Environmental Biosurveillance Design Framework

A framework for developing environmental biosurveillance for health (EBH) programmes

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AI Use Declaration

Anthropic Claude version 3 LLM was used to assist with initial brainstorming, report structure and refine some of the text.

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Contents

1. Executive summary

This document presents a comprehensive framework for developing and implementing environmental biosurveillance for health (EBH) programmes, be that human, animal, plant or environmental health. EBH encompasses surveillance using environmental matrices extraneous to the host-pathogen relationship, including wastewater, air, surface water, and sentinel species such as bivalves. The framework originated from experiences in using wastewater-based surveillance for COVID-19 in England.

The framework provides structured guidance for both reactive implementation during emergencies and proactive planning for future surveillance needs. This dual approach enables efficient resource allocation and helps identify capability gaps before they become critical during emergencies.

Key components of the framework include:

- **Programme Development:**
    - Systematic processes for defining surveillance questions and objectives
    - Clear protocols for establishing detection actions and response triggers
    - Comprehensive assessment of technical value and other considerations including cost, logistics, and ethics
    - Detailed guidance for roles, responsibilities, and stakeholder engagement
- **Operational Elements:**
    - Specific requirements for sampling programmes and sample handling
    - Data governance and quality assurance protocols
    - Standardised approaches for sample analysis and data interpretation
    - Research and development integration for method improvement
- **Quality Management:**
    - Robust quality control measures throughout all processes
    - Clear documentation requirements and templates
    - Standardised protocols for method validation
    - Regular review and optimisation procedures

The framework emphasises the importance of proper data management through the Public Health Environmental Surveillance Open Data Model (PHES-ODM) with a 'whole data lifecycle approach' and adherence to FAIR (Findable, Accessible, Interoperable, and Reusable) principles.

This guidance document serves as a practical tool for public health officials, laboratory managers, and surveillance programme coordinators. It enables the development of well-structured surveillance programmes that effectively support public health decision-making while maintaining scientific rigour and operational efficiency.

2. Introduction

Environmental biosurveillance for health (EBH) has emerged as a powerful tool for monitoring disease prevalence in populations. This approach began with wastewater-based surveillance for poliovirus as part of global polio eradication efforts, demonstrating the value of environmental sampling for public health surveillance. The COVID-19 pandemic marked a significant expansion of this approach, as wastewater surveillance was rapidly deployed worldwide to track SARS-CoV-2 transmission dynamics in communities.

EBH encompasses any biosurveillance that uses environmental matrices extraneous to the host-pathogen relationship for health surveillance purposes. While wastewater-based surveillance represents a prominent example, EBH includes surveillance using other environmental matrices such as air, surface water, and sentinel species, for example bivalves. This broader definition distinguishes EBH from traditional environmental monitoring programmes that focus on statutory requirements for water and food quality. Instead, EBH specifically aims to provide epidemiological insights into disease prevalence and dynamics within populations.

Historically, EBH has primarily focused on human health surveillance. However, the principles and approaches can be equally applied to monitoring disease in animal and plant populations. This versatility makes EBH a valuable tool across the spectrum of health surveillance needs.

The expansion of EBH applications, particularly wastewater-based surveillance, has led to diverse approaches and methodologies. The COVID-19 response in England exemplifies both the potential and challenges of rapidly implementing large-scale environmental surveillance. While the programme achieved significant successes, its rapid implementation in response to a public health emergency revealed areas where efficiency could have been improved through better coordination and planning.

This framework addresses these challenges by providing structured guidance for developing robust EBH programmes. It supports public health management decisions through two key approaches:

Reactive Implementation:

- Provides clear, systematic guidance for establishing surveillance programmes during emerging health threats.
- Ensures efficient resource allocation and coordination.
- Promotes standardised approaches that enhance data quality and comparability.

Proactive Planning:

- Enables advance development of surveillance frameworks for potential future needs.
- Reduces pressure during emergency responses.
- Allows identification of capacity and capability gaps before they become critical.
- Supports strategic resource allocation and development.
- Plans for and supports data management across the whole 'data lifecycle'.

By following this framework, organisations can develop well-structured surveillance programmes that effectively support public health decision-making. Whether used reactively during an emerging situation or proactively for future planning, the framework promotes efficient, coordinated, and scientifically robust approaches to environmental biosurveillance for health.

3. How to use this document

This framework provides specific guidance for developing environmental biosurveillance programmes but does not supersede established project and programme management practices. The guidance should be implemented within existing organisational project and programme management frameworks, under the direction of experienced programme managers. Programme managers should integrate these surveillance-specific requirements with standard project management practices including risk management, stakeholder engagement, resource allocation, and change control. The framework is designed to complement, not replace, these established management approaches.

This document is designed to guide users through the process of assessing the application of environmental biosurveillance for health. When a need to evaluate environmental biosurveillance arises, users should follow the processes outlined in this document systematically.

Overview Flow Diagram (Section 5): Begin by referring to the overview flow diagrams in Section 5. Figure 3 provides a visual representation of the workflow and serves as a reference throughout the assessment process. The user should familiarise themself with this diagram as it outlines the entire process from start to finish.

Starting Point - T01 Detection Challenge: Typically, users should start at the T01 Detection challenge and work their way through the workflow systematically. This initial step is crucial as it sets the foundation for the entire assessment process. By starting here, users can ensure that they are addressing the primary detection challenges before moving on to subsequent steps.

Element details: Each element in the Overview flow diagram corresponds to a specific section in this document. These sections provide detailed requirements and instructions for each element. Users should review these sections for detailed guidance on each workflow step. Each element is organised into one of seven categories for clarity: terminators, processes, decisions, documents, multiple documents, databases or data.

Parallel Processes: To improve efficiency, some parts of the workflow can be conducted in parallel, especially if multiple staff members are available. This allows for a more efficient assessment process without compromising the thoroughness of the evaluation. For example, while one team member is

working on sample collection, another can begin data analysis, thereby speeding up the overall process.

Documentation and Record-Keeping: It is important to maintain thorough documentation and records of all steps taken during the assessment. This includes recording any deviations from standard protocols, noting any challenges encountered, and documenting the outcomes of each step. Proper record-keeping ensures transparency and accountability and provides a valuable reference for future assessments.

Review and Feedback: After completing the assessment, it is beneficial to review the entire process and gather feedback from all team members. This review helps in identifying any areas for improvement and ensures that the process is continuously refined and optimised for future assessments. Any feedback on the effectiveness of this framework is also welcomed by the author to ensure that any future versions are as useful as possible for the user.

4. Glossary

Analytical Data: The measurements obtained from the analysis of samples, such as virus genome copies per litre (gc/l) of wastewater.

Analytical Metadata: Information related to the conditions and methods used during the analysis of samples, ensuring the reliability and reproducibility of the results, and enabling re-use.

Application Programming Interface (API): A protocol that allows data exchange and functionality between software applications.

Biobanking: The process of storing biological samples under controlled conditions for future analysis, typically involving preservation methods such as deep-freezing or chemical preservatives.

Bivalve molluscan shellfish (BMS): Shellfish belong to the class Bivalvia within the phylum Mollusca, characterised by a shell divided into two hinged valves. For example, oysters, mussels and cockles.

British Standards Institution (BSI): The national standards body of the United Kingdom, responsible for producing British Standards and other standards-related services.

Chain of Custody: A documented record that tracks the movement and handling of samples from collection through analysis and storage, including dates, times, and individuals involved in each transfer, ensuring sample integrity and traceability.

Composite Sample: A sample consisting of multiple portions collected over a defined time period and combined into a single sample for analysis, often used to obtain a more representative average of conditions over time.

Controlled Vocabulary: A controlled vocabulary is an established list of standardised terminology for use in indexing and retrieval of information. A collection of concepts used to populate a field in a data model.

Comma-Separated Values (CSV): A file format used to store tabular data in plain text, where each line of the file is a data record, and each record consists of one or more fields separated by commas.

Quantification Cycle (Cq): In qPCR; the cycle number at which the fluorescence generated within a reaction crosses the threshold, indicating the presence of the target DNA.

Data Management Planning (DMP): A strategy for managing data throughout its lifecycle, ensuring it meets FAIR principles.

Data Sharing Agreement (DSA): A formal document that outlines the terms and conditions under which data can be shared between organisations, including specifications for data usage, security, and confidentiality.

Environmental Biosurveillance Design Framework (EBDF): A framework (this document) for developing EBH programmes.

Environmental Biosurveillance for Health (EBH): Programmes aimed at monitoring and analysing environmental matrices extraneous to the host-pathogen relationship to detect and respond to health threats,

incorporating various environmental samples such as wastewater, air, or sentinel species.

European Committee for Standardisation (CEN): An organisation that develops and publishes European Standards (ENs) to ensure the safety, interoperability, and quality of products and services across Europe.

FAIR Principles: Guidelines to ensure that data are Findable, Accessible, Interoperable, and Reusable.

Grab Sample: A discrete sample collected at a specific time and location, providing a snapshot of conditions at that moment.

International Electrotechnical Commission (IEC): An international standards organisation that prepares and publishes standards for electrical, electronic, and related technologies.

International Organisation for Standardisation (ISO): An international standard-setting body composed of representatives from various national standards organisations.

ISO 9001: An international standard that specifies requirements for a quality management system.

ISO/IEC 17025: An international standard that specifies the general requirements for the competence of testing and calibration laboratories.

Laboratory Information Management Systems (LIMS): Systems used for managing laboratory data, ensuring data quality and integrity, and improving efficiency.

Matrix Facility Operators: Personnel or organisations responsible for managing and operating facilities where environmental samples are collected, such as wastewater treatment plants or air monitoring stations.

Material Transfer Agreement (MTA): A contract that governs the transfer of tangible research materials between organisations, defining terms for use, intellectual property rights, and other conditions.

Memorandum of Understanding (MOU): A formal agreement between parties that outlines shared goals and responsibilities without creating legally binding obligations.

Method Readiness Level (MRL): A scale from 0 to 9 that evaluates the maturity and reliability of analytical methods, ranging from basic concept to fully validated and standardised procedures.

One Sample, Many Analyses (OSMA): An approach where a single environmental sample is used for multiple different types of analysis, maximising the information obtained while minimising sampling costs and effort.

Operational Readiness Index (ORI): A weighted average of Method Readiness Levels for different aspects of a surveillance programme, used to evaluate overall programme readiness and identify areas needing development.

Passive Sample: A sample collected continuously over time using a device that accumulates the target analyte, often used to detect substances present at low concentrations.

Personal data: as defined in UK GDPR is information relating to an identified or identifiable natural person ('data subject').

