and interpretation of population and spatial data from public health experts
- knowledge of sanitation practices and systems, including in non-sewered settings from community representatives
- development of equitable communication systems that prioritize understandable, actionable and non-stigmatizing communication including community representatives.
- Other expertise depending on the particular WES use case, context and epidemiology

To ensure fit for purpose implementation, a national coordination structure encompassing all relevant actors should be established and led by public health actors such as the Ministry of health or national public health or environmental agency. Figure 2.2 illustrates sectoral roles.

The operational WES lead may be an environmental epidemiologist or similar and, together with their team they determine surveillance priorities as well as coordination and use of data. The WES team also collaborates with the public and/or private entities responsible for sanitation and designated laboratories. The WES team ensure internal coordination within or across health agencies involving those responsible for communicable disease, public health responses, public communications, community engagement, decentralized local public health units and other frontline responders. Researchers play a key role in addressing key knowledge gaps and driving innovations and program improvement across all areas and may play a hybrid implementation and research role.

Figure 2.2. Key sectors and their likely roles in WES design and implementation.

3 WES programme elements

A WES programme may include three inter-related and synergistic elements to meet current and future threats. These are:

- **Routine ongoing sentinel surveillance** of current priority targets
- **Agile time-limited surveillance responses** to emerging threats (if and when needed); and
- **Planning, preparation and continuous improvement** for any future or current WES (whether routine or agile)

Figure 3-1 provides a simple schematic of the interplay between the choice of WES target, the context, and surveillance use case with specific surveillance objectives that inform local public health actions. These local considerations then lead to WES programme design decisions for routine and/or agile WES surveillance. These vary widely from one location to another and may change over time in the same location based on adaptation and periodic review.

Prioritization of targets for WES should consider current and potential future threats. Current threats and triggers for agile responses can be quantified drawing on past evidence. Future epidemic and pandemic threats involve significant uncertainty about the likelihood of their occurrence and potential impact including the unknown pathogen X [13].

Table 3.2 summarizes the three elements together with surveillance targets, use cases and key activities.

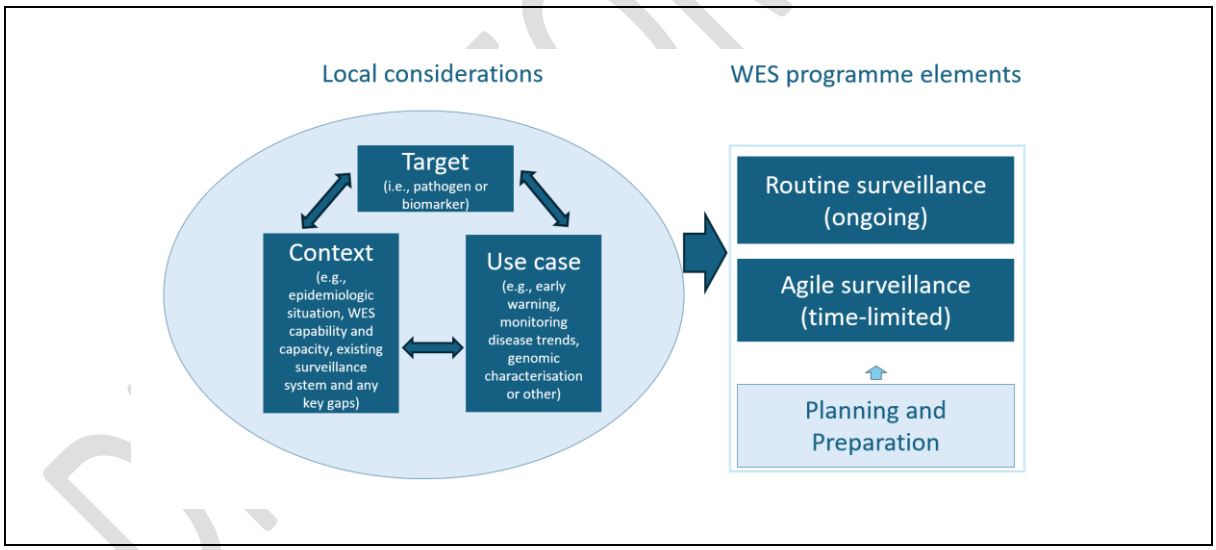


Figure 3.1. Interplay between choice of pathogen, context, current surveillance system, and use case.

Table 3.2. WES programme elements and associated use cases and key activities.

Program Elements	Targets	Use cases	Key Activities
Routine Sentinel Surveillance (ongoing)	Current threats	• Early warning which leads to response - which may include additional investigations such as agile WES Response • Monitor spatial and temporal trends to inform public health responses as well as to better characterise epidemiology +/- pathogenic genomics	• Ongoing sampling, analysis and reporting of selected priority targets at routine cadence. • Sentinel sites may include geographically representative large population centres, transport hubs or other strategic locations including consideration of at-risk and under-served populations if feasible • Laboratory analysis tailored to objective (e.g. presence/non-detect, quantitative, genomic characterisation) • Defined thresholds (e.g. absolute or relative increase/decreases in observed pathogen circulation) for agreed public health action • Note - Clear temporal patterns in diseases may justify dynamic sampling frequencies, such as reduced frequency during expected periods of low prevalence.
Agile Surveillance (time-limited response)	Specific emerging threats	• As for 2. above for time-limited period - focuses on implementing and scaling up WES to respond to an emergency situation • Response to specific trigger (from routine WES, case detection or other) OR • Response to new emergent threat with pathogen not included in routine WES program	• Integrate agile WES surveillance as part of outbreak/emerging threat response optimising synergies with any existing WES activities. • Activate agile WES response with governance, resourcing, and coordination. • Rapid laboratory method validation for target (if needed). • Implementation: adapt sampling frequency, locations, laboratory analyses, reporting, and response protocols, as required drawing on existing WES experience and programmes (if any).
Planning, preparation and continuous improvement	As above plus Likely future threats	• Planning and other activities to prepare for new or changed WES implementation to enable timely agile responses in the presence of an existing WES program • Program improvement and innovation activities aim to optimise program cost-effectiveness and current and potential utility • In the absence of an existing WES program, planning and other preparedness activities are required with a lead time to establish any WES (routine or agile)	• Define triggers for agile surveillance including from routine WES (e.g. as for polio) • Identify and plan for future threats in different scenarios (e.g. pandemic flu, expanded arboviral disease distribution) • Consider needed governance mechanisms, resources, research and development needs, capability, and partnerships, including leveraging existing WES. • Maintain operational preparedness for an Agile surveillance response. • Prepare and maintain capacity and supply chains for sampling and laboratory analysis for priority future threats, including relationships and governance. • Prioritise technical preparedness activities such as method validation and optimisation, assessment of sampling locations, and ability to access sampling locations. • Continuous program improvement and innovation (for any WES activities)

4 Selection and prioritization of one or more pathogen targets

4.1 Overview

This section outlines a sequential, evidence-informed, decision-making process for the local and contextual prioritization of one or more WES pathogen targets and their related use cases. The prioritization process is intended to enable implementation of WES systems to support public health decision-making that achieve improved outcomes to disease outbreaks. The prioritization process shown in Figure 4.1 and 4.2 is intended for use at country level. However it can also be applied at other geographic scales (including global, regional and sub-national) to consider WES for current, emerging and potential future threats. The approach requires:

- identification of locally relevant potential pathogens/targets for WES (Section 5.2)
- a sequential evaluation considering criteria of; Public health significance, Feasibility, Acceptability and Optimisation leading to prioritized pathogens/targets and use cases (Section 5.3)

The approach promotes structured contextualized consideration of the potential added value and actionability of WES results as part of cost-efficient multi-target WES Figure 4.2 illustrates the sequential steps starting with the locally relevant list of potential pathogens and ending with WES implementation including routine and agile surveillance and initial planning and preparedness activities as well as ongoing improvement and periodic evaluation activities.

This process likely requires a combination of desk research and deliberations in an interdisciplinary approach with local experts and decision makers. Periodic review and updates are needed, given the changing status of communicable disease and rapidly evolving field of WES evidence, technology and capacity.

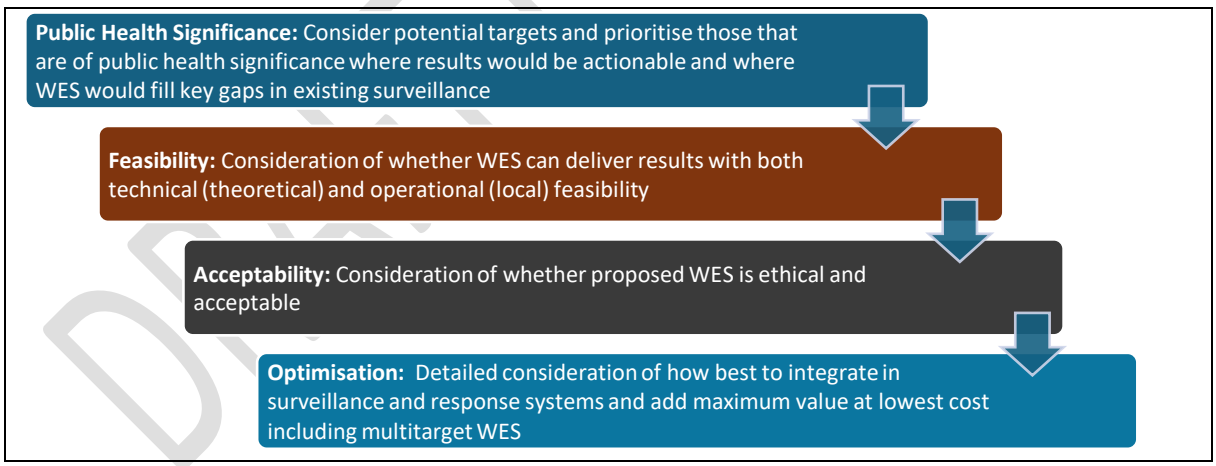


Figure 4.1. Overview of prioritization process for selecting targets for WES implementation

The proposed process and criteria (shown in Figures 4.1 and 4.2) draws on published conceptual frameworks [5,26–28] and were developed in consultation with global experts. These criteria explicitly require consideration of the overall disease surveillance and response system inclusive of any other current or planned surveillance (if any exist) and the added relative value (or lack thereof) of WES.

Figure 4.2 illustrates the sequential steps starting with the locally relevant list of potential pathogens and ending with WES implementation including routine and agile surveillance and initial planning and preparedness activities as well as ongoing improvement and periodic evaluation activities.

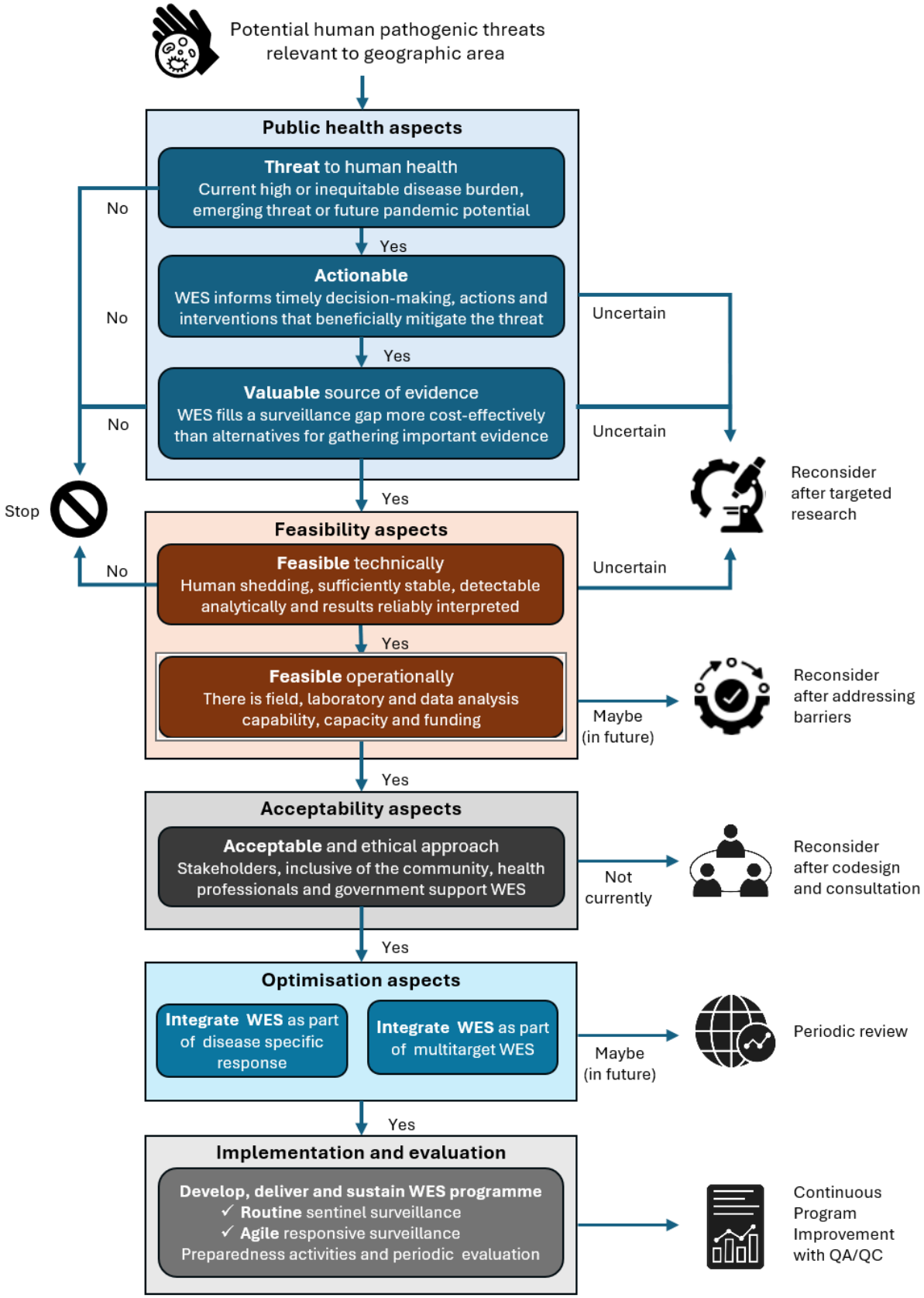


Figure 4.2. Prioritization process for selecting targets for WES implementation

4.2 Potential locally relevant pathogens

A preliminary step is to identify locally relevant priority diseases and associated pathogens for public health surveillance. These are not WES-specific and may already be available.. If not, they may be identified using expert opinion of public health and communicable disease experts, including animal health experts, together with global and local evidence to identify pathogens are of interest. The context specific list will draw on evidence as listed below and shown in Figure 4.3 and Table 4.4.