Public Health Environmental Surveillance Open Data Model (PHES-ODM): An internationally recognised framework providing a robust, relational schema for storing and sharing data. It supports FAIR principles with an emphasis on data quality.

Process Control: A known substance or organism used to monitor and validate analytical procedures, ensuring the reliability of results.

Programme Data and Information System (PDIS): A centralised system that integrates all aspects of an EBH programme, including sampling information, analytical results, quality control data, protocols, and reporting outputs, while enabling integration with external systems and stakeholders.

Quality-Focused FAIR (Q-FAIR): An approach that emphasises data quality and 'fit for purpose' in addition to the FAIR principles.

Quantitative Polymerase Chain Reaction (qPCR): A laboratory technique used to amplify and simultaneously quantify a targeted DNA molecule.

RFC 4180: A standardised format for representing tabular data, ensuring consistency, integration, and accessibility.

Sampling Metadata: Information related to the conditions by which samples were collected, ensuring that analytical data gathered from each sample can be interpreted and re-used robustly.

Transport Metadata: Information related to the conditions and methods used to transport samples from the collection site to the laboratory.

Unicode Transformation Format - 8-bit (UTF-8): A character encoding standard used for electronic communication.

5. Overview

The EBDF is a series of processes and decisions used to develop an EBH programme. The workflow for the EBDF is summarised in this section. It is important to note that the exact order suggested does not always need to be followed, and some processes can be carried out in parallel. Figure 1 shows a high-level summary of the EBDF, while Figure 2 presents a simplified version of the overall workflow. Figure 3 provides a detailed overview flow diagram of the EBDF. Each element within Figure 3 corresponds to a section in this document.

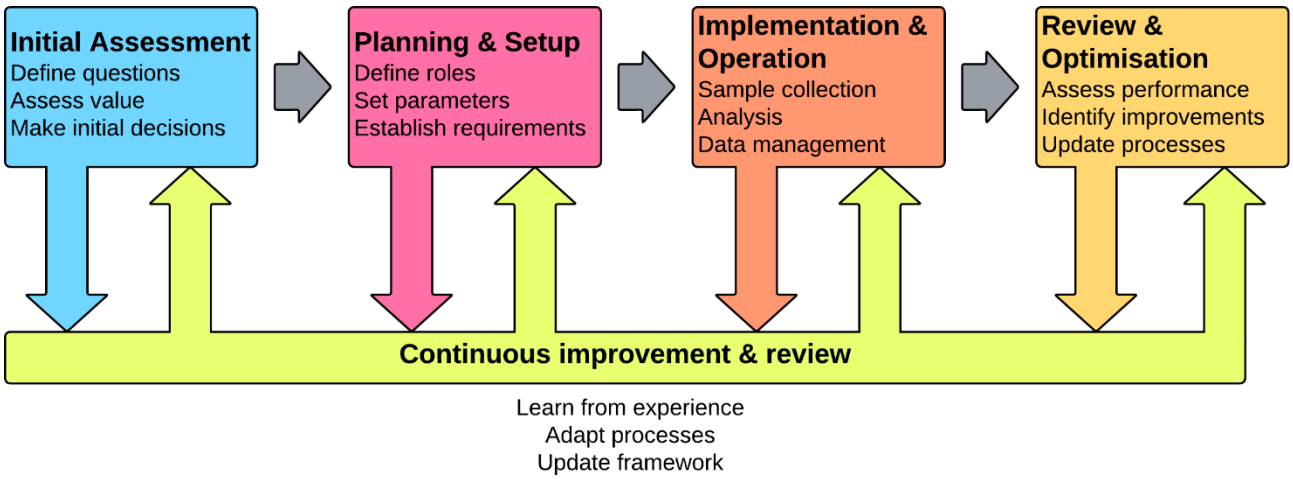


Figure 1: High-level overview of the Environmental Biosurveillance Design Framework (EBDF) showing the four main phases: Initial Assessment, Planning & Setup, Implementation & Operation, and Review & Optimisation. The coloured arrows indicate continuous improvement through learning from experience, adapting processes, and updating the framework.

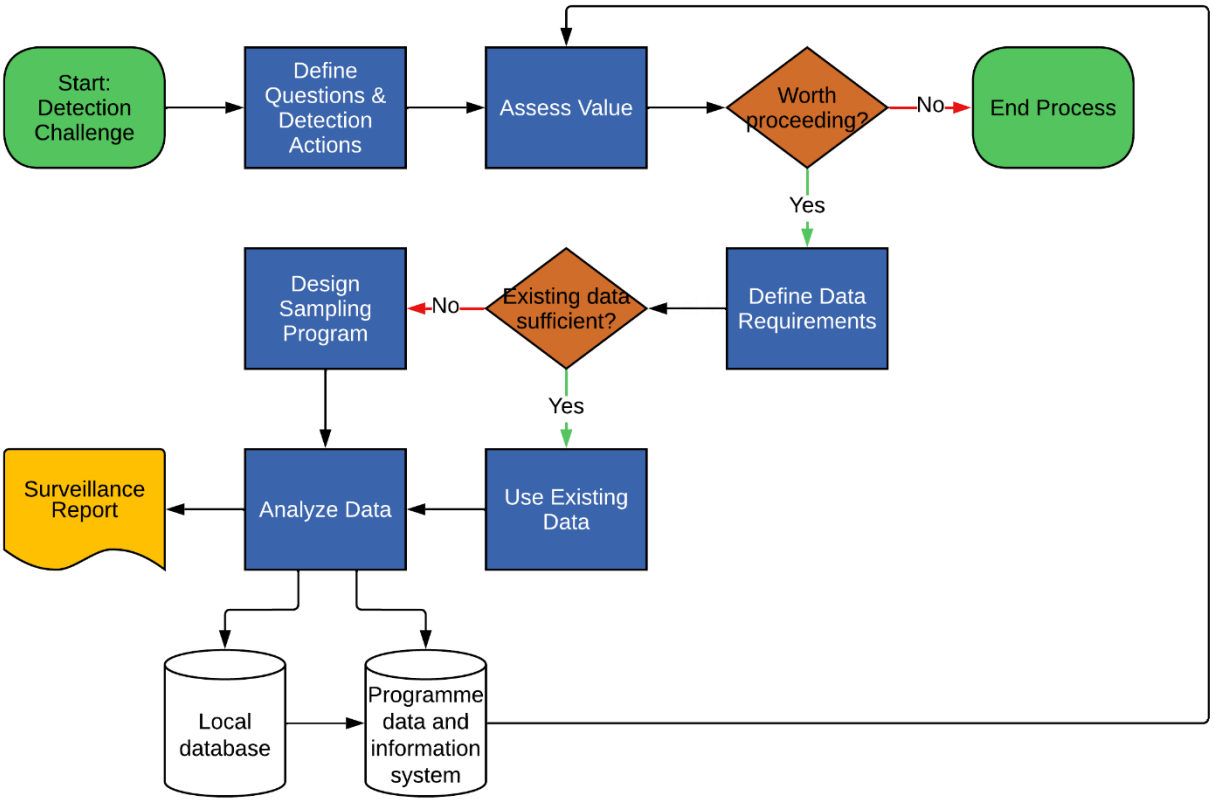


Figure 2: Simplified linear workflow of the Environmental Biosurveillance Design Framework (EBDF) showing the key decision points and processes from initial detection challenge through to data outputs. The workflow illustrates the two main pathways depending on data availability: using existing data or designing new sampling programmes. Decision points are shown in red, processes in blue, terminators in green, reports in yellow and databases in white.

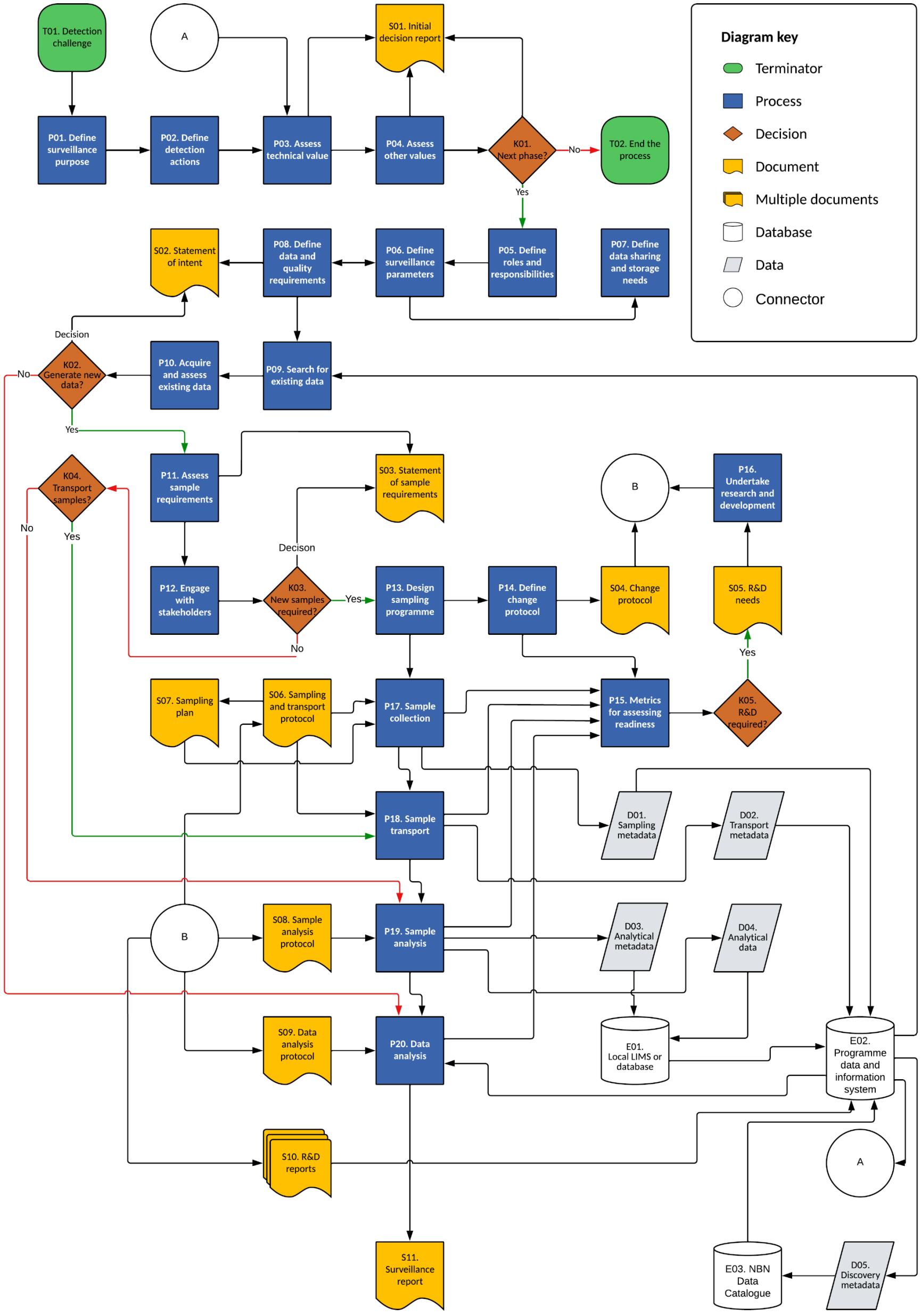


Figure 3: Detailed workflow diagram of the Environmental Biosurveillance Design Framework (EBDF) showing the interconnections between processes (blue boxes), decisions (orange diamonds), documents (yellow notes), databases (white cylinders), terminators (green boxes), and white circular connectors (A and B) that indicate continuation points where workflow paths connect across different parts of the diagram. The workflow illustrates the progression from initial detection challenge through to implementation, with decision points guiding whether to proceed, generate new data, or conduct research and development.

6. Data

Data are the key asset collected and produced from any surveillance programme. High quality data enable robust trend analysis, data-driven risk assessments and reliable interpretation. In turn, these data are the basis for decision making, early detection of health threats and enhanced capacity to respond to emerging environmental and public health threats. In EBH, two types of data are used: 1) analytical data that are the measurements of specific target analytes and 2) metadata that provide essential context (such as sampling methods, geographic locations, or temporal details) ensuring that the analytical data are interpretable, reproducible. Both data types are essential to building a complete picture of environmental conditions and trends. All data should preferentially be recorded in digital formats throughout the sample-data lifecycle to minimise the likelihood of transcription errors and data loss.

To maximise the utility of EBH data, they must adhere to open standards and established models, including FAIR data principles (Wilkinson *et al.*, 2016). Open standards, consistent formats and controlled vocabularies from such models and principles, ensure interoperability and machine readability, enabling seamless integration across datasets and organisations. This aligns with the UK Government's Open Standards principles (Cabinet Office, 2018), which emphasise transparency, accessibility, and the use of widely adopted frameworks to ensure flexibility and promote value for public sector investments. Clarification of conditions and licensing also ensures that recognition is given to each data provider and that any terms and conditions are known and shared. By integrating these principles and standards early, programmes can efficiently enable data sharing within and beyond government organisations; support evidence-based public health interventions and reduce costs associated with integrating disparate data sources.

PHES-ODM

The Public Health Environmental Surveillance Open Data Model (PHES-ODM) is recommended for use in EBH programmes (PHES-ODM, 2024). PHES-ODM is an internationally recognised framework and provides a robust, relational schema for storing and sharing data. PHES-ODM supports FAIR (Findable, Accessible, Interoperable, and Reusable) principles, with additional emphasis on data quality, aligning with the Q-FAIR (Quality-Focused FAIR) approach. It ensures accuracy, completeness, and consistency by defining clear standards for both analytical data and metadata.

PHES-ODM has been developed to support wastewater-and environmental monitoring of public health threats. At the time of writing, PHES-ODM is in its second version and supports multiple sample types (such as fomites, wastewater, faeces, sludge and air filters), several sampling location types (such as wastewater treatment plants, holding tanks, aeroplanes and schools) and almost 200 analytes including several named pathogens, antimicrobial resistance, and environmental measures (such as sample temperature, humidity and pH).

Where data already adhere to existing, widely adopted data standards it is highly recommended to undertake a mapping exercise to ensure that the requirements of the PHES-ODM are met to ensure consistency and interoperability. Where such mappings are undertaken, it is desirable to make the outcomes of these openly available to increase transparency and re-usability.

RFC 4180 – common data exchange format

RFC 4180 (CDDO, 2023) provides a standardised format for representing tabular data. To enhance data accessibility and interoperability, PHES-ODM-based datasets should be exportable in non-proprietary formats conforming to RFC 4180. This ensures:

- Consistency: Uniform rows and handling of special characters in accordance with RFC 4180.
- Integration: Compatibility with diverse systems and software environments that support comma separated values (CSV).
- Accessibility: Adoption of UTF-8 encoding to meet international standards for data exchange.

Chain of custody

Recording the chain of custody for samples is crucial for maintaining sample integrity and ensuring accountability by documenting every individual who has handled the samples and the conditions under which they were transported. This practice supports compliance with standards, enhances data quality, and provides a clear history for addressing any issues that may arise during transport. While retention of personal data (named individuals) is important for chain of custody, onward data sharing may require anonymity to adhere to privacy requirements.

Q-FAIR data principles

Embedding Q-FAIR principles (Wilkinson *et al.*, 2016) into surveillance data management is crucial for unlocking the value and usability of the data collected and generated. Following these principles ensures:

- High-**Quality** data that are
- **Findable** and discoverable by surveillance teams (both machine-readable and understandable by humans),
- **Accessible** (sharable between partners and publicly open digitally wherever possible),
- **Interoperable** (facilitating combinations for reuse across different purposes and systems),
- and promote **Re-Use**.

Controlled Vocabulary

Differences in terminology across datasets can create numerous issues, but using controlled vocabularies can help mitigate these problems and ensure consistency. Below are examples of relevant controlled vocabularies that are adopted both in the UK and internationally. Embedding these vocabularies in each dataset or sample collection enables recognised international data aggregation and comparable multilingual data and sample collection.

Table 1: Controlled vocabularies applicable to EBH and One Health data management, providing standardised terms for use in databases.

Vocabulary/Standard	Description	Link
Chemical Abstracts Service (CAS) Registry	Authoritative collection of unique identifiers for chemical substances.	https://www.cas.org/cas-data/cas-registry
DCAT-3	W3C recommendation providing standardised terms for describing datasets and data catalogues to facilitate discovery.	https://www.w3.org/TR/vocab-dcat-3/
EMBL-EBI Ontology Lookup	A repository of biomedical ontologies	https://www.ebi.ac.uk/ols4/
EnvO	Environment Ontology - Controlled vocabulary for environmental features and habitats standardizing descriptions of environmental samples.	https://sites.google.com/site/environmentontology/home
EPPO Global Database	European and Mediterranean Plant Protection Organisation codes - Standard terminology for plant pests and diseases.	https://gd.eppo.int/
GAZ	Gazetteer - Geographic location terminology important for standardising location data in surveillance.	https://environmentontology.github.io/gaz/
ICES Controlled Vocabularies	For marine data management and are referenced in the MEDIN data guidelines.	https://vocab.ices.dk/
ITIS	Integrated Taxonomic Information System - Authoritative taxonomic information essential for standardising species names in environmental monitoring.	https://www.itis.gov/

Vocabulary/Standard	Description	Link
LOINC	Logical Observation Identifiers Names and Codes - Standard for identifying medical laboratory observations including environmental testing parameters.	https://loinc.org/
NERC Vocabulary Server (NVS)	Includes the NERC parameter codes, used to standardise environmental and scientific measurement.	https://vocab.nerc.ac.uk/
OIE-WAHIS	World Organisation for Animal Health terminology - Standardised terms for animal diseases and surveillance.	https://www.woah.org/en/what-we-do/animal-health-and-welfare/disease-data-collection/world-animal-health-information-system/
PHES-ODM dictionary	Standardised vocabulary tables for environmental surveillance including terms for sample types, measurement units, methods, and quality indicators.	https://github.com/Big-Life-Lab/PHES-ODM/tree/main/dictionary-tables
Pathogen Transmission Ontology	The Pathogen Transmission Ontology outlines how human disease pathogens are transmitted between hosts, reservoirs, or sources, either directly or indirectly, using animate vectors or inanimate vehicles.	https://www.ebi.ac.uk/ols4/ontologies/trans
SNOMED CT	Systematized Nomenclature of Medicine Clinical Terms - Comprehensive clinical healthcare terminology including terms relevant to disease surveillance and environmental health.	https://digital.nhs.uk/services/terminology-and-classifications/snomed-ct

Vocabulary/Standard	Description	Link
WHO ICD	International Classification of Diseases - Standardized terminology for epidemiology and health management.	https://www.who.int/standards/classifications/classification-of-diseases
World Register of Marine Species (WoRMS)	For species identification and classification.	https://www.marinespecies.org/

6.1. Sampling metadata (D01)

All the information that relates to the conditions by which samples were collected are referred to as the "Sampling metadata". The accuracy of these metadata ensures that the analytical data gathered from each sample can be interpreted in a robust way. It is therefore important that these metadata are collected and recorded consistently. Table 2 outlines the minimum information required for sampling metadata.

Additionally, metadata must adhere to established standards with controlled vocabulary use where possible as this allows the interoperability of data and makes it machine readable and comprehensible, and so facilitates the effective management, sharing, and analysis of environmental data.

The specific metadata that must be collected alongside each sample will be dictated by the specific requirements of the surveillance needs. A list of metadata that will be recorded for each sample must be documented and matches within PHES-ODM should be highlighted. It is also recommended that for all programmes, detailed chain of custody records are maintained, documenting those who collected the samples, along with the date and time of each transfer to other individuals in line with ISO 22095:2020.

All sampling metadata must be made available as quickly as possible to all those working on the programme. This is important to ensure that any data obtained from samples can be interpreted appropriately. In some cases, such as where the sampling was not carried out according to the established protocols, samples may be rejected by laboratories before analysis commences. The ability for laboratories to reject samples is important, because it reduces wasted resources and enables rapid resampling if necessary. However, this is only possible if the sampling metadata are shared in a timely fashion.

Table 2: A list of the minimum metadata required for sample collection and how they should be recorded in line with PHES-ODM.