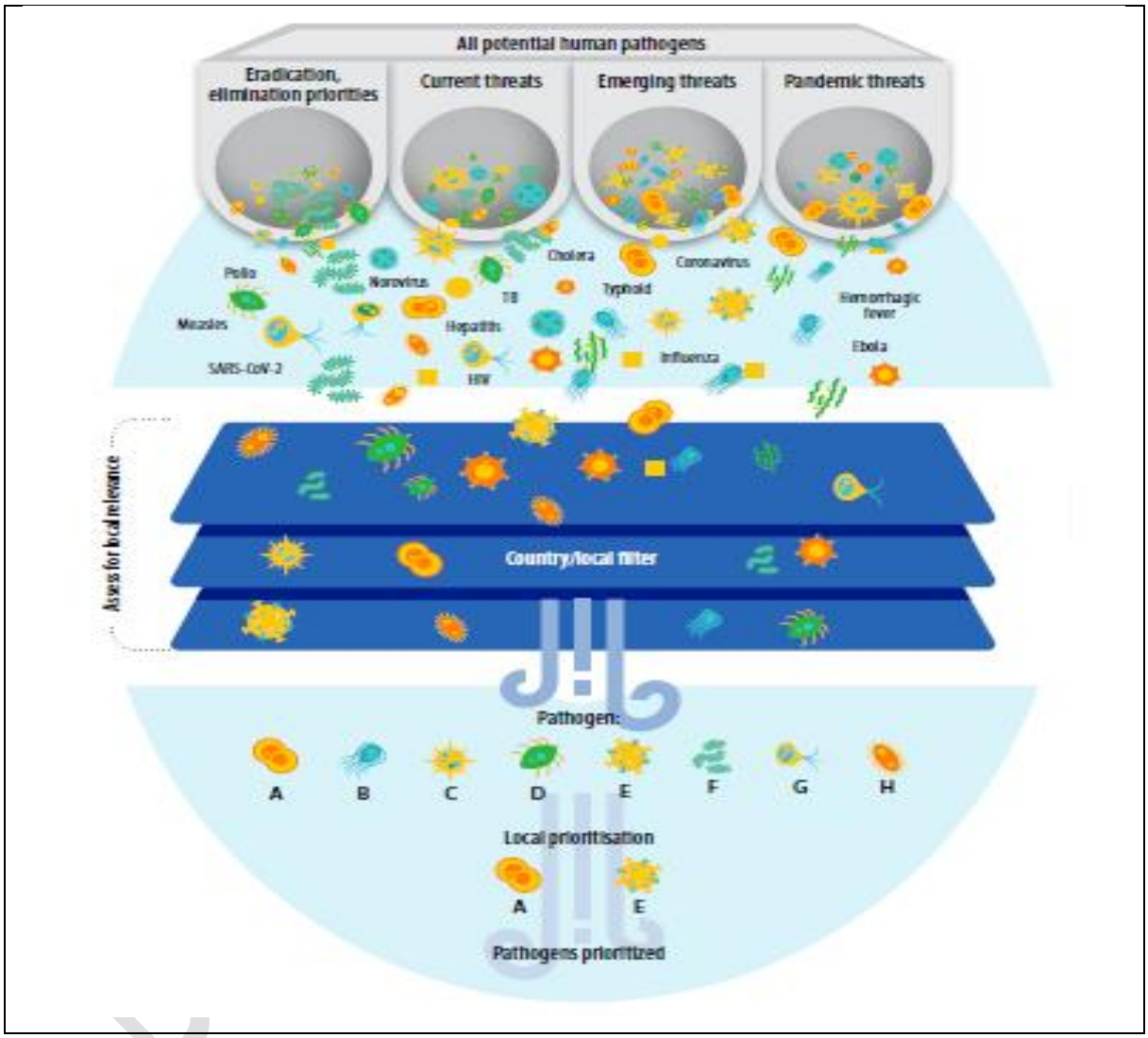


Figure 4.3. Schematic of prioritization process for selecting locally relevant list of candidates.

Other general disease prioritization efforts (e.g. One Health or Genomic prioritization workshops) and the results of those activities should be incorporated into this portion of the evidence generation exercise.

Country evidence (or geographic area under consideration) such as: Diseases prioritised for elimination, reportable diseases, Other surveillance and vaccination data (e.g. syndromic, hospitalisations, under-5 and overall mortality, zoonotic surveillance, vaccination coverage and susceptible populations), Cross-border evidence from adjacent/epidemiologically linked countries.

Global current evidence including:

- WHO priority pathogens and/or diseases
  A. Pathogens targeted for eradication or elimination[29]
  B. Specific pathogens listed as of high regional and/or global priority
    - epidemic/pandemic potential [13]
    - endemic priority[30]
    - bacterial priority pathogens[31]
    - fungal priority pathogens[32]
    - Antimicrobial resistance burden[33]
  C. Zoonotic pathogens relevant to human health
  D. Vaccine preventable diseases
- Other pathogens as advised by global and regional experts working in WES target prioritization
  - drawing on reportable diseases, high burden of disease, evolving threats
  - considering current WES evidence (e.g., in accompanying WES summaries for pathogen(available here) and EC encyclopaedia-cloacae.

Research knowledge base – publications and credible reports

Table 4.4 provides a curated list of human pathogens categorised by attributes of interest with relevance for public health surveillance. It includes human pathogens which have been, or are currently included, in WES with public reporting at scale in one or more countries, as well as categories A-D listed above and as advised by the global WES expert review group. This table is non-exhaustive. Pathogenic bioterrorism threats are not included but may also be considered. Annex 1 summarizes the same list of pathogens but is organised by both disease and syndromic presentation.

Table 4.4. Human pathogens of interest for potential WES evaluation (non-exhaustive) [1]

Viruses	
Viral pathogen - "Disease X"[2]	Influenza B [1,6]
Viral pathogen - * antiviral resistance of concern [8]	Lassa fever virus[2,3,4]
Chikagunya virus[3,4,5]	Marburg virus[2 ,3]
Coronavirus group (including SARS-CoV-2[1,2,6,7,] MERS-CoV2,3 and other alpha and beta coronaviruses)	Measles virus [6]
Crimean-Congo haemorrhagic fever orthonaivirus[2,3,4]	Monkeypox virus [1,6]
Dengue virus [3,5,6]	Mumps virus[6]
Enterovirus (D681 and other non-polio enteroviruses)	Nipah virus[2 ,3,4]
Ebola virus [2,3,4,6]	Norovirus[1]
Hendra virus [4]	Parainfluenza (1-4)[1]
Hepatitis A Virus [1,6]	Polio [1,2,6]
Hepatitis B Virus [6]	Respiratory Syncytial Virus [1,6]
Hepatitis E Virus [6]	Rift Valley fever virus [2 ,3,5]
Human adenovirus F [1,6] (and other adenovirus spp)	Rotavirus [1,6]
Human immunodeficiency virus [7,8]	Rubella [6]
Human metapneumoviruses [1]	West Nile virus [5,6]
Human papilloma virus [1,6]	Yellow fever virus [5,6]
Japanese encephalitis virus [3,4,5,6]	Varicella zoster virus [6]
Influenza A virus (including seasonal human) [1,2.4,6] and avian influenza [2,4]	Zika virus [1,2,5]

Bacteria	Funghi
Bacterial pathogen - "Disease X"[2]	Fungal pathogen -* antifungal resistance of concern [8]
Bacterial pathogen - * antimicrobial resistance of concern [8]	
Bordetella pertussis and B. parapertussis [6]	
Camplyobacter spp 4 (including C. jejuni))	Candida albicans and Candida auris [1,7,8]
Chlamydia trachomatis [7,8]	Cryptococcos neoformans [4.7,8]
Corynebacterium diphtheriae [6,7]	
* Carbapenem resistent enterobacteriaceae spp [8]	
Eschirichia coli (including Shiga toxin producing (STEC)) [4]	**Parasites: protozoa, helminths and ectoparasites**
Legionella spp (including L. pneumophila)	
Leptospira spp [4,7]	Cryptosporidium spp (including C. parvum)[4]
Mycobacterium tuberculosis complex [6,7,8]	Cyclospora cayatenensis [3]
Neisseria spp (including N. meningitidis and N. gonorrhoeae) [7,8]	Echinococcus spp (including E. granulosus)[4]
Salmonella enterica spp (including serovar typhi [3,6,8] and paratyphii [3,8]	Entamoeba histolytica
Shigella spp [7,8] (including S. sonnei, S. flexneri, S. boydii, S. dysenteriae)	Giardia duodenalis [7,8]
Treponema pallidum [7]	Plasmodium spp (including P. faliciparum) [3,7,8]
Vibrio cholera [3,6]	Sarcoptes scabiei var hominis (Scabies) [3,7,8]
Yersinia spp (including Y. enterocolitica) [4]	

Key

*1. - WES use at scale in one or more countries **(bolded)***
2 - inclusion as pathogen with moderate or high pandemic potential
3 - public health significance is not global but is more localized to specific climate zones or geographic areas
4 - known zoonotic host or hosts
5 - indicates vector borne
6 - human preventive vaccine available
7 - specific therapeutics available
8 - AMR is of concern affecting therapeutic options
Blue text - indicates an unidentified pathogen X or an emerging one with antimicrobial resistance of concern
**Antimicrobial resistance and antimicrobial resistance genes (AMR/ARG) may be relevant to multiple pathogens*

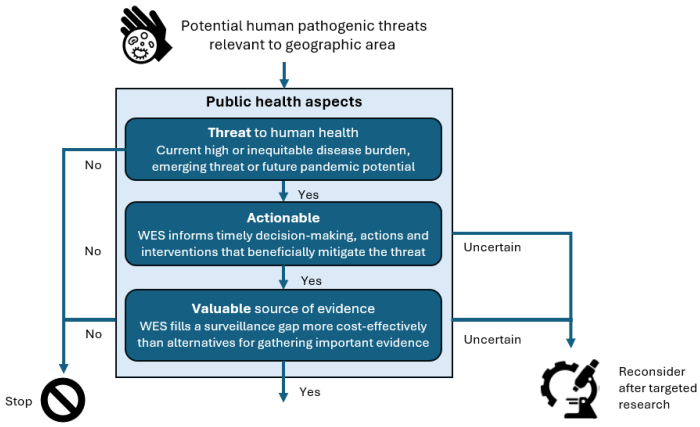
[1] * *This list is drawn from the published literature, publicly available dashboards, GLOWACON's Encyclopaedia Cloacae, and information shared at regional and global WES workshops and community of practice meetings. However, as the field is developing so rapidly, including with use of multi-pathogen arrays and metagenomics, this is not intended to be exhaustive and does not capture all pathogens evaluated for WES in research studies.*

4.3 Prioritising pathogens and targets for WES

The following sections describe key characteristics of each step summarized in Figure 4.2.

Step 1: Public Health criteria

The Public Health Significance step considers potential pathogens or targets and prioritises those of public health significance where results would be actionable and where WES would strengthen existing surveillance and fill key gaps (which may be a lack of surveillance).