Metadata type	PHES-ODM partID	PHES-ODM partLabel	Notes
Unique identifier for a sample	sampleID	Sample ID	
Collection date and time	collDT; collDTEnd; collDTStart	Collection date time; Collection date time end; Collection date time start	For grab samples this is the date, time and timezone the sample was taken. For composite or passive samples this is the date, time and timezone the sample was started and finished being taken. Use ISO 8601 notation i.e. yyyy-mm-ddThh:mm+hh
Unique sampling location identifier	siteID	Site ID	
Sampling geographical location co-ordinates	geoLat; geoLong	Latitude; Longitude	Expressed as decimal degrees according to ISO 6709. e.g. +51.5081; -0.1281. Use WGS84 as the coordinate reference system (CRS)
Sample matrix	saMaterial	Sample material	
Sample collection type	collType	Sample collection type	
Relevant environmental conditions	Variable	Variable	Includes examples such as UV index and water temperature. Relevant partIDs are listed under the "measurement" partType in PHES-ODM

Metadata type	PHES-ODM partID	PHES-ODM partLabel	Notes
Sampling and transport protocol version	protocolID	Protocol ID	
Protocol deviations	notes	Notes	Free text to describe any deviations from the SOP
Chain of custody information	NA	NA	Not covered by PHES-ODM. This should be recorded in line with ISO 22095:2020 and must be treated as sensitive when sharing with 3rd parties

6.2. Transport metadata (D02)

Transport metadata includes all the information related to the conditions and methods used to transport samples from the collection site to the laboratory. This includes any metadata recorded by those transporting the samples as well as metadata records from the laboratory receiving the samples (such as arrival times). Accurate and consistent recording of these metadata ensures the integrity and reliability of the analytical data obtained from the samples. Table 3 outlines the minimum information required for sample transport metadata.

Typically, all samples will be transported according to an established protocol. This will ensure that transport is consistent between samples, thereby assuring the quality of samples on arrival and minimising the individual pieces of metadata that must be recorded for each sample. However, any deviations from the transport protocol must be noted as part of the transport metadata. The specific requirements for transport metadata will vary depending on the needs of the programme. However, it is recommended that for all programmes, detailed chain of custody records are maintained, documenting everyone who handled the samples during transport, along with the date and time of each transfer.

As with sampling metadata, transport metadata must be made available as quickly as possible to all those working on the programme.

Table 3: A list of the minimum metadata required for sample transport and how they should be recorded in line with PHES-ODM.

Metadata type	PHES-ODM partID	PHES-ODM partLabel	Notes
Unique identifier for a sample	sampleID	Sample ID	
Date sample was dispatched from sampling site	sentDate	Date sample was sent	Use ISO 8601 notation i.e. yyyy-mm-dd
Relevant environmental measurements	NA	NA	Transport conditions are not covered by PHES-ODM. Examples include temperature on arrival or temperature logger data
Sampling and transport protocol version	protocolID	Protocol ID	
Protocol deviations	notes	Notes	Free text to describe any deviations from the SOP
Chain of custody information	NA	NA	Not covered by PHES-ODM. This should be recorded in line with ISO 22095:2020 and must be treated as sensitive when sharing with 3rd parties

6.3. Analytical metadata (D03)

Analytical metadata includes all the information related to the conditions and methods used during the analysis of samples. It is important to record all the information on factors which may impact the results of sample analyses. If laboratories conform to the principles of ISO/IEC 17025 and ISO 9001, these metadata are expected to be collected as part of the compliance to those standards. It is therefore recommended that laboratories seek accreditation/certification to these international standards where possible. Table 4 outlines the minimum information required for analytical metadata.

Analytical metadata may may not be of relevance to all stakeholders in the programme that do not work directly with the laboratory analyses. The analytical metadata should nonetheless be maintained to allow auditing of the analytical processes and to provide quality assurance. Where anomalies in data occur, analytical metadata can be used to trace the source of those anomalies.

Table 4: A list of the minimum metadata required for sample and data analysis and how they should be recorded in line with PHES-ODM.

Metadata type	PHES-ODM partID	PHES-ODM partLabel	Notes
Unique identifier for a sample	sampleID	Sample ID	
Date analysis	aDateStart; aDateEnd	Analysis date start; Analysis date end	Use ISO 8601 notation i.e. yyyy-mm-dd
Identity of analyst	contactID	Contact ID	Must be treated as sensitive when sharing with 3rd parties
Specific instruments and equipment used, including their calibration details.	instrumentID	Instrument ID	
Reagents and standards used, along with their sources, lot numbers, and expiration dates	NA	NA	Not covered by PHES-ODM. This should use controlled vocabulary such as CAS RN unique identification reference where possible.
Protocol deviations	notes	Notes	Free text to describe any deviations from the SOP
Chain of custody information	NA	NA	Not covered by PHES-ODM. This should be recorded in line with ISO 22095:2020 and must be treated as sensitive when sharing with 3rd parties

6.4. Analytical data (D04)

The analytical data are the measurements obtained from the analysis of samples, for example, virus genome copies per litre (gc/L) of wastewater or presence/absence of a virus in a specified volume of water. Maintaining the quality of the analytical data guarantees reliability and reproducibility of the results. The following key points must be considered:

- **Consistency of units:** Data must always be reported using the same units to ensure comparability between datasets and minimise confusion and errors when comparing datasets.
- **Raw and calculated data:** If data are calculated from raw data, the raw data must be recorded alongside the calculated data. The calculations must be recorded within the analytical protocols. An example of this is the calculation of virus concentration from C_q values in qPCR analyses. This calculation requires C_q data from the samples as well as the quantification standards. The C_q data for both the samples and the standards must therefore be recorded alongside the calculated virus concentration.
- **Quality checks and validation:** All data must be checked for accuracy, provenance and reproducibility before they are reported. Automated validation, for example via databases, may augment traditional QA/QC checks. Manual checking can be particularly time-consuming and so automation can both drive efficiencies in addition to ensure that all quality control measures have been followed and that the correct data are reported for individual samples.
- **Consistent and efficient formatting:** Data should be reported in a format that is compatible with existing data standards (such as PHES-ODM). The formatting must remain consistent between reports and any changes in formatting must be reported alongside the data. This will enable automated data handling, reduce the need for data analysts to clean datasets prior to analysis, and support rapid and efficient data analysis.

6.5. Discovery metadata (D05)

Discovery metadata provides essential context about datasets, data products and outputs, documenting ownership, licensing, conditions of use, and other key information needed to enable discovery and appropriate reuse of data.

For new EBH programmes developed using the EBDF, the UK Cross-Government Metadata Exchange Model (based on DCAT-3) should be used as the primary standard for discovery metadata. This ensures compatibility with the NBN Data Catalogue and promotes consistent documentation across government programmes.

In cases where well-established domain-specific metadata standards exist and are more suitable for the particular data type (such as MEDIN for marine environmental data), these may be used instead. However, when using alternative standards, programmes must ensure their metadata can be mapped to and integrated with the UK Cross-Government Metadata Exchange Model to maintain cross-government data discoverability.

Metadata may be recorded and managed through various systems, depending on the infrastructure available to data providers. Organisations should utilise existing in-house metadata catalogues and portals when available, particularly if these systems already support efficient metadata sharing with external national and global aggregators. Where such infrastructure is not available, external tools and support services may be used to facilitate metadata creation and sharing.

7. Databases

7.1. Local LIMS or database (E01)

Appropriate capture and storage of analytical data alongside their associated metadata is vital for enabling adherence to Q-FAIR practices (Jamieson, 2023). It is important that the data can be analysed easily and rapidly so that deviations from expected norms can be investigated in a timely manner. It is recommended therefore, that data are stored within a properly maintained relational database; the most suitable application of which are Laboratory Information Management Systems (LIMS).

A LIMS is an electronic system comprised of front-end software linked to underlying databases which facilitates the management of laboratory data and sample metadata. LIMS typically include the ability to track samples, allow data entry, workflow automation, and reporting.

There are several benefits to using a LIMS for tracking laboratory operations and data management. LIMS centralise data storage, making data and technical information easier and more ready to manage, access, and share. Allowing all relevant data to be readily available to authorised users for example, improves efficiency in data delivery and reduces the risk of data loss and corruption.

A LIMS can enhance data quality and integrity through automated data entry and validation, reducing human errors. It helps ensure consistent data capture with embedded standards and controlled vocabularies. LIMS also supports compliance with quality standards by providing auditing tools for tracking laboratory operations. By simplifying laboratory tasks like sample tracking, data entry, and report generation a LIMS can allowing scientists to focus on technical work and improving overall productivity.

However, implementing and maintaining a LIMS comes with significant challenges. The initial cost of LIMS software, infrastructure, and training can be substantial, particularly for smaller laboratories. System downtime can severely impact laboratory operations, making robust IT support essential. Users often face a steep learning curve, and resistance to workflow changes can hinder effective adoption. Additionally, customisation to meet specific laboratory needs can be time-consuming and expensive which may limit flexibility to adapt the system as requirements change.

To maximise the benefits of a LIMS, it is essential to ensure the system setup meets all functional and regulatory requirements, as well as specific use cases

and user needs. A clear understanding of the specific requirements for surveillance purposes is crucial to ensuring a system is fit for purpose. Requirements, existing workflows, and proposed workflows must be comprehensively mapped out and documented. This stage requires time and cooperative engagement between scientists and technical teams.

Many laboratories will already be operating a LIMS. though any new requirements for surveillance will need to be planned and implemented. Where laboratories do not currently operate a LIMS for delivery of part of the programme, it is vital that there is transparency about how they store data, maintain data integrity and ensure that reports are accurate.

7.2. Programme data and information system (E02)

A comprehensive data and information system forms the backbone of any successful environmental surveillance programme. This centralised system encompasses all aspects of the programme, including sampling information, analytical results, quality control data, protocols, and reporting outputs. The system should serve as the authoritative source of programme information whilst enabling integration with external systems and stakeholders.

The programme data and information system (PDIS) provides a unified platform for data integration across multiple laboratories (where relevant) and sampling locations. This integration enables programme-wide analysis and reporting, allowing rapid identification of trends or anomalies that might not be visible when examining individual laboratory results in isolation. The system also maintains standardisation across the programme by enforcing consistent data formats and metadata requirements, thereby ensuring compatibility between different data sources.

Document management should also be a key function of the PDIS. The system should maintain current and historical versions of all programme protocols, standard operating procedures, and analytical methods. This centralised approach to documentation ensures that all stakeholders work from the same approved versions of procedures whilst maintaining a clear audit trail of any changes. The system should also track the relationships between specific data sets and the protocols used to generate them, supporting both operational needs and quality assurance requirements.

Proper implementation of access control and security are vital for an effective system. The system must support different levels of access for different stakeholder groups, from laboratory staff requiring detailed technical information to public health officials needing summary reports. These access controls must be specific enough to protect sensitive information whilst still enabling appropriate data sharing and collaboration. The system should also include an auditing function which records data access and modifications.

A robust and effective data and information system must be able to receive data from LIMS, field collection devices, discovery metadata catalogue, and other data sources through appropriate APIs or data transfer protocols. It should also make use of an Application Programming Interface (API) or similar links of data where it has been made openly accessible. Similarly, the system should be capable of providing data and metadata to external systems, such as public health databases or reporting websites. These integration capabilities

ensure that the system can support both routine operations and emergency response scenarios effectively.