- **Significance of public health threat**: The target represents a significant current or potential public health threat, factoring in the size of the susceptible population, the potential severity of disease and disease burden, outbreak potential, and other socioeconomic harms or disruptions, as well as equity considerations (disproportionate burden). Criteria may include considering the potential impact of climate change, increased international travel, population migration, antimicrobial resistance, and ageing populations, as well as emerging and re-emerging diseases.

- **Actionable:** WES results would be likely to contribute to useful, timely information to support public health decision-making – with consideration of specific objective/s, implementable interventions at specific triggers, and likelihood for benefit and harm

- **Valuable:** WES results fill a surveillance need in the most cost-effective way - adding value to overall pathogen or disease specific surveillance. WES may be the only source of surveillance information, or more typically, supplements case-based and other surveillance.

These criteria relating to the public health threat and the potential useful public health actions arising from WES are the primary considerations. If a target does not meet these criteria, it is not a suitable WES candidate for the purposes of infectious disease surveillance at this time.

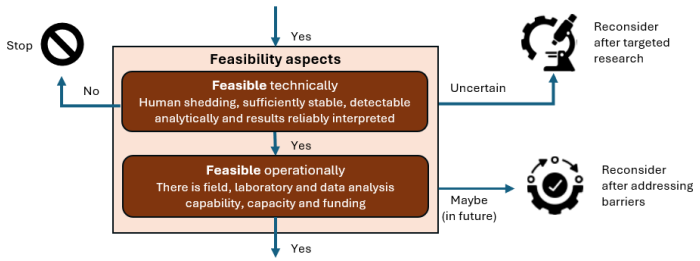
This ensures that meaningful public health action is planned from the outset prior to any WES activities.

Assessing these criteria requires input and leadership from health authorities with multidisciplinary relevant expertise. The process of implementing WES is iterative and with experience gained through operating WES (as well as through research) further relevant actions may be defined or thresholds for action adjusted.

Step 2: Feasibility criteria

The Feasibility step considers whether WES can deliver results with both technical (theoretical) and operational (local) feasibility in the given context.

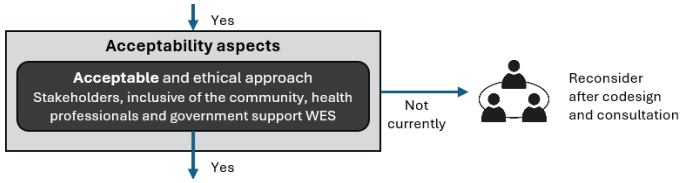
- **Technical feasibility**: The ability for the target to be detected in samples derived from wastewater or environmental water (including faecal sludge) and measured effectively for the purpose required (i.e., the target is shed in wastewater or environmental water, is sufficiently stable that it can be detected, and can be reported and interpreted reliably with respect to its presence, concentration, and/or genomic characterisation, as is relevant to the surveillance objective/s). This criterion requires evidence that the target of interest is shed from human (or other target hosts/vectors), persists in wastewater or human-impacted environmental waters and laboratory analytical methods are sufficiently sensitive and specific that results can be interpreted with confidence with respect to a useful metric aligned to the surveillance objective; for example quantitative absolute indicator or relative indicator or qualitative indicators such as shown for SARS-CoV-2 community infection trends, relative abundance of SARS-CoV-2 variants or detection of SARS-CoV-2 RNA providing early warning in presence of low case numbers. The evidence may be categorised objectively as "adequate and supportive" (illustrated as "Yes", proceed to next step), "adequate but unsupportive" (illustrated as "No", stop), or insufficient evidence currently available (illustrated as "uncertain", triggering consideration of an expanded review of the evidence and/or targeted research). An evidence gap for a target with high burden and actionable ratings should be prioritized for rapid research to address this knowledge gap. This information may largely be drawn from global evidence, but there also needs to be consideration if the evidence is relevant to the local context (e.g. for population distribution and movement, local sewage, climate, disease epidemiology, etc). Of note, evidence may be empirical without requiring each aspect to be evaluated; for example detection of mpox clade IIb in sewage demonstrated it was shed, sufficiently stable to be detected and relevant to the surveillance objective to identify local circulation.



- **Feasible operationally:** The surveillance required is feasible in terms of site selection (sanitation system mapping, site assessment, understanding relationship to contributing human population etc), sampling and transport (sampling type, site access, frequency, safety, etc.), laboratory capability for required analysis and reporting, public health use (review, action, integration, communications and response etc.) and resource availability to support WES. This criterion requires consideration of end-to-end workflows in the local context including integration with any existing WES for other pathogens and is expected to vary by location and over time. If there is no current feasibility, (e.g. inadequate skilled staff or lab resources to ensure adequate quality of assays and biosafety, inadequate ability or capacity to take timely public health action on findings, or other constraints), it could merit preparedness activities and investments to build capacity and capability for future WES.

Step 3: Acceptability criteria (Ethical and social licence, decision makers and legal)

The Acceptability step considers whether proposed WES is acceptable considering ethical issues, social licence, buy in of decision makers and legal issue to ensure WES is acceptable to key stakeholders.



- **Social licence:** This criterion requires meaningful consultation and codesign with key stakeholders, including community representatives, to ensure ethical, human rights and social licence aspects are effectively and transparently considered and optimised in planning and implementation. This requires identification and engagement with relevant key stakeholders specific to the proposed WES use cases and associated activities.

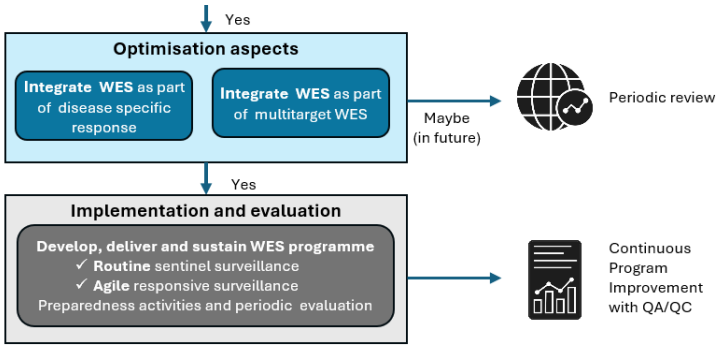
- **Ethics:** Principal considerations include privacy, data sharing, program effectiveness, potential for harm and approaches to mitigate harm. Approaches need to consider protection of groups who may be at risk of stigma and in difficult social or legal contexts.
  Community-level WES typically covers a large pooled-population and therefore has advantages from an ethical perspective over individual data. Individually identifiable data may be relevant in the context of hyper-localised WES (e.g., vessels, other small geographically defined populations) where individuals may be identifiable now or in the future with technological advancement. Other issues may arise though; the absence of opt-in or out options, monitoring borders and travelers through transport hub and vessels, the complexity of interpreting WES results) with potential for misinterpretation, sample ownership and potential use of samples, infrastructure or data for purposes beyond that of the primary public health use case (e.g., for law enforcement or private interests).
  The WHO Guidelines on Ethical Issues in Public Health Surveillance [34] guides users to carefully and transparently weigh 17 specific and interrelated ethical guidelines. These emphasize the common good, equity and respect for people and highlights the critical role of good governance. A specific ethical framework for WES based on the general ethical guide for surveillance is being developed by WHO. This section will be updated once the new WES ethical framework is published.

- **Decision makers**: Acceptability to government decision makers is also critical to secure needed initial and ongoing resources and approvals involving the health and finance ministries and any others involved; this likely includes political, economic, social as well as health related considerations. If acceptability is low, then the proposal should be reconsidered if effective mitigation and acceptable alternatives cannot be identified.

- **Legal:** Legal considerations intersect with ethical ones. Ownership of wastewater and environmental samples is a legal matter; sewage derived samples often lies with the entity owning the sanitation services (or water body from which samples are taken). A special case is the aviation or maritime companies which own the vessels and ports from which samples may be collected. Use of these samples including testing, storage, disposal and the data and information arising from them are also legal matters that may be unclear, lack legal precedent and differ between jurisdictions. Privacy protections and separation between law enforcement and public health entities for data use also differs between jurisdictions.

Step 4: Optimisation criteria

The Optimisation step considers how to integrate in disease surveillance and response systems and add maximum value at lowest cost including multi-target WES.



- **Integrated in disease specific surveillance and response**: building on the deliberations in the 1st step where WES was judged to add value to existing surveillance, this step now requires detailed consideration of what, when, how and by who that would optimally happen and any key limitations or issues which must be addressed. As illustrated in Fig 2.1 this requires consideration of how integration can be effected at initial and end stages with relevant design, integrated analysis and decision-making steps including:
  - Overall and WES specific surveillance objectives, detailed response protocols including how results from WES or another source trigger any actions (including agile WES with changes in WES sampling, analysis or turn-around-time); and
  - integration of information from WES with other sources to provide timely combined intelligence to inform public health decisions and other relevant actions.

- **Integrated in multi-target WES program :** if there is an existing WES program or ongoing activities (such as for polio, SARS-CoV-2 and/or other targets) consider how the addition of the new WES activities can be optimally integrated in a cost-efficient way and, where possible, strengthen the WES program in relation to all surveillance objectives.
  - Addition of a target may simply require additional laboratory analysis and little to no change in sampling or transport activities and related costs and leverage existing laboratory infrastructure and capability.
  - However, there may be pathogen-specific requirements at each step of the process which require careful evaluation of where there are synergies and where there are pathogen-specific needs and a need for trade-offs between cost and performance. Additional detail on multi-target WES considerations follow in the next section.

- **Capability, sustainability and the greater good**: there are also cross-cutting and longer-term considerations such as whether the inclusion of the additional target in WES promotes system capability and sustainability including ongoing program performance and to be better prepared to implement agile WES if the need arises. Consider whether local WES results are relevant to other populations at the global or regional level, i.e. sentinel transport hubs for regional or global levels, or have valuable linkages to other One Health programs, such as biosecurity, diseases of livestock or wildlife, and zoonoses with benefits beyond human health.

At the end of step 4 WES team should have a short list of pathogens prioritized for implementation in routine or agile mode. They should also have identified research, capacity and consultation needs for pathogens that did not advance through the previous steps.

4.4 Combining multiple pathogens

The preceding criteria (which focus on public health significance, feasibility, acceptability and optimisation) principally relate to prioritizing a single target and associated use case with integration in an ongoing WES program (if one exists).

There is also merit in considering the value of a synergistic, multi-target WES program; it may be useful and cost-effective, rather than to select a single target, to select a combined set of compatible targets, for instance a group of high priority respiratory pathogens such as SARS-CoV-2, influenza A and B viruses, and RSV to track individual and combined respiratory infections in order to project combined health system burden, and inform planning and mitigation through vaccine campaigns, public awareness and behavior change communications and other public health actions.

Technical and operational considerations for optimally combining targets as part of a multi-target WES program include considering the degree of alignment of sampling locations, seasonality or other temporal or event-related drivers, sampling and transport methods, analytical techniques, data management, methods of communication, interpretation and shared human, infrastructure and other resources. The more these variables align, the more opportunities there are for gaining cost-efficiencies through multi-pathogen surveillance as well as considering trade-offs. However, WES may be considered for a single pathogen and parallel processes may be required for some steps in a multi-target program. Nevertheless, potential for future additional targets and other changes should also be included in preparedness in the design phase, especially as they relate to data management and reporting as this can greatly reduce barriers and costs for additional targets in the future.

The following additional aspects may be qualitatively or quantitatively evaluated to inform optimization decisions, and these may in turn influence decisions on whether to ultimately include a set of targets within a WES program.

When deciding whether there is value in combining targets, there are five major drivers of decisions:

- **Epidemiological** - Epidemiological considerations have meant that targets have been grouped according to the principal syndromic presentation of the disease (respiratory, gastrointestinal, hemorrhagic fever, mucocutaneous etc). The transmission pathways, clinical presentation, risk factors for severe disease and public health responses for diseases with similar symptoms are often aligned, and hence so are the surveillance needs.

- **Sampling** - Alignment on populations of interest and sampling type and frequency between targets. WES programs can be optimized around the most efficient and effective way to collect samples which are relevant to the population at risk (of interest), the prevailing sanitation treatment system, the target of interest and the timeliness of results (through frequency of sampling and turnaround time).

- **Analytical** - Analytical considerations have meant that targets are broadly grouped according to the group of pathogens (e.g. viruses, bacteria, other). The methods for sampling, sample matrix, extracting, and analyzing the targets and their genetic material are typically tailored to pathogen group at this kingdom level. However other considerations in relation to pathogen size, presence of envelope, charge and other factors may influence specific methods and compatibility between pathogens.

- **Context** - Contextual considerations have meant that targets have been grouped according to the intervention context. For instance, for some parameters, such as *V. cholerae* and Typhoid and Paratyphoid *Salmonella*, there is limited value in sampling from transport hubs in locations with good WASH coverage since the conditions that would permit diseases such as cholera and typhoid to become endemic are absent. On the other hand, there is value in testing samples from transport hubs for respiratory pathogens, such as influenza viruses and coronaviruses, since these are readily transmitted in all settings, regardless of WASH coverage. In other cases, vaccination coverage might be the principal contextual driver of relevance.

- **Administrative** - The way in which the targets/diseases/interventions are grouped within the health agency organizationally may be important. This may include how data is reviewed, monitored, and acted upon. Whilst in theory WES is most efficient as a "horizontal" system that considers all targets together and that are wastewater and environmental water focused, disease monitoring and mitigation programs are often more "vertical" programs that are disease-focused. Polio has, as an eradication target, a strong vertical disease program, which provides a model for integrated polio WES and clinical surveillance but has, not to date integrated other pathogens as part of multi-target WES. SARS-CoV-2, arguably, tends toward the inverse, i.e. it is not an eradication target, and WES programs have often been integrated with other respiratory pathogens, such as RSV and IAV/IBV. Therefore, understanding that horizontal and vertical matrix of how diseases are managed and targets are monitored for surveillance influences how WES is understood, funded, delivered, and used. The horizontal character of WES stems from the fact that it is based on a collaboration between public health, wastewater, and environment sectors, (rather than the vertical character of disease-targeted public health programs), which provides more opportunity to target multiple pathogens simultaneously.

4.5 Costs and benefits: Realizing WES benefits through implementation

Prioritization for WES outlined in Figure 5.2 is grounded in providing awareness to enable more effective responses to disease outbreaks that present a substantial threat to public health. Realizing these benefits requires implementing systems that provide information that provides value that exceeds the costs incurred to obtain it[35,36]. Analysing the value of information from WES involves consideration of how benefits of WES are realized and maximized; the full range of costs incurred for WES; and deep uncertainties about when and how disease outbreaks and responses to them will unfold.

The benefits from WES derive from whether actions are taken with the additional information from WES that is not available elsewhere result in improved outcomes. Information only has value when it spurs changes in action or behaviors that lead to beneficial results. For example, modelling studies posit that WES could provide awareness of disease outbreaks that can[37]:

- inform decisions to implement public health interventions earlier than if reliance on clinical data alone,
- provide information that enables targeting of medical countermeasures and non-pharmaceutical interventions towards subcommunities or disease variants, or
- provide information that enables the public to adopt preventive or protective actions themselves such as adopting transmission reducing measures or seeking prophylactic medical countermeasures

Consideration of benefits should include a broad range of outcomes including:

- reduced morbidity and mortality, both from direct consequences of improved response to the disease outbreak and reduced comorbidities due to disruptions to healthcare and public health delivery created by the disease outbreak; and
- reduced economic burdens and societal disruptions induced by public health interventions resulting from the potential to adopt less stringent, less broad, or shorter duration interventions.

WES cost analysis should consider the broad range of expenses incurred. Costs begin with fixed costs for acquiring equipment and establishing WES collection, analysis, and reporting systems. Costs also include on-going operating expenses that can scale with the intensity of operations to cover the personnel and supplies required to conduct sampling, analysis, and sharing of information. Cost analysis need also include expenses to sustain capacity: such as maintenance and replacement of equipment and recruiting, training, and retention of personnel. Finally, cost analysis must also consider how WES performs when incorporated into decision-making and how it can reduce costs in public trust and support from overreaction to disease outbreaks and failure to respond adequately.

The context within which WES prioritization decisions are made is deeply uncertain. The timing, types, geographic location and extent, severity, and potential for mitigation of disease outbreaks creates the potential for practically uncountable scenarios. Analysis of the value of WES that considers the breadth and nature of these uncertainties supports implementation of WES within a resilient and robust public health intelligence function[37].

4.6 Quality assurance and continuous improvement

[placeholder covering M&E]

5 Cross-cutting aspects

5.1 Program planning considerations

A target-agnostic overview of a WES workflow is given in Figure 5.1. When evaluating whether one or more pathogens or other health-related targets can be efficiently combined, the figure illustrates some of the considerations to be evaluated.

The use case, context, and existing WES program, influence how a target is incorporated within an integrated WES program.

When assessing the compatibility or incompatibility of various targets is it important to clarify for each; the context, use case, existing and proposed sampling and analytical approaches, and response.

Incompatibilities can arise at one or more of the process steps in the workflow. For instance, if a sufficiently timely and sensitive workflow for one target is not consistent with another, the two targets might not be amenable to combining within one program. Some targets warrant higher frequency sampling than others, or they may have different optimal sampling sites, frequencies and methods, or very different processing and analytical methods.

This section provides a target-agnostic summary of some of the process steps in this workflow and illustrates the diversity of approaches that have been adopted in WES. The pathogen-specific target summary sheets (prepared separately) highlight current or predicted recommended approaches for pathogen specific aspects of the workflow.

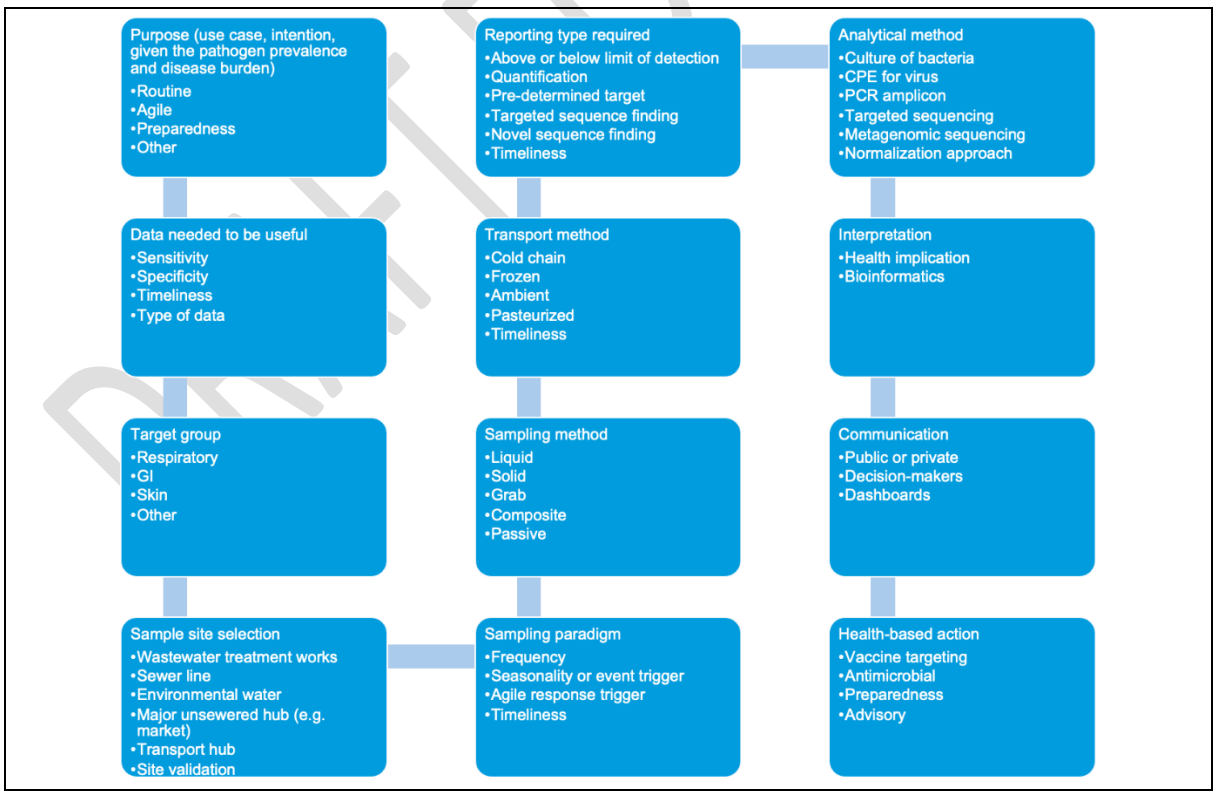


Figure 5.1. Illustrative target-agnostic overview of a WES workflow

Table 5-2. Illustrative comparison of typical scenarios to demonstrate potential alignments and incompatibilities for three WES targets

Target / Use case	Poliovirus	SARS-CoV-2	*S.* Typhi and Paratyphi
Example objective	Demonstration of polio-free status in contexts with local elimination	Tracking trends in COVID-19 incidence and variants in circulation	Identification of circulation of typhoid (and assessment of AMR in a setting with limited blood culture capacity
Example public health action informed by results	Vaccination campaigns and screening for cases	Vaccination campaigns and healthcare facility readiness	Vaccination campaign advocacy and healthcare facility readiness
Example reporting level	Global	National	Local
Sampling			
Example field sampling location	A small number of nationally significant sentinel sewered and unsewered sites representing major populations based on GPEI guidelines	Sentinel sewered and unsewered sites as hubs representing targeted populations based on locally derived priorities including manholes and/or wastewater treatment plants	Sentinel sewered and unsewered sites as hubs representing targeted populations based on locally derived priorities including manholes and/or wastewater treatment plants
Example sampling frequency	Bi-Monthly	Weekly	Monthly
Example field sampling method	Grab or composite	Composite or passive	Passive
Example storage and transport of samples	Cold chain, not frozen	Cold chain, not frozen	Cold chain, not frozen
Example seasonality of sampling	Year-round	Year-round	Year-round
Example agile responses	Step up spatial and temporal resolution in response to detection to identify hotspots and sources	Not applicable since this program is used for trending and public information	Step up spatial resolution in response to detection to identify hotspots and sources
Analytical			
Example laboratory analytical method	Culture then PCR	PCR	Culture enrichment then PCR
Multi-target Compatibility			
Sampling	Weekly routine samples could be collected from all relevant sites for SARS-CoV-2 testing Samples could be tested monthly from some sites for poliovirus and *S.* serotype Typhi and/or Paratyphi testing		
Transport	All samples would be shipped and stored via cold chain		
Analysis	Some samples may be tested using culture-based methods in some circumstances, others direct PCR. If culture-based methods are used, the PCR reactions for all three targets would be run separately. . The analytical laboratory would need to be set up for culture-based virology and bacteriology, and PCR methods, to test for all three pathogens separately. Sequencing capacities may also be required to estimate the proportion of circulating variants and to characterize isolates for antimicrobial resistance properties.		

5.2 Site selection and sampling strategies

Sampling locations, type (e.g., wastewater from sewers, environmental drainage), frequency, time-to- reporting after sampling and other aspects must be tailored to the local context. This included consideration of sanitation systems (i.e. sewered or non-sewered systems) as well as human and financial resources and local regulations. The objective is to set up the sampling program so that sentinel sites are sufficiently informative that the results can complement other surveillance systems and inform public health action for the use case and context. This requires expert knowledge of the population of interest as well as of the sanitation system characteristics and coverage. Populations can be dynamic, and in considering WES one must consider contributions from individuals who live, work, recreate or visit the sanitation catchment. Population dynamics (demography, mobility, and migration) and health statistics can be brought into the understanding of WES sample point catchment population dynamics. Locations which include hospitals, schools, transport hubs, gathering points for prayer, shopping, festivals, seasonal work or other special locations may have complex population dynamics and relationships with local communities. For some zoonotic targets it is important to understand animal host dynamics within the catchment of the sample.

It is also important to consider both what is known about the population directly sampled at sentinel sites and whether sentinel site results can reasonably be generalised to the broader population of interest. Mapping of sewer and environmental water networks and their catchment populations may be poor and maps may not exist or be incomplete. An understanding of the network and represented population is needed prior to sample site selection to enable interpretation of results.

The sampling strategy for a WES program is related to the incidence of the target in the community of interest and the surveillance objective(s):

- **In contexts where clinical testing is being practised, and locally acquired cases are being reliably diagnosed**: In principle, direct surveillance for the target in clinical samples, such as stools, blood, or respiratory secretions, from persons presenting with symptoms through public health surveillance remains important in areas with elevated incidence of disease, in situations where the diagnostic testing is sufficiently reliable. However, costs and other factors limiting the reach of clinical diagnostic testing or preventing individuals from accessing health systems, combined with the presence of asymptomatic infections, practically mean that representative surveillance data are often lacking, particularly in many low-income countries where the target of interest may be most prevalent. In such contexts, WES can provide supplemental, community-scale representative data to fill gaps in clinical data. To realize the value of such a WES program it must be undertaken with sufficient spatial coverage, at sufficient frequency, and be ongoing or available as and when required, and able to be adapted to need, albeit potentially with very low positive predictive value.
- **In contexts where clinical testing is being practiced, and no locally acquired cases are being diagnosed -** Even when there are no cases being routinely reported and the target is no longer thought to be circulating within the local population, a WES program can provide early warning of introduction and re-emergence. This can present challenges since interest in, and funding for, surveillance for a disease can wane as incidence drops. In addition, the lower the incidence of a disease, the more extensive (in terms of spatial extent and frequency of sampling) the WES program may need to be to detect positive samples earlier to provide earlier warning. However, WES objectives do not necessarily require the frequent detection of positive samples to be achieved. Objectives such as providing early warning for new pandemics, detecting early signals of new variants, monitoring the global spread of infectious diseases, and demonstrating local

eradication, are important applications of WES even if most or all samples return non-detection for the target. Targeted WES testing, such as at sea and airports and other transportation hubs and points of entry, of high risk communities, or in areas receiving large inflows of persons from endemic areas, can help prioritize the WES program.