7.3. NBN Data Catalogue (E03)

The NBN data catalogue is an online resource which will provide users across the UK government with the ability to search for government biosurveillance datasets. It does not hold the data themselves but instead holds discovery metadata such as where the data are held, who the owner is and how it can be accessed if needed.

At the time of writing, the NBN data catalogue was under development and so all information given here is provided on the assumption that development of the Data Catalogue has continued as planned at the time of writing and it is available for use.

If the NBN Data Catalogue is not available, then it is advised that the user discusses data availability with data managers within their organisation and with external collaborators. However, even if the Data Catalogue is available, it is still advisable to search other data catalogues for data and information relevant to the EBH programme under development. Table 5 lists some existing data catalogues which may be useful in this scenario.

Table 5: Overview of Data Catalogues for Environmental and Public Health Surveillance

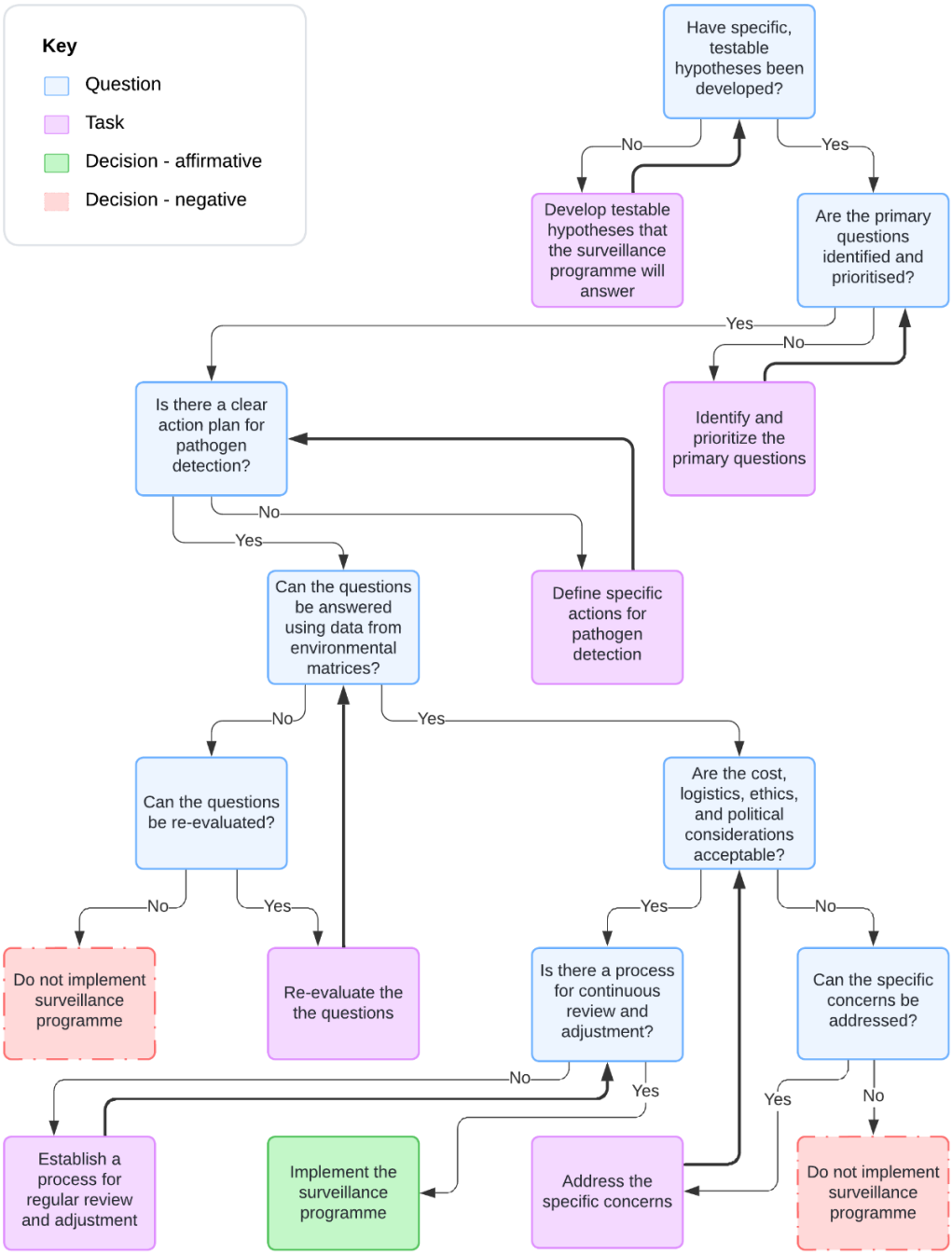
Name of data catalogue	Description	Link
APHA Vet Gateway	Veterinary surveillance data including notifiable diseases, antimicrobial usage in livestock, and animal health monitoring.	https://www.apha.gov.uk/vet-gateway
Cefas Data Portal	Repository for marine and aquatic science data, including fish health, water quality, and marine environmental monitoring. Essential for the environmental and aquatic health component of One Health.	https://data.cefas.co.uk/
Defra Data Services Platform	Comprehensive environmental data platform covering air quality, soil health, biodiversity, and agricultural statistics. Critical for monitoring environmental health factors.	https://environment.data.gov.uk/
Environmental Agency Open Data	Environmental monitoring data including water quality, pollution incidents, and ecological assessments.	https://environment.data.gov.uk/
Food Standards Agency Data	Food safety data, including foodborne illness surveillance, antimicrobial resistance in food chain, and food hygiene ratings.	https://data.food.gov.uk/
MEDIN	Collaborative network providing marine environmental data, including water quality, marine biodiversity, and coastal health monitoring.	https://medin.org.uk/data
Public Health Scotland Open Data	Scottish public health surveillance data including zoonotic diseases and environmental health.	https://www.opendata.nhs.scot/
The official portal for European data	Broader European context data including cross-border disease surveillance, environmental monitoring, and public health statistics.	https://data.europa.eu/en

Name of data catalogue	Description	Link
UK Biobank	Large-scale biomedical database including health, environmental exposure, and genetic data.	https://www.ukbiobank.ac.uk/
UK Government Data Portal	Central hub for UK public sector data, including health, environmental, and agricultural datasets. Contains valuable cross-sector data relevant to One Health surveillance.	https://www.data.gov.uk/
UKHSA Data Dashboard	Public health surveillance data including infectious diseases, antimicrobial resistance, and environmental health impacts. Key resource for human health surveillance.	https://ukhsa-dashboard.data.gov.uk/
Wales Environmental Data Portal	Welsh environmental and ecological monitoring data.	https://naturalresources.wales/evidence-and-data/

8. Decisions

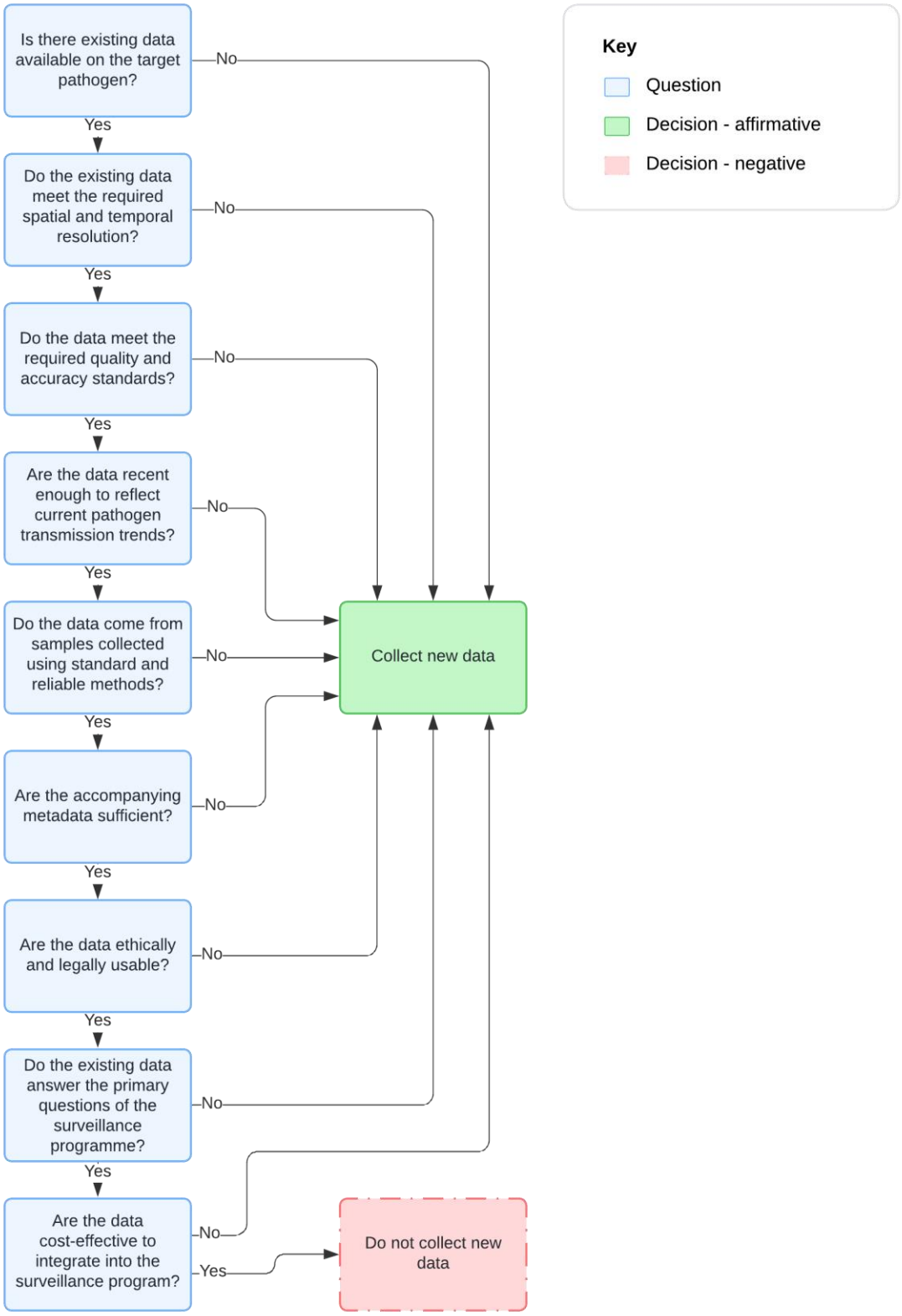
8.1. Next phase? (K01)

Once the initial processes (P01 to P04) of the surveillance design framework have been carried out, use the decision tree below to decide whether to continue to the next phase of the surveillance design framework. This decision should be recorded in the Initial decision report (S01).



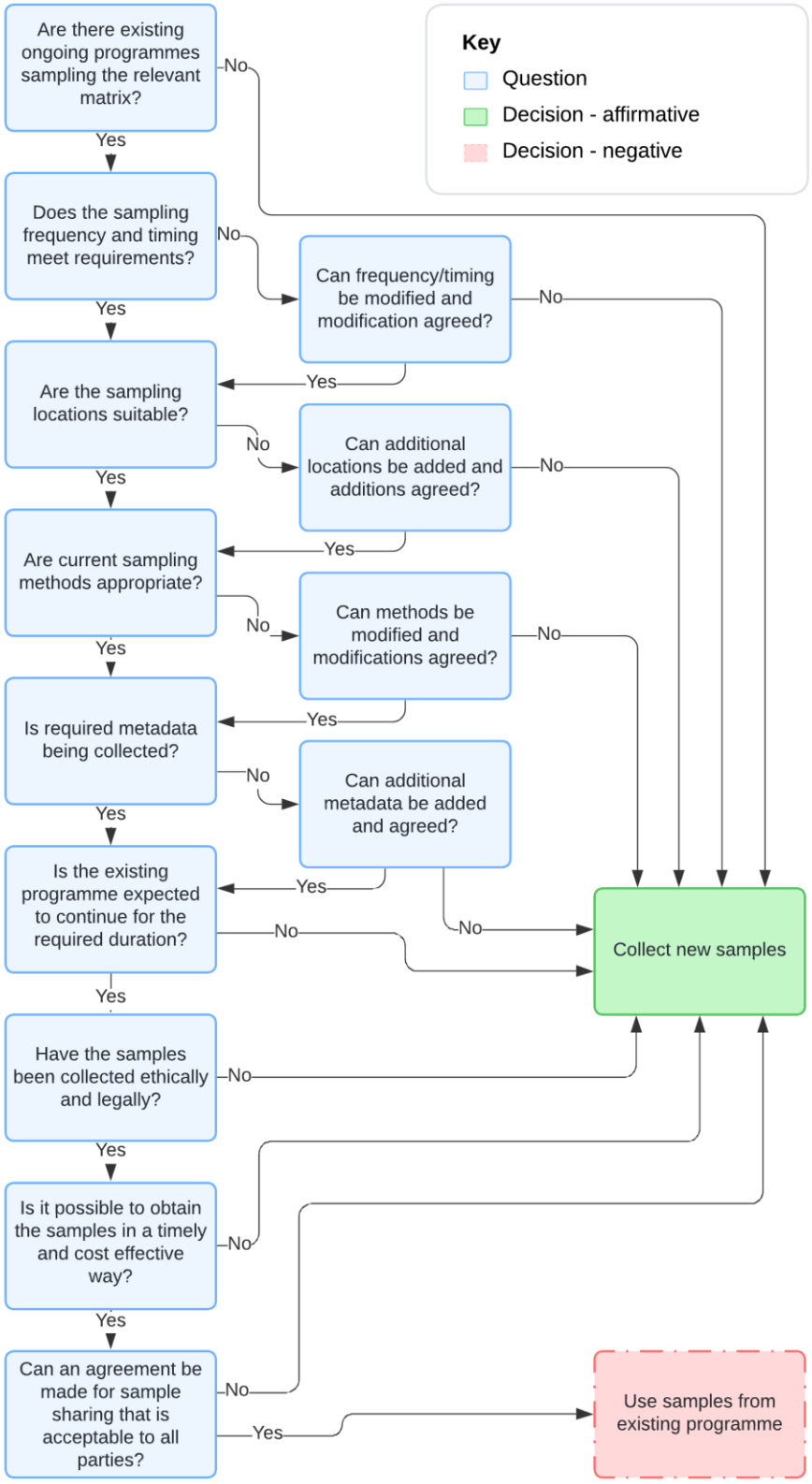
8.2. Generate new data? (K02)

Once an assessment of the existing and available datasets has been conducted (P10), use the decision tree below to determine whether new data need to be collected.



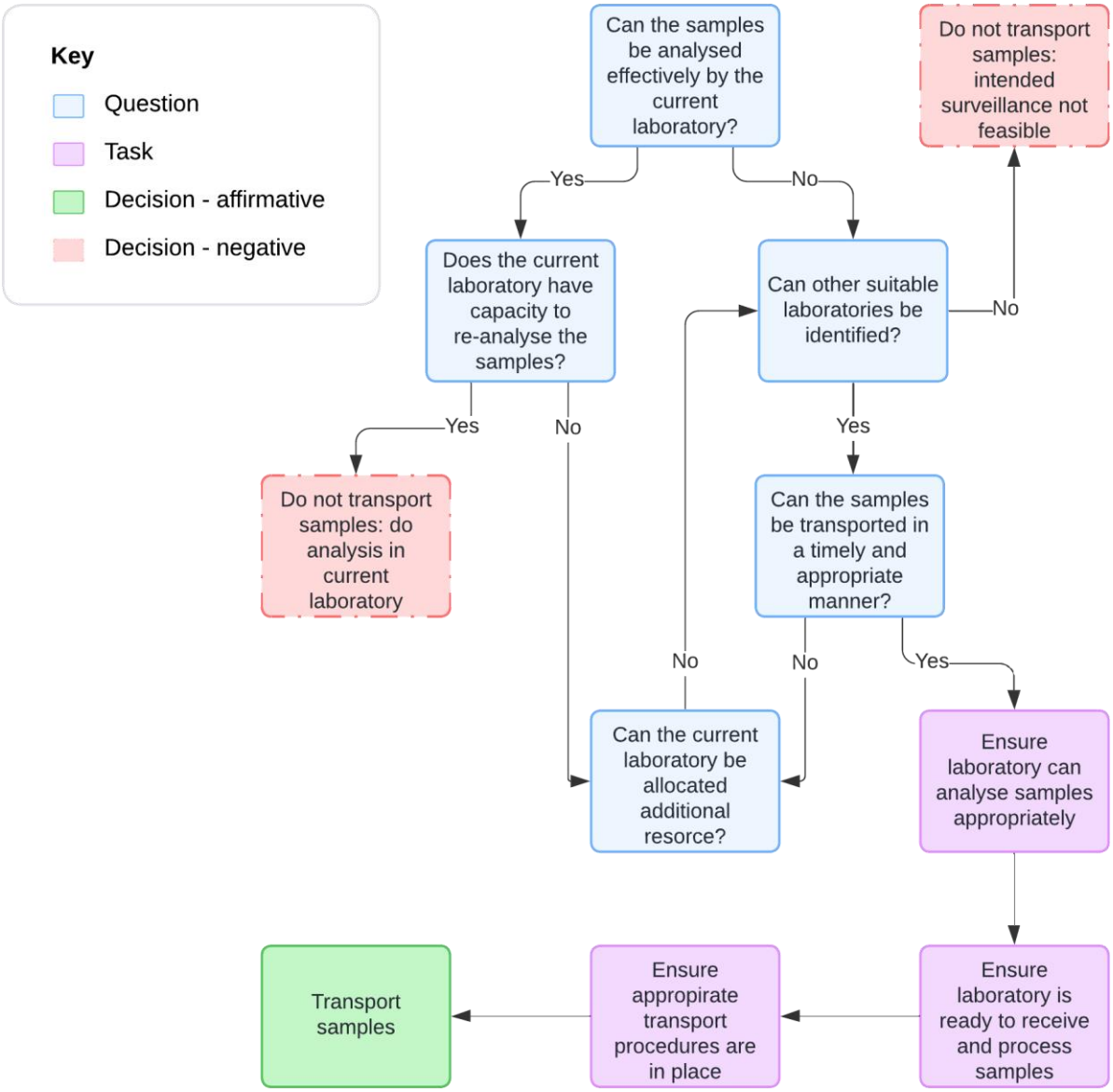
8.3. New samples required? (K03)

Once an assessment of the requirements for samples has been made (P11), use the decision tree below to determine whether new samples need to be collected.



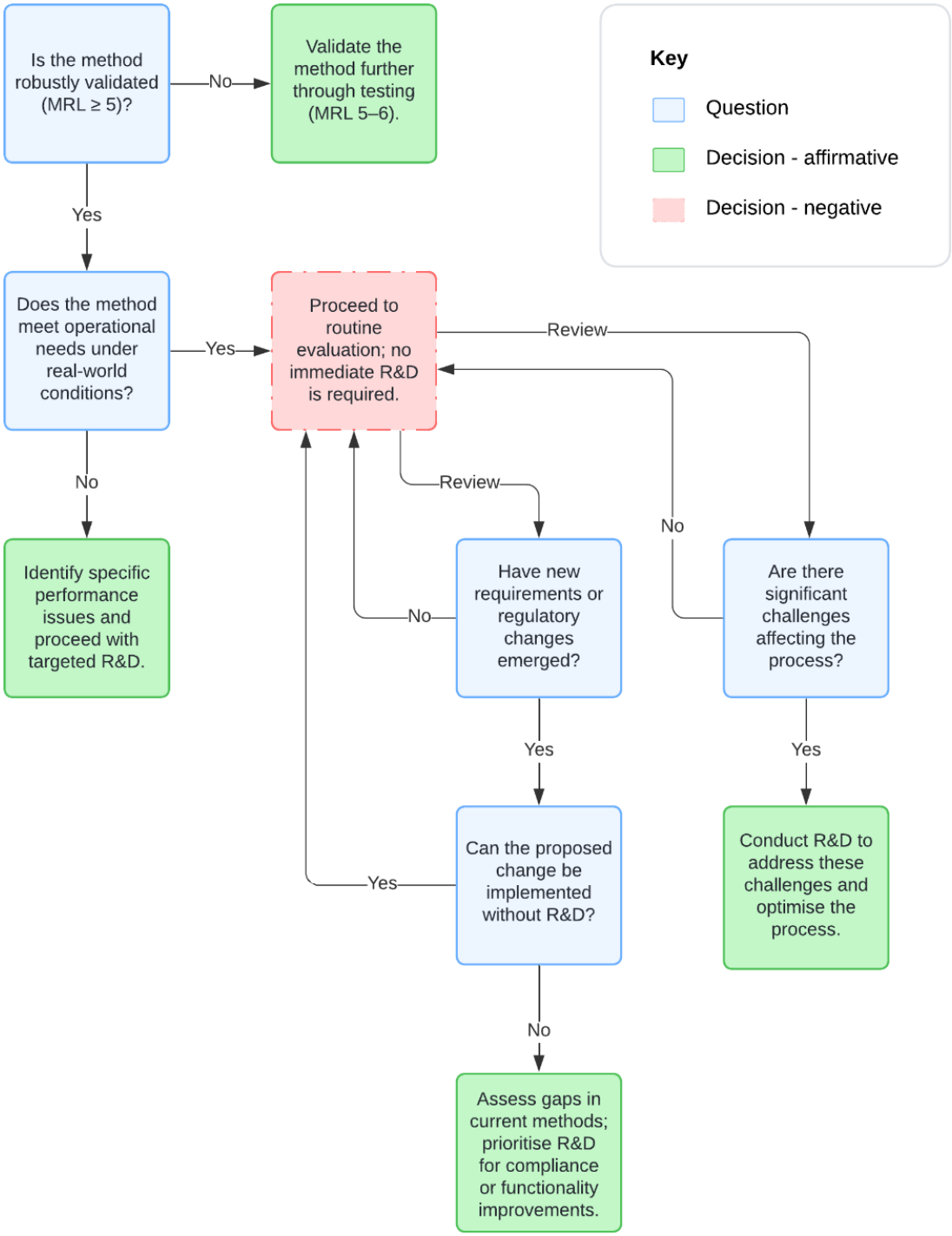
8.4. Transport samples for further analysis? (K04)

This decision must be made following engagement with stakeholders (P12) and a decision not to collect new samples (K03). This will determine whether the existing samples can be analysed within the laboratory that holds the existing samples, or whether they must be transported to a different laboratory for further analysis.