- **Sampling strategies may differ within one jurisdiction** - . The geographical, geopolitical or administrative areas may not align with the wastewater and environmental water catchments within which diseases are circulating. The administrative boundaries of the public health systems are not aligned with under-ground wastewater system infrastructure or environmental water catchment boundaries. However, administrative areas are defined, and hence wastewater and environmental water catchments can be attributed to populations, who can be assigned to one or more public health administrative districts, using diagrams, system descriptions, modelling and geographic information system tools. In such circumstances, different WES sampling strategies may be occurring within the same jurisdiction. Similarly, there may be under-represented or vulnerable groups for whom special WES programs are undertaken that differ from those in the surrounding areas.
- **Sampling strategies may differ over time** - There may be a routine baseline sampling and testing program, with limited sites, frequencies, and targets. In addition, agile surveillance options may be in place, to enable enhanced surveillance to occur to expand the coverage of sites, frequencies, and/or targets, in response to changing circumstances.

Increased sampling locations and frequency and shorter turn-around time all contribute to higher quality timely data but also to higher program costs. Integration with existing sampling and sample transport processes reduces costs and resource requirements.

The sampling frequency and reporting of data is heavily linked to the surveillance objectives and should be considered alongside the frequency of public health and surveillance coordination meetings, surveillance data review, ability to act on data, and surveillance from sentinel or supersite hubs, etc., to optimise alignment and decision-making. The sampling frequency must be of value to those decision-makers.

Sampling at consistent sites (strategic sentinel sites with reasonable population coverage or corresponding to population of interest) at a consistent frequency provides a baseline sampling strategy, derived based on consideration of the variability in duration of disease, shedding level and pattern, and the ability of public health agencies to act on the data. At its lowest, the sampling frequency is likely to be no less than monthly. For most targets, once the sampling frequency drops below monthly the results are of limited value since targets are likely to follow seasonal or evolutionary patterns that vary over at least monthly timeframes. Exceptions might be targets that vary more slowly over time, such as antimicrobial resistance genes, or periodic cross-sectional data, e.g. to track genomic changes over long timeframes that do not require frequent sampling. Sampling less than monthly would be of little actionable value for routine surveillance.

At the upper end of sampling frequencies, to provide early warning, or detect rapid changes, an acceptable strategy involves sampling at least two times per week with turnaround of results on a weekly basis. This frequency is a function of analytical capacity of the labs, cost, and the ability of public health agencies to interpret the data. This is recommended when there is a high utility to detect rapid changes in circulation of the targets, and noting that the composition in a catchment can differ between weekends and normal working days, due to the commuting pattern of persons in the catchment. If the sole target of the WES programme is subject to gradual change in a population, an alternative and less frequent sampling frequency may be sufficient Optimal sampling periods and frequencies are also significantly affected by tourism seasons, extended national holidays, and

cultural gatherings. Therefore, it may be especially valuable to collect samples at different frequencies during these times to facilitate early warning.

Importantly, for most WES programs, the purpose is to target sentinel sites which provide generalizable information to the broader population, not to exhaustively sample wastewater and human impacted environmental water to try to achieve coverage of the entire community. Understanding the local sanitation systems permits targeting of sampling to more centralized systems that more efficiently capture larger populations. Where large populations are not connected to centralized systems, representative samples of decentralized systems, and samples of environmental water impacted by pathogens shed from humans, can be targeted to provide some coverage. This can include pooled wastewater from septic and sludge collection sites if collection of such samples can be routine and timely for public health impact; however, it is likely that environmental water impacted by open defecation, discharge from septic systems, or unsafely managed onsite sanitation systems may provide a more realistic and timely collection site given that emptying of onsite systems is often less than monthly. However, for ethical reasons, there is a need to avoid inadvertently identifying individuals. In contrast to individual clinical specimens, these environmental samples represent contributions from populations, and the goal is to efficiently provide a pooled and not an individual sample, to efficiently provide information about pathogen circulation in larger populations or sub-populations over time.

Recommendations regarding sampling sites, sample types, and sampling frequency, can be adjusted to meet jurisdiction-level surveillance objectives. A flexible approach is encouraged to define a feasible core sampling strategy and specific triggers for heightened or reduced surveillance as best required to inform actions. For example, increased frequency to identify the peak of a wave (and inform heath system planning); increased frequency and variant testing if signal of an uptick in cases (to inform modelling for size and duration of wave); decreased frequency if the purpose is simply to follow broad trends over time.

Sampling sites are typically limited to points that represent wastewater catchments (e.g. from the inflows to sewage treatment plants) or human-impacted environmental water catchments (e.g. from downstream of unsewered areas that capture substantial amounts of human waste). Sampling from individual septic tanks that capture individuals or very small populations, and similar sites, can represent ethical challenges, especially around identifiability of individuals, as well as lacking sensitivity. There is more value, and there are less ethical concerns, in sampling from community septic tanks, or in certain contexts (e.g. transient populations like travel hubs, or particularly vulnerable populations like refugee camps).

5.3 Sampling capacity requirements

In general, sampling analysis falls into four categories as summarised in Table 5.3.

- **Grab sample**. Depending on location and access, such sampling may not require specialist sampling equipment and can simply entail collecting a liquid sample in a conventional water sampling container. Repeat grab samples taken at different times may be pooled to form a manual composite sample.

- **Active time-weighted or flow-weighted composite sampling**. These collect and composite a sequence of liquid samples at intervals, either based on flow (flow intervals) or time (time intervals) over a defined sampling period, typically 24 hours as part of a periodic sampling program. The devices are specialized and require a pump and sampling line, a receptable, and a battery or mains powered sampling devices, and ideally they are refrigerated during the sampling period.

- **Passive (or trap) composite sampling**. This involves placing a matrix in a liquid medium to attract and retain the target over time, with flexible timing typically ranging from one day to one week, followed by retrieval of that target. As such, this requires two visits to the sampling site to collect each sample. The sampling devices need to be installed in locations that are unlikely to be interfered with due to tampering or theft. There can be limitations to the adsorptive capacity of the capture medium that are relevant if retention periods longer than one day are used. This shares some advantages with active composite sampling but is cheaper and less technically and logistically complex since power and costly refrigerated equipment is not required. This method of sampling is typically applied in situations where continuous sampling with superior temporal coverage is desired as well as in a greater range of locations where the use of active composite samplers is not feasible (due to power or security conditions or the lack of available composite samplers). Comparative evidence regarding the concentration of inhibitory substances on the sampling matrix, the differential attachment and persistence of primary targets and other targets used for normalization are required. Unlike grab samples, quantitation is not possible to calculate with flow, and there are uncertainties introduced when using biomarkers to provide some basis for normalization. Cost, feasibility as well as the pathogen specific evidence on the appropriate sampling material are considerations in deciding which sampling method to use.

- **Sampling of solids**. This entails collecting a sample in a conventional sludge sampling container, including faecal sludge from, for instance, septic tanks, primary sludge from wastewater treatment plants, or sediment from environmental samples. There can be challenges accessing certain types of sludge in treatment plants. In general, pathogens are more concentrated in solids than liquid, and evidence on the liquid:sludge partitioning ratio of specific pathogens, where available, is noted in the target summary sheets. The concentration of inhibitory substances in solids, and the differential partitioning and persistence of the primary targets and of other targets used for normalization if undertaken, are considerations in deciding between liquid and solid samples. A further consideration is the relationship to the underlying population and pathogen circulation as if the pathogen persists for a long duration in solids it compromises the interpretation of the result as it cannot differentiate between a recent case/s and those which are not.

All forms of sampling require some form of regular, reliable access to a sampling point and this is not always simple. Sampling from locations that don't have readily accessible sample points can prove

technically and logistically challenging. Tailored bespoke sampling devices may be required, even for collecting grab samples, such as those created to access aircraft sullage tanks during the COVID pandemic.

For environmental samples, sampling sites that draw from ephemeral streams or that are located in areas prone to flooding may present challenges. Ideally, the sampling sites will be accessible when relevant, taking into consideration wet and dry seasons and floods, to enable year-round surveillance.

Ideally, the best sites for sampling are those that provide optimal evidence when interpreted by the public health agency. However, as should become evident when considering the above constraints, sampling site selection is often a compromise between various logistical and practical factors and the sites that would provide the best evidence to inform the WES program. Sampling sites should be evaluated for utility over time, as described in the polio WES guidance. Nonetheless, it is important to evaluate and document sampling site characteristics so that samples collected can be related, to the extent that is reasonably practicable, to a catchment, contributing resident, working or visiting population, and timeframe, in a meaningful and informative manner, albeit this is not always simple or reliable.

Sampling needs to be carried out safely, and this includes personal security, traffic safety, safety from exposure to potentially hazardous solids, liquids and gases found in wastewater and associated infrastructure, safety from drowning, avoidance of becoming trapped, handling of infrastructure to collect samples, and so forth. These safety requirements are consistent with conventional wastewater access, handling, and sampling requirements, and specific guidance during the COVID-19 pandemic has been prepared [38]. Traffic safety and personnel security considerations can limit where samples can be collected or where samplers can be placed.

Additional data and information are essential to help with interpretation, and potentially with interpretation of data. This can include information on date and time of day, liquid flow rates, recent rainfall (for systems combined with drain water), temperature at point of collection and when received by the lab, origin of material in the catchment contributing to the sample, and recent events or activities that may have influenced the persons present in the catchment (such as gatherings), and other information (oily sheen, scums, colour, or other unusual observations). Additional physical, chemical, and microbiological data, or data on flows into the catchment from inputs not related to human waste, such as industrial or other water flows, can also assist with normalization. Further discussion on more advanced endogenous normalization markers is given in the next section.

The pathogen or target sought, use case, methods available, biosafety considerations, etc., influence the choice of method applied. Table 5.4 summarizes example cases and associated sampling frequencies and methods typically applied.

Table 5.3. High level summary of sampling approaches and requirements

Sampling method / Requirements	Liquid grab	Liquid active composite	Passive composite from liquid	Solid grab from faecal sludge or wastewater or environmental solids
Sample collection device	Simple sample container holder or access to a sample tap	Specialized time and/or flow-proportional sampling device with sample line and pump requiring power and refrigeration (unless creating a composite from grab samples collected over time)	Means to suspend material in wastewater or environmental water, permitting flow past and contact with the material (often housed in protective casing & material optimized for pathogen of interest)	Simple solid sample collection device
Sample container	Standard microbiological sample container			
Temporal coverage	• Single point in time • Periodic sampling	• Usually 24-hours • Periodic sampling	• Variable duration (short to 7 days) • May provide continuous coverage	• Single point in time • Periodic sampling
Representative of catchment population	• Low • Small volume • Sample can be made more representative by well-targeted timing and location of sample	• High • Can be set up to capture wastewater in proportion to either time or flow rate. The latter is more technologically challenging but is technically achievable and is routinely practiced.	• Moderate-High • Captures target in proportion to its pathogen specific uptake properties over time • Requires pathogen specific validation • Normalisation markers can be utilised (refer to main text)	• Low - High • Captures material in proportion to its solid phase partitioning over time • Limited by potential for selectivity in solids-phase apportioning and degradation over time once in solids phase • Some targets will be more solids-associated than others
Need for reliable power	Not required	Required, either mains power or battery	Not required	Not required
Security considerations	Not required	Required as equipment is costly and readily vandalised	May be required. While not costly, may be stolen if visible	Not required
In-stream flows from within or at the effluent discharge point of buildings	Yes, if accessible	Yes, if secure and accessible	Yes, if accessible	No
In-stream flows from piped networks	Yes, if accessible	Yes, if secure and accessible	Yes, if accessible	No
In-stream flows from drainage channels	Yes	Yes, if secure and accessible	Yes, if accessible	Yes, if there is a sedimentation point
Treatment plant inflows	Yes	Yes	Yes	Yes, if there is a sedimentation point, e.g. primary sludge
Sludges and solids	No	No	No	Yes, if there is a sedimentation point, e.g. faecal sludge in a septic tank
Transport vessels and hubs	Yes	No	Yes (at port/airport)	Yes, if waste includes settled solids
On-site decentralized systems such as septic tanks	Yes	No	Sometimes	Yes
Costly equipment	No	Yes (can be thousands of US$ per autosampler, plus ongoing operating cost)	No	No

* This refers to refrigeration during sampling. Sample storage during transport still requires cold chain as far as reasonably practicable, for most targets, with the exception of targets for which cooling may induce dormancy which may inhibit culture-based detection.

Table 5.4. Illustrative example of some use cases, sampling frequencies, and analytical methods.

Use case	Example sampling frequency	Example analytical method	Example result
Baseline trends	Weekly	Depends on the target, typically genetic, and sometimes culture-based, or a combination	Quantification and normalization may provide additional information if undertaken appropriately, but may introduce unnecessary complexity and confounders
Early warning	Twice weekly	Depends on the target, typically genetic for the most rapid results	Detection is the priority (quantification adds extra value but is not necessarily undertaken)
Preparedness for pandemics	Sufficient to maintain capacity and relationships between stakeholders	Both culture-based and genetic methods need to be ready to apply	Evidence of adequate process performance and reliability
Tracking or detecting known variants	Weekly	PCR for specific target variants	Variant identification, detection and, if required, quantification
Novel variants	Weekly	NGS of whole genome or target genes	Sequence library of variants present, with detection and evaluation of novel variants.

5.4 Laboratory capacity requirements and quality management

High-quality and reliable laboratory data are a core component of a successful WES program. Building or strengthening environmental microbiology laboratory capacity is one of the first steps in WES program development. A landscape assessment of environmental microbiology laboratory capacity can provide stakeholders a clear understanding of the scope of investment needed. Existing environmental microbiology laboratories or polio environmental surveillance laboratories may already be present and may be utilized to include new or expanded WES testing. If these types of laboratories are not present, underutilized clinical or other types of laboratory spaces may need to be modified prior to use. Developing and sustaining WES laboratory capacity can require substantial financial investment; efforts may need to start small and scale up over time.

Strengthening environmental microbiology laboratory capacity is essential where it is not already sufficient. Public health labs might have no prior experience on working with environmental (water) samples. Water laboratories on the other side, might not have experience in pathogen detection in the context of WES.

5.4.1 Personnel

For sampling, it is not necessary to involve environmental microbiologists. In wastewater treatment plants (WWTPs), many staff are trained in both wastewater and sludge sampling. This sampling is part of their daily routine which is conducted to monitor the treatment plant processes.

Trained environmental microbiologists that are familiar with analysing wastewater, sludge, and environmental water, as applicable, for pathogens, are critical for the analytical septs to ensure the collection of accurate and reliable WES data. These scientists have specialized knowledge of the physical, chemical, and biological interactions between microorganisms and their surrounding environment and of the dynamic nature and complexities of environmental matrices. They possess expertise in collecting, processing, and testing environmental samples using various microbiological and molecular techniques and are proficient in interpreting environmental data and recognizing methodological limitations. Clinical detection methods often serve as a starting point for development of methods for environmental samples; environmental microbiologists are adept at

adapting and optimizing clinical methods to improve their sensitivity and specificity for application to environmental samples. Environmental microbiology is a specialized discipline that may not be available for study in many universities and there is a need for training and professional development to increase this capacity globally to support the growth of WES. An important major difference between clinical microbiology and environmental microbiology is that usually environmental microbiology is trying to quantify the target pathogen (or indicator organism) in the environmental sample. For most clinical microbiology, the focus is on detection (presence/absence) of a pathogen in a clinical specimen. For WES, sometimes information on presence is sufficient, but not always.

The number of laboratory personnel needed for WES programs is governed by efficiency of sample collection and transport, methodological approaches used, and resource availability. Each context has its own organizational structure. Therefore, it is not straightforward to provide effective guidance on the necessary laboratory personnel. In principle, Sufficient full-time experienced bench-level microbiologists are required for conducting pre-analytic (sample receipt, equipment maintenance, materials preparation), analytic (sample processing, sample analysis), and post-analytic (data entry, clean-up/decontamination) activities. Similarly sufficient senior laboratory staff are required for laboratory administration, quality and safety management, and reporting activities. Additional bench-level microbiologists allow for enhanced efficiency and specialization in responsibilities, which can lead to increased sample throughput, higher quality data, and sustainable staff workloads. The APHL SARS-CoV-2 Wastewater Testing Guide[39] and the Polio Laboratory Manual[40] provide additional guidance for laboratory personnel needs for WES programs.

In addition to upfront training, ongoing refresher training, assessment, and competency tracking is required.

5.4.2 Facilities and safety

Laboratories tasked with analysing WES samples encounter biosafety risks due to both potentially high concentrations and diversity of pathogens in samples. Prior to conducting WES laboratory activities, a biological risk assessment should be conducted to identify potential site-specific hazards and to select appropriate building and equipment requirements, barriers, laboratory practices, and mitigations to protect both laboratory staff and the environment.

Sample transportation needs special consideration, particularly where samples need to be transported over long distances, such as in remote or rural areas. Sampling can introduce cost, logistical, and technical challenges. Sample transportation and storage should entail keeping the sample temperatures low in most cases, with refrigeration temperatures being recommended (but not frozen), and the time between sampling and the start of analysis as short as possible. For some targets, such as some pathogens (e.g., *V. cholerae*), ambient temperature is preferred to stop the pathogens moving into a non-cultivable physiological state in response to the cooling.

WES samples may be analysed directly or may first need to undergo processing steps to concentrate the target, increase the target of interest, or remove interfering substances from complex matrices. Initial WES sample processing should not take place in rooms used for the following activities:

- Media and materials preparation
- Tissue culture for viral infectivity assays
- Molecular reagent preparation
- Molecular analysis (e.g., polymerase chain reaction (PCR), sequencing)
- Administrative functions (offices, breakrooms)

It is also highly advised that WES sample processing not take place in rooms used for clinical diagnostic testing, as there is a risk of cross-contamination. If use of separate rooms is not possible, care must be taken to reduce cross-contamination between environmental samples and clinical specimens. This can include using separate benches and equipment, installing physical barriers, varying timing of sample processing to minimize overlap (e.g., separate days), employing different staff for WES activities, and performing rigorous cleaning and disinfection procedures between tasks.

Adequate biosafety control is required for collection and analysis of wastewater from WES. The level of personal protective equipment and vaccination required for sampling and analysis, and recommended laboratory biosafety controls for collected for wastewater, depend on the pathogens under investigation. In the polio program, there is guidance on these aspects. Laboratories should adhere to Biosafety Level 2 (BSL2) standards as a minimum requirement for processing WES samples. This includes (but is not limited to) ensuring controlled access to laboratory spaces, providing sufficient handwashing facilities with running water and soap, utilizing impervious surfaces that are easy to clean and disinfect, maintaining adequate lighting conditions, and having essential decontamination equipment and procedures in place. Additionally, due to the potential for high concentrations in WES samples, laboratories should utilize biological safety cabinets (BSCs) during processing steps that could create infectious aerosols or splashes (e.g., pipetting, mixing, sonication, manipulating cultures). In situations where a BSC is not available, appropriate personal protective equipment (PPE) should be used alongside other administrative controls as determined by the biological risk assessment. Standard and special microbiological practices, safety equipment (including PPE), and facilities recommendations for BSL2 laboratories can be found in Biosafety in Microbiological and Biomedical Laboratories[41]. The biosafety level of the testing laboratory, and testing of target pathogens should be guided by local regulations on biosafety[41].

5.4.3 Methodological approaches

5.4.3.1 Overview of approaches

There are two general categories that WES methodological approaches fall in to; culture-dependent approaches and culture-independent approaches. Each is detailed below.

Culture-dependent approaches

Culture-dependent approaches involve utilizing artificial laboratory conditions to cultivate a pathogen of interest from a sample. Currently, the most widely demonstrated culture-dependent approach for WES is that used for poliovirus. While information on pathogen viability is not necessary for WES, a culture-dependent approach helps increase the concentration of the pathogen, while also suppressing the growth of non-target microorganisms, thereby making the pathogen more likely to be detected. Another advantage of using a culture-dependent approach is that it can result in production of isolates, which are usually required for definitive confirmation of the pathogen via morphological, biochemical, serological, antimicrobial susceptibility, and/or molecular-based (e.g., PCR, sequencing) tests.

However, there are limitations to utilizing culture-dependent approaches. Some microorganisms enter a viable but non-culturable (VBNC) state within the environment and therefore are not able to be cultured. For those that can be cultured, selective media are often not selective enough, especially when used for complex WES samples that may contain a diversity of closely related species; the number of isolates that must undergo confirmatory testing may be cost- and time-prohibitive. Additionally, artificial laboratory conditions may select for certain strains of microorganisms over others, thereby biasing results. Furthermore, culture-dependent approaches

that include a broth-based enrichment step eliminate the ability to quantify the pathogen of interest in the original sample, which is needed for assessing trends in WES. This can be circumvented by using a most-probable-number (MPN) enrichment format, however this approach can also be cost- and time-prohibitive. Lastly, confirmatory testing of enrichment broths alone (and not of isolates) may be misleading or inconclusive if multiple gene targets are required for definitive confirmation and these targets exist independently in other species or strains.

Culture-independent approaches

Culture-independent approaches involve direct testing of a WES sample, generally via PCR or sequencing, without inclusion of a culture step. Currently, the most widely demonstrated culture-independent approach for WES is that used for SARS-CoV-2. Culture-independent methods are faster and more cost-effective than multi-step culture and confirmation methods and may more easily facilitate implementation of multi-target WES. Additionally, culture-independent approaches can allow for bypassing challenges with VBNC microorganisms and with microorganisms for which culture-based approaches have not been developed.

There are also limitations to utilizing culture-independent approaches. These approaches work best if there is a single gene target specific to the pathogen of interest. Because an isolate is not generated for further testing (as is the case with testing enrichment broths alone, as discussed above), results may be misleading or inconclusive if multiple gene targets are required for definitive confirmation and these targets exist independently in other species or strains. An example of this would be the challenge posed when attempting to link antimicrobial resistance (AR) genes with their host pathogen, as AR genes are often on mobile genetic elements which can exist outside of cells or be transferred between bacteria. Culture-independent approaches might also not be sensitive enough to detect targets when they are in low levels in the environment, even when concentration methods are utilized.

5.4.3.2 Method selection considerations

In the absence of standard WES methods, as is currently the case for many targets of interest, the choice of methods selected for each application depends on the context and information sought. There are several considerations to keep in mind when selecting methods for individual- and multi-target WES:

- **Pre-analytical processes** may be performed on WES samples prior to analytical methods to homogenize the sample, concentrate or enrich the target of interest, remove interfering substances, or facilitate analytical workflows. Pre-analytical processes can include:
  - Sonication
  - Centrifugation
  - Filtration
  - Affinity capture
  - Pre-enrichment and selective media enrichment
  - Chemical precipitation
  - Enzymatic treatment
  - Nucleic acid extraction
  - Nucleic acid purification

- **Analytical processes** for WES samples are performed to detect and/or characterize specific pathogens or genes. The choice of which to use depends on the specific target(s) of interest, the concentration of target(s) anticipated, and whether information sought is detection of specific serotypes, sequence types, or antimicrobial resistance profiles, or concentration of target(s) to

assess trends. There are certain features that are common between some targets, and understanding those differences and commonalities assists in designing versatile and sustainable WES programs. Analytical processes can include:
  - Microscopy
  - Direct plating or plating membrane filters on selective media
  - Biochemical, serological, and antimicrobial susceptibility testing of bacterial isolates
  - Cell culture infectivity assays
  - Immunological tests
  - PCR, including conventional, real-time (qPCR), reverse-transcriptase (RT-PCR), digital (dPCR) or droplet digital (ddPCR), and multiplex PCR
  - Sequencing, including whole genome, 16S rRNA, targeted amplicon, and metagenomic sequencing

- **Use of clinical methods** - Care must be taken when applying gold standard clinical methods to WES samples. Clinical methods are usually sufficient for pathogen identification in human specimens, especially when combined with symptomatology. However, WES samples will generally contain a much larger variety of microorganisms than clinical samples, including atypical or environmental species and strains that may cross-react with clinical tests. Furthermore, WES sample matrices can be very distinct from clinical sample matrices (e.g., blood, sputum, stool) so clinical methods usually will need to optimized to improve their sensitivity and specificity for WES samples.

- **Sequencing** can be used to uncover additional information on targets, such as sequence types and variants. Direct sequencing approaches may in the future play a prominent role, although they are currently still in their infancy for this application in WES.
  - Interpreting sequence information requires the use of bioinformatic software tools. These tools combine and align the raw sequencing data and compare it to reference sequences to identify known sequences for specific genes or pathogens and help identify lineages and variants. The value of these tools improves over time as more sequence information is uploaded. There are a number of tools for uploading, sharing, and querying genetic information derived from WES (e.g. PHA4GE).

- **Proprietary test kits** and platforms have been developed for wastewater surveillance to detect specific gene targets without the need for fully equipped laboratories or highly trained staff. These kits can be useful in resource-limited settings and remote locations. However, they come with disadvantages, including high initial costs, routine procurement challenges, and limited sustainability due to high cost per sample. Additionally, these platforms restrict flexibility in target addition and may limit the understanding of result outputs. Their sensitivity and specificity may also be limited, and they are not always optimized or validated for environmental samples.

- **Practical considerations** should also be acknowledged when selecting methodological approaches, including the capability and capacity of the analytical facility and human resources.