8.5. R&D required? (K05)

The decision to carry out research and development (R&D) needs to be made once the methods for the operational processes (P17 to P20) have been selected. Following an assessment of the method readiness level (MRL) of each method (P15), the following decision tree can be used. The appropriateness of each method should be reviewed periodically and following any triggers for assessing the need for change (P14).



9. Preparatory processes

9.1. Define surveillance purpose (P01)

Once the decision has been made to proceed with the initial stages of the EBDF for a new detection requirement, the first step involves clearly defining the purpose of the surveillance by developing the questions that the programme is attempting to answer.

This will ensure that any subsequent steps are carried out in the correct context and that all individuals involved in developing a new programme understand the overall objectives. Without this information, there is a possibility of multiple interpretations regarding the reason for conducting surveillance. This can lead to inefficient resource use and the collection of data that may not achieve their intended purpose. (Calba *et al.*, 2015; Rivers *et al.*, 2023). These questions not only guide the design of the surveillance system but also ensure that the data collected can effectively inform public health decisions and interventions.

Examples of questions that environmental data may be used to answer are:

- Is the presence of a specific pathogen in the environment is linked to the outbreak of a particular disease in the population?
- Do certain environmental conditions, such as temperature or rainfall, increase the prevalence of a pathogen in the environment?
- Has the implementation of a new sanitation policy or infrastructure project caused changes in the concentration of a specific pathogen in the environment?
- Does the genetic diversity of a pathogen in wastewater correlate with the rate of new infections in the community?
- Do vaccination campaigns lead to a decrease in the environmental load of pathogens causing vaccine-preventable diseases?

It should be noted that these are just examples, and other questions may be developed.

It is likely that in some cases, the programme may be used to answer multiple questions and therefore it is essential to prioritise the primary questions that the EBH programme aims to answer. Prioritisation will allow the development of a focused approach, where the most critical questions are addressed first, while secondary questions can be acknowledged but treated as supplementary.

In many cases, the detection or quantification of a pathogen or sub-type of pathogen should lead to an action (covered by P02). However, if no immediate action is required upon detection, the purpose of the surveillance must be

explicitly defined, such as establishing a baseline dataset for future reference (Walker, Wade, *et al.*, 2024).

An example of the latter may be establishing a baseline for pathogen prevalence, diversity of (potential) pathogenic lineages or genes associated with virulence/pathogenicity to inform future surveillance.

9.2. Define detection actions (P02)

Establishing clear actions following the detection of pathogens in environmental samples ensures effective responses. This proactive strategy enables rapid interventions, thereby minimising outbreak risks and improving community and environmental health outcomes. For example, if a target pathogen is detected or it is detected at levels above a predetermined threshold, specific actions should be defined. These actions may include, but are not limited to, enhanced clinical surveillance, vaccination campaigns, or public health education initiatives (Weidhaas *et al.*, 2021). The actions following detection must be defined according to existing practices, policies and legislation for the target pathogen or group of pathogens in collaboration with the relevant competent authorities where they exist.

The importance of defining actionable public health interventions can be illustrated in the case of poliovirus detection in wastewater, which serves as an early warning system and enable timely public health responses to prevent outbreaks. In this scenario, upon detection of poliovirus in UK wastewater, UKHSA will work closely with the NHS to reduce the spread of polio within communities. Early detection means that vaccination campaigns will be rapidly implemented in response to wastewater findings. If environmental samples indicate the presence of wild or vaccine-derived poliovirus, targeted vaccination efforts will then be initiated (Klapsa *et al.*, 2022). Detection of poliomyelitis due to wild-type poliovirus must also be reported to the World Health Organisation within 24 hours (WHO, 2022; UKHSA, 2024).

In determining the relevant actions to take when a pathogen is detected, it is important to draw upon the broader policy on control of that pathogen, in particular whether the aim is eradication or control. However, it should be noted I n this case, that the feasibility of this aim will only be known once data collection has begun. The decision for defining that policy and its broad implications to is beyond the scope of this guidance and should be assessed by relevant policy experts.

If the simple detection of a pathogen does not warrant immediate action, alternative criteria should be established based on the surveillance programme's defined purpose. This purpose could be either operational (enabling specific public health responses) or investigative (answering specific research questions about disease patterns). For operational purposes, monitoring temporal or geospatial trends can help identify emerging threats that require intervention. For example, a consistent increase in pathogen levels in wastewater over time or in specific areas may prompt public health responses, such as intensified clinical surveillance or targeted interventions

(Diemert and Yan, 2020; Carmo dos Santos *et al.*, 2024). For investigative purposes, the surveillance data can help determine which response approaches are most effective - for instance, whether broad community interventions or targeted local responses better control disease spread. The surveillance design must clearly link to these intended uses, with data collection and analysis methods specifically chosen to support either operational decision-making or hypothesis testing. This has been demonstrated in practice, as wastewater monitoring has successfully identified regions with high levels of otherwise undetected infections, enabling evidence-based decisions about where and how to implement public health interventions (Weidhaas *et al.*, 2021).

9.3. Assess technical value (P03)

When developing an environmental surveillance programme for biological threats, assessing the technical value of the surveillance is essential for ensuring its effectiveness and relevance. This assessment involves addressing several critical questions before programme implementation.

It is vital to determine whether the target pathogen is shed in a way that allows reliable detection in wastewater or other environmental matrices. Understanding the shedding dynamics of the pathogen is therefore essential, as certain pathogens may not be consistently shed in faeces or other bodily fluids or may exist in low concentrations in the environment. Brouwer et al. (2022) highlighted the importance of understanding viral shedding patterns in modelling environmental surveillance for public health, particularly for enteric pathogens like poliovirus.

There must be a clear understanding of what detection in wastewater implies about the presence of the pathogen in the population. This includes knowledge of the pathogen's environmental behaviour and its correlation with clinical cases. Wastewater surveillance can provide insights into community transmission dynamics; however, the implications of detected pathogens must be carefully interpreted. For example, wastewater data can identify areas where viral shedding is not declining, indicating ongoing transmission that may not be captured by clinical data. In this sense, DeJonge (2023) found a positive correlation between wastewater surveillance and emergency department visit data for respiratory syncytial virus (RSV) and influenza, further supporting the interpretation of wastewater findings in relation to clinical trends. Walker et al. (2024) showed a correlation between norovirus levels in wastewater and clinical cases at a national level, as well as a possible trend of changing norovirus levels coinciding with national COVID-19 related lockdowns.

The questions outlined in P01 should be answerable using data from environmental matrices. The feasibility of answering these questions is therefore vital for the programme design and implementation. The acquisition of data must be conducted in a timely manner to ensure the effectiveness of any necessary actions. Data must therefore be collected and analysed within a timeframe that allows for an appropriate response to the target threats. For example, high-frequency monitoring of wastewater is usually necessary to correlate pathogen concentrations with community incidence rates effectively (Weidhaas et al., 2021).

The technical value of environmental surveillance should be reviewed intermittently to ensure that the programme remains relevant and effective. Establishing a frequency for these reviews is important for adapting to changing epidemiological landscapes and emerging threats. The review process should be guided by several factors, as discussed below.

Pathogen Dynamics

The frequency of reviews should be influenced by the characteristics of the pathogens being monitored. For pathogens with known seasonal patterns or those that exhibit rapid changes in prevalence, more frequent reviews may be needed. During the COVID-19 pandemic, rapid mutations in the SARS-CoV-2 genome required frequent adaptations to methodologies to adapt to the evolving epidemiological landscape (Ulhuq *et al.*, 2023). This approach allows for adjustments to surveillance strategies based on real-time data.

Integration of Multiple Data and Information Sources

The availability of other data and information sources, such as clinical case reports or syndromic surveillance data, can inform the review process. When clinical data indicate an increase in cases, it may trigger a more immediate review of environmental surveillance findings to correlate pathogen detection in wastewater with clinical trends (DeJonge, 2023). This integration enhances the overall understanding of pathogen dynamics.

Technological Advancements

As new techniques become available (such as improved sequencing or biosensors) these should be evaluated to determine whether they can be incorporated into the EBH programme.

Regulatory and Policy Changes

Changes in policies or regulatory frameworks may also influence the frequency of reviews. If new guidelines are issued regarding pathogen monitoring or reporting, the programme should be evaluated to ensure compliance and alignment with these standards.

Stakeholder Feedback and Performance Metrics

Gathering feedback from stakeholders involved in the EBH programme can provide valuable insights into its effectiveness and areas for improvement. Performance metrics, such as detection rates and response times, should be analysed regularly to determine if the programme meets its objectives.

Review frequency

Based on these considerations, a recommended frequency for reviewing the technical value of an EBH programme could be established as follows. However, it should be noted that this is just a guide, and the review frequencies must be decided by the needs of each programme:

- **Quarterly Reviews:** For pathogens with known seasonal patterns or during periods of heightened surveillance needs, quarterly reviews may be appropriate to ensure that the program remains responsive to emerging threats and epidemiological changes.
- **Biannual Reviews:** For pathogens with stable epidemiological profiles or when no significant outbreaks are detected, biannual reviews may be sufficient to assess the ongoing relevance and effectiveness of a surveillance programme.
- **Annual Reviews:** An annual review should be conducted to evaluate the overall performance of the surveillance programme, incorporating stakeholder feedback, technological advancements, and changes in risk and policies.