- **Research and innovation** - There are a number of promising methodological approaches currently being evaluated for WES. Overall, more research is needed to develop and validate approaches for individual- and multi-target WES before standardized methods are available.

5.4.4 Quality management

A Quality Management System (QMS) is a set of policies, processes, and procedures for designing and operating a laboratory and producing timely data that meets high quality standards. There are 12 Quality System Essentials (QSE) (pop out box) that are the building blocks of a laboratory QMS.[i] While all components of a QMS are important, there are several that require special mention as they are vital for the success of a WES program.

- **Purchasing and inventory**: Equipment and supplies for some WES procedures are specialized and their availability in specific regions of the world is often limited or difficult. Availability of equipment and supplies should be considered early in WES program development when targets and protocols are being selected. Laboratories should establish robust supply chains with reliable vendors, anticipate supply needs through careful forecasting, and maintain updated consumables inventories. Additionally, developing contingency plans for supply chain disruptions or equipment failures is essential to minimize downtime and maintain continuity of laboratory operations.

- **Training**: Comprehensive laboratory training to ensure consistent and accurate testing practices is essential for staff in laboratories undertaking WES activities given the specialized and evolving nature of the field. It may be difficult to find qualified environmental microbiologists trained in WES procedures. Clinical microbiologists can be cross-trained to conduct WES processing and testing; in low resource settings where clinical microbiology expertise is often limited, care should be taken to ensure that bandwidth for clinical testing is not adversely impacted. To interpret and utilize the data, data scientists will need specialised training in data and informatics for WES. To communicate the results, data visualisation and communication training will be required to enable personnel to translate and communicate the results into formats suitable for use by public health practitioners.

- **Quality control for testing:** As with any laboratory testing, quality controls should be included in WES sample processing. The APHL SARS-CoV2 Wastewater Surveillance Testing Guide for Public Health Laboratories explains controls that should be included for wastewater samples generally, such as endogenous controls to determine the human faecal input and help to normalize, as well as matrix spike controls to help determine recovery efficiency. The guide also explains controls that should be included during the molecular analysis steps to help with understanding of contamination and interpretation, such as method blanks, extraction blanks, no template controls, and quantitative positive controls. All controls should be selected to incorporate methodological considerations, resources, and target specifics. Importantly, the raw untransformed data should be reported since normalization can introduce confounders and additional variability[39,40].

5.5 Normalization strategies

The raw untransformed data should be reported since normalization is complicated and can introduce unnecessary complexity and variability that can be confounding to the use case. In addition, to enhance data interpretation in some situations, standardization and normalization can be undertaken by adjusting measured target concentrations for markers of the extent of human influence on the sample or population size covered etc. Physical, chemical and microbiological normalization options are noted below, including advanced normalization markers that have been utilized, albeit with variable reports on the value of doing so.

Most of the normalization markers utilized to date have been related to fecal and/or urine inputs to the material being sampled. These may be of most value for normalization of fecal-oral pathogens, but of less value for targets shed via respiratory, skin, or other pathways.

Normalization markers recently reviewed in the context of SARS-CoV-2 surveillance (Parkins et al 2024) include chemicals (creatinine, ammonia, 5-Hydroxy-indoleacetic acid, caffeine, paraxanthine), non-pathogenic viruses (pepper mild mottle virus (PMMoV), crAssphage), non-pathogenic bacteria (bacteroides HF 183, Lachnospiraceae), and human markers (human-specific 18 S rDNA, human-specific mitochondrial NADH dehydrogenase). The review did not single out any specific marker as being the most suitable, although PMMoV is one of the most used. Many of these markers require advanced analytical chemical or microbiological methods, and the behaviour of the markers and their analytical processing streams are likely different than the targets of interest. If multiple targets, use cases, and contexts are being considered, more than one marker may need to be considered. Therefore, the selection and use of such markers requires careful, specialist consideration.

5.6 Data to action

The WES program is undertaken to inform public health actions. Therefore, it is important that data are shared with public health authorities in a timely manner. The data may originally be obtained from environmental, commercial, private, wastewater utility, NGO, academic, or other non-health agency laboratory sources, and governance and data transfer to public health agencies needs to be managed. Care should be taken in the format in which these data are shared. Due to the nature of WES methodology, which includes detection limits, the statistical variability of environmental sampling, and the potential for false negatives, detect/non-detect language should be used to represent WES data as opposed to presence/absence terminology often used for referencing clinical test results. Additionally, as explained previously, WES data is often less intuitive than results obtained from clinical surveillance and often requires back calculation from the direct test output to be shared as concentration per original sample volume in order to be more easily interpreted. In general, it is unexpected or surprising results that trigger public health actions, i.e. information provided by the WES program that was not evident from the existing surveillance programs or understood from a priori assumptions. Often, but not always, it is necessary to assess data from WES alongside other existing forms of surveillance to understand the full picture of disease transmission and inform public health action. Because of this, it is suggested that WES data for a given target be aligned, managed, and/or shared with the public health subject matter group responsible for that target.

Examples of triggers for action include:

- Detection of a target that was not known to be circulating in the community as an early warning of its emergence in the population represented by the catchment of the sample. This can provide early warning of an emerging or re-emerging target.
- Elevated target detected, at levels significantly above those in comparable points in time or space, and indicative of a hotspot, cluster, or outbreak.
- Initiating enhanced clinical surveillance in a high-risk regions or contexts.
- The need for a monitoring and evaluation of programme such as during a vaccination campaign.

Examples of public health actions in response to WES results may include the following:

- Promoting non-pharmaceutical interventions such as:
  - Application of public health and social measures (PHSM) guidance frameworks
  - Drinking water, sanitation and hygiene (WASH) promotion
  - Wearing masks
  - Physical distancing
  - Reduced movement in the community
  - Encourage isolation of at-risk groups
  - Encourage clinical testing
  - Vector-control

- Enhancing vaccination programs
  - Spatial targeting
  - Promoting vaccine uptake
  - Accelerating or bringing forward vaccination activities
  - Selecting the specific pathogens to include in vaccines
  - To inform vaccine development, e.g. identifying and tracking variants of concern can inform vaccine development priorities
  - Understanding vaccine impact, and targeting catch-up campaigns

- Ramping up healthcare facility preparedness

6 Research needs and future updates

6.1 Priority research needs

A comprehensive global research agenda is needed to accelerate at-scale implementation WES. This includes transfer of research and pilots into strategy, plans, resource mobilization, monitoring and evaluation to advance the effectiveness, scale and equity of global WES activities to protect public health.

Priorities relate to; key scientific knowledge gaps which require applied (sometimes basic) research to address, unlocking technological and implementation barriers that require innovation and translation from research to practice, and tools, capability building, integration, and linkages to the public health actions.

The Global Consortium for Wastewater and Environmental Surveillance for Public Health (GLOWACON) has been established to advance such an agenda. The research and development objectives and priorities described below draw from this GLOWACON Technical Working Group's draft technical working paper and deliberations at global and regional WES conferences, as well as from published literature, WES expert advice including that from the WES Expert Review Group.

Of note, knowledge gaps which are pathogen-specific applied research priorities are documented in the pathogen/disease specific WES target summaries (those of cholera, influenza, mpox, polio, COVID-19 and typhoid).

Eight priority areas were identified using a thematic analysis, these are formulated as objectives below:

1. Identify priority pathogens for WES
2. Develop improved cost-effective, robust tools and techniques for the sampling, detection and analysis of priority targets
3. Develop improved cost-effective, robust tools and techniques for the interpretation of WES data
4. Promote integration of WES results as part of collaborative surveillance into mainstream public health decision-making and public communications
5. Promote ethical practice for WES for public health purposes
6. Enhance the use of WES in non-sewered settings
7. Strengthen WES capability and capacity including in human resources
8. Identify other potential use cases for WES for public health and One Health purposes to inform future program development priorities

Priority areas within each objective are as follows:

1. Identify priority pathogens for WES

- Evaluation and refinement of framework for contextual prioritization of potential WES targets given the public health need
- Relevant evidence to inform prioritization: e.g. pathogen specific WES evidence synthesis for priority (and potential high-priority) pathogens
- Effective linkages with cross-cutting and pathogen specific initiatives and evidence; e.g. collaborative surveillance gaps and priority areas which WES may help address

2. Develop improved cost-effective, robust tools and techniques for the sampling, detection and analysis of priority targets

2a. Sampling

- Development, evaluation and optimization of cost-effective harmonized sampling strategies for sentinel sites related to population of interest for the specific surveillance objectives and context (inclusive of sanitation systems, population distribution and movement). This relates both to local/country or subnational surveillance and global or supranational surveillance through transport networks.
- Development, evaluation and optimization of cost-effective harmonized sampling methods (inclusive of type, frequency/duration, in-field concentration etc) considering individual and combined priority pathogens as well as context specific needs (sewage system, temperature, capacity etc) : this includes consideration of feasibility and affordability and requires head to head comparative evaluations on key attributes (including sensitivity and quantification if applicable).
- In particular, sampling innovations and applications for non-sewered settings including those in hotter climates is a priority to address global equity given understudied/underserved populations
- Sampling evidence synthesis which includes description and comparison of sampling methods overall and by pathogen with strengths and limitations

2b. Method validation

- Development, evaluation, validation and refinement of cost-effective harmonized (or standardized) methods to optimize sensitivity and quantification: cross-cutting and pathogen specific approaches. Note this relates in part to sampling methods (2a) as well as in-laboratory preparation, extraction, concentration, enrichment, recovery and related methods prior to analysis. This includes consideration of feasibility and affordability and requires comparative evaluation of methods against one another and/or reference standards to assess key attributes.

2c. Analysis (laboratory)

- Development, evaluation and refinement of cost-effective harmonized (or standardized) WES analytic protocols for priority pathogens for specific surveillance objectives considering individual and combined priority pathogens as well as context specific needs (laboratory capacity/capability and local prevalence of inhibitory substances and zoonotic contributions). This requires consideration of feasibility and affordability. Note this covers a very wide range of laboratory methods spanning molecular detection, culture and sequencing as is relevant to priority WES pathogens and antimicrobial resistance. This includes consideration of feasibility and affordability and requires head-to-head comparative evaluations on alternative methods on key attributes.
- In particular, innovations and applications for affordable, decentralized analysis, close to point of sample collection, which do not require highly skilled operators, and/or other cost-effective innovations which decrease time from sample detection to result, would expand potential WES applications and timeliness of results.

2d. Cross-cutting : Quality management, supply chain and biorepository

- Improve WES quality management: Establishment of cost-effective WES quality assurance systems and networks in support of individual laboratories building on existing local, regional or global systems: includes, inter alia, identification and distribution of verified WES controls and external quality assurance panels to laboratories and structured support for intra-laboratory WES quality management systems and processes.

- Improve supply chain management and reduce recurrent program costs including at country, regional and global levels. Specifically, following harmonization of sampling, pre-analytic and analytic methods, negotiated bulk-procurements, securing a contingency stock and/or enhanced local manufacturing may become possible to take advantage of economies of scale and increased certainty in future procurement needs.
- Guidelines to establish cost-effective WES biorepositories for stored samples at the national or supranational level which protect the integrity of the samples and associated metadata. Complements data management and stewardship below.

3. Develop improved, robust tools and techniques for the interpretation of WES data

- Provide relevant evidence to inform interpretation of laboratory results related to specific pathogens, surveillance objectives, intended actions and context.
  This covers a wide range of priority areas which requires robust data, statistical analysis +/- modelling with goal to provide evidence, and if possible harmonized if not standardized approaches. These include:
  - definition of threshold for action
  - source and direction of biases (sampling, laboratory, other)
  - uncertainty estimates and sources
  - normalisation and adjustment for known and unknown confounders (for inter-sample comparisons and/or aggregation)
  - lead time of WES over clinical cases (theoretical and actual)
  - genomic analysis (NGS and metagenomics) in all its complexity including comparison to reference databases and provision of meaningful understandable evidence for public health use
  + including antimicrobial resistance genes and potential or known relationship to source pathogen of interest
  + including potential zoonotic source
  + phylogenetic relationships
  - optimal and minimal WES datasets (standardized)
  - extrapolation to other populations from sentinel site data (analytic modelling)
- Required evidence for above such as pathogen specific shedding profiles. Many of these also relate to, and can inform sampling frequency and method.

4. Promote integration of WES results as part of collaborative surveillance into mainstream public health monitoring and evaluation and decision-making and public communications

- Effective linkages and codesign with cross-cutting and pathogen/disease specific decision makers: develop fit for purpose data (to information to intelligence) to decision making pathways with supportive and scalable dashboards or other tools and associated training and documentation
- Develop guidelines and best practices for public facing communications in support of priority use cases considering priority stakeholders such as medical/health practitioners and individuals and communities at most risk and those targeted for behavior change

5. Promote ethical practice for WES for public health purposes

- Develop WES specific ethical guidelines for pathogens, antimicrobial resistance and related targets and disseminate to WES practitioners and researchers. Encourage use, feedback and documentation of case-studies within communities of practice

6. Define the value of collaborative surveillance components inclusive of WES

- Conduct (an updated) cost-benefit analyses of Collaborative Surveillance systems for different geographies and use cases inclusive of WES as one component
- Identify targeted priority diseases/pathogens where the current surveillance system is weak and estimate the potential value of WES and WES innovations

7. Strengthen WES capability and capacity including in human resources

- Promote WES specific capability across the interdisciplinary workforce through enhanced guidelines and resources, preservice and in-service training. This is a multi-faceted area spanning all the different skillsets required.
- Infrastructure including transport, laboratory, data management etc is also required with initial and ongoing investments. Development of guidelines and standardized protocols as well as preferred product and preferred pathway characteristics are needed to guide and inform large investments by host governments, private laboratories and development partners.

8. Identify other potential use cases for WES for public health and One Health purposes to inform future program development priorities

- Identify possible use cases for WES for public health purposes beyond human infectious diseases and their potential value, limitations and synergies with WES. Whilst outside the scope of this document, there may be value in identifying other uses of wastewater and environmental monitoring beyond human public health and potential for synergies as well as limitations or harms (e.g. illicit drug monitoring).

6.2 Future updates

This version is a draft for pilot application at regional and country level . The draft will be finalized incorporated feedback and experience from pilot application. WES is rapidly evolving globally alongside emerging discussion on the wider application of WES for other existing or future programs and public health emergencies. The anticipated shelf-life of this document will be three years consistent with PRC rules for a Scientific Brief. Updates after this time will occur with timing depending on the level or substantive new evidence and experience on and concrete evolution of the wider WES discussion.

7 Methods

The proposed process and criteria were developed in consultation with global experts and drew on conceptual frameworks to prioritise pathogens for WES in the published literature [5,26–28]. The choice of the six viral and bacterial pathogens which were initially prioritized for individual WES summaries may not be prioritized in all settings. Additional individual WES target sheets will be developed as additional needs are identified and resources allow. A sub-group of WES and disease expert completed the structed evaluation of evidence in the at a glance assessment for each of the prioritized pathogens.

7.1 Evidence review and quality appraisal

Evidence used was sourced from:

- Rapid, systematic narrative review, supported by currently unpublished work and information on technical sharing specialist forums.
- Expert opinion from the expert review group
- Findings from sharing drafts of the document with an External Review Group (ERG).
- Inputs from a series of targeted qualitative expert interviews to harvest practical experience, lessons learned and needs, with disease experts from WHO and CDC.
- Literature review methods - A hybrid method was employed involving:
    - structured search queries tailored to the subject of interest (noting these were many for each pathogen target sheet)
    - review of abstracts of articles found to identify those pertinent
    - further review of references in selected articles to identify any additional pertinent references

7.2 Evidence to decision process

Evidence was synthesized into the document based on quality assessment and evidence to decision criteria and presented to the expert group for decision by consensus via online meetings and email exchange of draft text.A consultation process via pilot workshops in four regions was completed with written and verbal feedback from national level WES implementors considered in the final version.

Final decisions were by consensus among expert review group members using decision criteria of: feasibility for immediate or staged implementation, intervention/option(s) acceptable to all stakeholders, balance between benefits and harms, impact on equity. Final review was completed by the WHO and CDC steering groups.

7.1 Expert selection and declarations of interest

Expert group members were selected via research and practitioner networks working on WES and disease surveillance globally. Selection aimed for a balance of academic, implementation and disease specific surveillance experience, as well as gender and regional representation. All members of the expert group signed declarations of interest, which was reviewed 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.

Annexes

(Note: Annexes are maintained separately and are in various stages of preparation. Annex will be shared for regional pilot events in 2025.

The decision support tools are intended to be used by each jurisdiction (country, region, locale) likely in a multi-disciplinary workshop to help guide consideration of potential pathogens (curated long list) and their public health prioritization as a WES target (prioritized short list).

Annex 1. Long list of potential pathogens for WES

[placeholder]

Annex 2: Table of pathogens with detailed WES summaries

[placeholder]

Annex 3. Public Health Significance - Decision Support Tool

[placeholder]

Annex 4. Feasibility Assessment - Decision Support Tool

[placeholder]

Annex 5 Acceptability Ethical and social licence, decision makers and legal - Decision Support Tool

[placeholder]

Annex 6. Optimization - Decision Support Tool

[placeholder]

Annex 7. Assessment of at a glance criteria – 6 priority pathogens

[placeholder]

References

1. WHO. Defining collaborative surveillance: a core concept for strengthening the global architecture for health emergency preparedness, response, and resilience (HEPR). Published online 2023. https://iris.who.int/bitstream/handle/10665/367927/9789240074064-eng.pdf?sequence=1

2. UNEP, WHO, FAO, WOAH. One Health Joint Plan of Action (2022 - 2026) | UNEP - UN Environment Programme. October 17, 2022. Accessed August 4, 2024. https://www.unep.org/resources/publication/one-health-joint-plan-action-2022-2026

3. World Health Organization, Food and Agriculture Organization of the United Nations, World Organisation for Animal Health. *Taking a Multisectoral, One Health Approach: A Tripartite Guide to Addressing Zoonotic Diseases in Countries*. World Health Organization; 2019. Accessed August 4, 2024. https://iris.who.int/handle/10665/325620

4. World Health Organisation. Environmental surveillance for SARS-CoV-2 to complement other public health surveillance. 2023. Accessed August 26, 2024. https://www.who.int/publications/i/item/9789240080638

5. WHO. Field guidance for the implementation of environmental surveillance for poliovirus. Published online 2023.

6. GPEI. Global Polio Eradication Initiative (GPEI). https://polioeradication.org/

7. WHO. Guidelines for environmental surveillance of poliovirus circulation. Published online 2003. https://polioeradication.org/wp-content/uploads/2016/07/WHO_V-B_03.03_eng.pdf

8. WHO. Global Polio Eradication Initiative. Guidelines on environmental surveillance for detection of poliovirus. Published online 2015. http://polioeradication.org/wp-content/uploads/2016/07/GPLN_GuidelinesES_April2015.pdf

9. World Health Organization. *Status of Environmental Surveillance for SARS-CoV-2 Virus: Scientific Brief, 5 August 2020*. World Health Organization; 2020.

10. WHO. Environmental surveillance for SARS-CoV-2 to complement public health surveillance. Interim guidance. Published online 2022. Accessed April 14, 2022. https://www.who.int/publications/i/item/WHO-HEP-ECHWSH-2022.1

11. World Health Organization. *Environmental Surveillance for SARS-CoV-2 to Complement Other Public Health Surveillance*. World Health Organization; 2023.

12. Archer BN, Abdelmalik P, Cognat S, et al. Defining collaborative surveillance to improve decision making for public health emergencies and beyond. *The Lancet*. 2023;401(10391):1831-1834. doi:10.1016/S0140-6736(23)01009-7

13. World Health Organisation. Pathogens prioritization: a scientific framework for epidemic and pandemic research preparedness. Accessed August 5, 2024. https://www.who.int/publications/m/item/pathogens-prioritization-a-scientific-framework-for-epidemic-and-pandemic-research-preparedness

14. WHO. Strengthening the global architecture for health emergency prevention, preparedness, response and resilience. 2023. Accessed November 7, 2024. https://www.who.int/publications/m/item/strengthening-the-global-architecture-for-health-emergency-prevention--preparedness--response-and-resilience

15. WHO. WHO global genomic surveillance strategy for pathogens with pandemic and epidemic potential 2022-2032. 2022. Accessed November 7, 2024. https://www.who.int/initiatives/genomic-surveillance-strategy

16. Carter LL, Yu MA, Sacks JA, et al. Global genomic surveillance strategy for pathogens with pandemic and epidemic potential 2022–2032. *Bull World Health Organ*. 2022;100(4):239-239A. doi:10.2471/BLT.22.288220

17. Global Health Security Agenda. Global Health Security Agenda. Global Health Security Agenda. 2023. Accessed November 7, 2024. https://globalhealthsecurityagenda.org/

18. Global Health Security Agenda. Global Health Security Agenda. Global Health Security Agenda. 2023. Accessed November 8, 2024. https://globalhealthsecurityagenda.org/

19. WHO. Global action plan on antimicrobial resistance. 2016. Accessed November 7, 2024. https://www.who.int/publications/i/item/9789241509763

20. WHO. Expanded Global Influenza Surveillance and Response System (GIRS). 2022. https://www.who.int/initiatives/global-influenza-surveillance-and-response-system

21. WHO. WHO Coronavirus Network (CoViNet). 2020. Accessed December 3, 2024. https://www.who.int/groups/who-coronavirus-network

22. WHO. Global Arbovirus Initiative. WHO - Global Arbovirus Initiative. 2022. Accessed December 3, 2024. https://www.who.int/initiatives/global-arbovirus-initiative

23. Coalition Against Typhoid (CaT). Take on Typhoid. CaT - Take on Typhoid. 2018. https://www.coalitionagainsttyphoid.org/

24. Global Task Force on Cholera Control (GTFCC). Global Task Force on Cholera Control (GTFCC). Global Task Force on Cholera Control (GTFCC). 1992. Accessed December 3, 2024. https://www.gtfcc.org/

25. Li J, Hosegood I, Powell D, et al. A global aircraft-based wastewater genomic surveillance network for early warning of future pandemics. *The Lancet Global Health*. 2023;11(5):e791-e795. doi:10.1016/S2214-109X(23)00129-8

26. Gentry Z, Zhao L, Faust RA, David RE, Norton J, Xagoraraki I. Wastewater surveillance beyond COVID-19: a ranking system for communicable disease testing in the tri-county Detroit area, Michigan, USA. *Front Public Health*. 2023;11:1178515. doi:10.3389/fpubh.2023.1178515

27. Diamond MB, Keshaviah A, Bento AI, et al. Wastewater surveillance of pathogens can inform public health responses. *Nat Med*. 2022;28(10):1992-1995. doi:10.1038/s41591-022-01940-x

28. National Academies of Sciences, Engineering, and Medicine, Committee on Community Wastewater-based Infectious Disease Surveillance, Water Science and Technology Board, Board on Population Health and Public Health Practice, Division on Earth and Life Studies, Health and

Medicine Division. *Wastewater-Based Disease Surveillance for Public Health Action*. National Academies Press; 2023:26767. doi:10.17226/26767

29. Khawar L, Donovan B, Peeling RW, Guy RJ, McGregor S. Elimination and eradication goals for communicable diseases: a systematic review. *Bull World Health Organ*. 2023;101(10):649-665. doi:10.2471/BLT.23.289676

30. Hasso-Agopsowicz M, Hwang A, Hollm-Delgado MG, et al. Identifying WHO global priority endemic pathogens for vaccine research and development (R&D) using multi-criteria decision analysis (MCDA): an objective of the Immunization Agenda 2030. *eBioMedicine*. 2024;0(0). doi:10.1016/j.ebiom.2024.105424

31. World Health Organisation. WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. 2024. Accessed November 20, 2024. https://www.who.int/publications/i/item/9789240093461

32. World Health Organisation. WHO fungal priority pathogens list to guide research, development and public health action. 2022. Accessed November 20, 2024. https://www.who.int/publications/i/item/9789240060241

33. Murray CJL, Ikuta KS, Sharara F, et al. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet*. 2022;399(10325):629-655. doi:10.1016/S0140-6736(21)02724-0

34. World Health Organization. *WHO Guidelines on Ethical Issues in Public Health Surveillance*. World Health Organization; 2017. Accessed June 11, 2024. https://iris.who.int/handle/10665/255721

35. Willis HH, Moore M. Improving the value of analysis for biosurveillance. *Decision Analysis*. 2014;11(1):63-81.

36. Awan J, Faherty LJ, Willis HH. Navigating Uncertainty in Public Health Decisionmaking: The Role of a Value of Information Framework in Threat Agnostic Biosurveillance. *Health security*. 2024;22(1):39-44.

37. Nascimento de Lima P, Karr S, Lim JZ, et al. *The Value of Environmental Surveillance for Pandemic Response*. RAND Corporation; 2024. Accessed August 6, 2024. https://www.rand.org/pubs/working_papers/WRA3263-1.html

38. Water Environment Federation, ed. *Protecting Wastewater Professionals from COVID-19 and Other Biological Hazards*. Water Environment Federation; 2020.

39. Association of Public Health Laboratories (APHL). *SARS-CoV-2 Wastewater Surveillance Testing Guide for Public Health Laboratories*.; 2022. Accessed December 3, 2024. https://www.aphl.org/aboutAPHL/publications/Documents/EH-2022-SARSCoV2-Wastewater-Surveillance-Testing-Guide.pdf

40. WHO. Polio laboratory manual, 4th edition. Published online 2004. https://polioeradication.org/wp-content/uploads/2017/05/Polio_Lab_Manual04.pdf

41. Meechan PJ, Potts J. Biosafety in microbiological and biomedical laboratories. Published online 2020.