9.4. Assess other values (P04)

When assessing the value of an environmental surveillance programme for pathogens and biothreats, it is essential to consider several factors beyond technical value, including cost, logistics, ethics, and political considerations. Each of these factors may not be fully quantifiable, necessitating the use of best estimates based on available data and expert opinions. If a decision is made to continue developing the surveillance plan, more accurate estimates should become available, and the value may need reassessment before commencing any surveillance. Regular reviews of these factors are important to ensure the program remains effective and relevant.

Cost

It is unlikely that accurate costs for a programme can be calculated at this stage. However, an indicative cost should be calculated as accurately as possible to allow a basic cost-benefit analysis to be carried out. An estimate of how confident the costings are should also be noted.

The cost of implementing and maintaining an EBH programme can vary significantly based on the programme's scale, technologies used, and pathogens monitored. Key cost considerations include:

- **Initial Setup Costs:** Expenses related to equipment, laboratory facilities, and personnel training. For example, wastewater surveillance for SARS-CoV-2 has been noted to be relatively low-cost compared to traditional clinical methods, as it can cover large populations with fewer resources (Parkins et al., 2024).
- **Operational Costs:** Ongoing expenses such as sample collection, analysis, and data interpretation must be factored in. Regular sampling is often necessary to provide actionable data, which can increase operational costs (Weidhaas *et al.*, 2021).
- **Cost-Benefit Analysis:** Estimating potential savings from early outbreak detection can help to justify costs. For example, early identification of pathogens through wastewater surveillance can prevent larger outbreaks, incurring significant healthcare costs. Other examples of factors to consider include (but are not limited to) impacts on wild habitats, farming and job losses.

Logistics

Logistics are vital for the success of an EBH programme:

- **Sample Collection and Transportation:** Samples must be collected in a timely manner and transported under conditions that preserve their integrity (Wade *et al.*, 2022; Holm *et al.*, 2023). Assess whether it is possible and feasible to carry out sample analysis in the field (i.e. close

to the sampling location) in a way that fulfils the quality and data needs of the programme.

- **Laboratory Capacity:** The ability of the right type of laboratories to process samples quickly and accurately is vital. Variability in technical capabilities and methods used across different laboratories can affect data quality and reliability (Chik *et al.*, 2021; Wade *et al.*, 2022; Walker, 2024). The ability of laboratories to handle pathogens safely and legally is also a vital factor in determining laboratory capacity (*Anti-terrorism, Crime and Security Act*, 2001; *The Specified Animal Pathogens Order*, 2008; *The Specified Animal Pathogens (Wales) Order*, 2008; *The Specified Animal Pathogens (Scotland) Order*, 2009; ACDP, 2023).
- **Integration with Existing Systems:** The programme should integrate with existing public health surveillance systems, including clinical data sources, to enhance overall effectiveness.

Ethics

Ethical considerations must be addressed to ensure public trust:

Privacy Concerns: Environmental surveillance may raise concerns about individual privacy, especially if data can be traced back to specific communities or sub-communities. Clear communication about data usage and protection is therefore essential.

Equity in Surveillance: Consideration must be given to ensure that surveillance efforts do not disproportionately target specific populations. Programmes should aim for equitable monitoring across diverse communities to avoid stigmatisation.

Informed Consent: Ethical considerations regarding informed consent and community engagement should be emphasised.

Policy Considerations

Policy factors can significantly influence the implementation and sustainability of EBH programmes:

Support from Government and Stakeholders: Gaining political support is crucial for securing long-term funding and resources. Engaging stakeholders, including local authorities and public health officials, can therefore enhance long-term programme viability.

Policy Alignment: Where possible, the programme should align with existing public health policies to ensure coherence and facilitate integration into broader health initiatives.

Public Perception and Trust: Building public trust through transparent communication about the goals and benefits of surveillance can minimise resistance and enhance community participation (Lyon, 2021).

Review Frequency

Given the dynamic nature of these factors, regular reviews are necessary to assess the ongoing value of the environmental surveillance.surveillance programme Suggested review frequencies include:

Quarterly Reviews: To assess cost-effectiveness, logistical efficiency, and ethical considerations considering emerging data and public health needs.

Biannual Reviews: To evaluate political support and stakeholder engagement, ensuring alignment with public health policies and community needs.

Annual Reviews: To conduct a thorough assessment of all factors, allowing for strategic adjustments to the programme.

9.5. Define roles and responsibilities (P05)

It is important to establish and designate a list of roles and responsibilities for the programme. While some of these roles will already have been established before this point, others will need to be considered once the decision to develop the EBH programme is made. Some of the key roles include:

Programme Director: Responsible for oversight and plans and ensuring quality control measures are integrated into all aspects of the programme.

Programme Manager: Responsible for ensuring that projects are (i) delivered safely to time and within budget; (ii) overseeing financial management; and (iii) driving senior level stakeholder engagement, including scientific/technical staff time allocation.

Project Manager: Responsible for the day-to-day management of specific projects within the programme. This includes planning, executing, and closing projects, as well as managing the project team, resources, and budget to ensure that project objectives are met.

Technical Lead: Oversees scientific and technical aspects, including the development of sampling protocols and laboratory methods and outlining data requirements.

Field Coordinators: Manage logistics for sample collection and transport, including site selection and scheduling.

Laboratory Analysts: Conduct laboratory analyses of samples, including pathogen detection and quantification.

Quality Control Officer: Oversees all quality control activities within the programme.

Data Manager: Oversees the curation of all datasets including sharing and publication requirements.

Data Analysts: Interpret surveillance data and identify trends.

Community Outreach Coordinators: Facilitate communication with local populations and gather community feedback.

Epidemiologists: Advise on key aspects of epidemiology (such as pathogen dynamics). Analyse data in the context of public health and identify patterns and potential outbreaks.

Public Health Officials: Integrate surveillance findings into broader public health strategies.

Regulatory Compliance Officer: Ensure adherence to all relevant regulations and guidelines.

Communication Specialists: Develop materials to inform the public and stakeholders about the programme's findings.

Research Collaborators: Include academic researchers or external partners who contribute expertise in specific areas.

Logistics and Supply Chain Manager: Manage the procurement of necessary supplies and equipment for sampling and laboratory analysis.

9.6. Define surveillance parameters (P06)

When establishing an EBH programme, several key parameters must be considered to enhance the programme's effectiveness and sustainability. These parameters include the programme's duration, spatial and temporal resolutions, stakeholder engagement and the roles of various participants involved in the program.

Define the sample matrix

The specific type of sample matrix must be made clear to enable accurate planning. For example, it is not enough just to specify "wastewater" or "air". This is because there are many different sub-types of these matrices which are not equally suited to answering specific biosurveillance questions. Additionally, the specific matrix sub-types will impact on the sampling and analytical methods. Table 6 shows a list of some environmental matrices that might be used for biosurveillance and breaks down some of their sub-types. It should be noted that there may be other suitable matrices that are not covered in this table. The nature of the surveillance that will be undertaken will determine which of the specific types of matrix is required. It should also be noted that some of these matrices have been studied in more detail for their applicability to EBH than others.

Table 6: Examples of sample matrices that may be used for EBH

General matrix	Sub-type	Description
Wastewater	Raw influent	Wastewater (usually sewage) entering a treatment works before any treatment. This is a highly variable matrix. It might be collected before or after coarse screening depending on experimental requirements. This is the type of water typically used for population level pathogen surveillance.
	Effluent	Wastewater exiting a treatment work having undergone some form of treatment. The level and type of treatment will affect pathogen prevalence significantly. Analysis of this type of water will usually be to understand the input of pathogen into the environment rather than disease prevalence in the population.
	Overflows	Usually untreated wastewater (typically sewage) mixed with surface water that is discharged

General matrix	Sub-type	Description
		directly into a watercourse when sewerage networks are at full capacity.
	Near source	Wastewater from close to a specific source. This type of water is usually analysed to give insights into localised (e.g. building or street) level disease prevalence. It is particularly important to thoroughly assess the ethical implications of using this type of water as a surveillance matrix.
	Industrial / agricultural	Wastewater associated with industrial or agricultural processes. The nature of this matrix will be highly specific to individual sources. It may contain pathogens or other biothreats directly associated with the process or biothreats that were already present before processing but have been concentrated in this location. It is important to consider impacts to individual businesses when using this matrix.
	Tanks	This might include aeroplane sewage, portable toilets, cesspits or any other wastewater that is stored in some form of tank or container prior to being treated and disposed of. The exact nature of this matrix will vary depending on the purpose of storage. Ethical considerations are particularly important here due to the limited population that these facilities serve.
Other waters	Surface water	Surface waters such as streams and rivers may contain run-offs from a wide catchment area that might not otherwise be captured within enclosed networks such as sewers. Additionally, these waters may contain pathogens and other biothreats of direct relevance to aquatic animal health. Depending on the sampling location within a river network, these waters may contain pathogens associated with local agriculture, wildlife, human conurbations and any other inputs to water.
	Seawater	Like surface waters, seawater may contain pathogens from a broad range of sources both from the terrestrial and the aquatic environment.

General matrix	Sub-type	Description
Air	Indoor air	Air samples can be used to detect airborne pathogens. This may be particularly useful in enclosed spaces where there is little airflow. Air samples may provide data both on the levels of a pathogen within a localised population (such as those using a specific facility), as well as data on potential hotspots for disease transmission (i.e. areas with high levels of airborne pathogens may be hot spots for transmission).
	Outdoor air	Outdoor air may provide data about the wider prevalence of pathogens within a defined geographic area. Many factors will affect the accuracy of results from such samples (e.g. wind conditions and UV index).
Sediment	Sewage sludge	Sewage sludge is distinct from raw influent wastewater in that it is a semi-solid by-product of wastewater treatment. Sludge has been found to contain higher levels of pathogens such as SARS-CoV-2 relative to wastewater, but may be less representative of the temporal trends in disease dynamics within a community
	Terrestrial soil and sediment	Terrestrial soils and sediments may be long-term reservoirs for pathogens and parasites and other biothreats. The application of sewage sludges as fertilisers may contribute to the micro-biological loads of soils.
	Aquatic sediment	Sediments in aquatic environments (e.g. riverbeds and seabeds) are similar to terrestrial soils and sediments in that they can be reservoirs of biothreats. However, aquatic sediments may contain pathogens that have originated from terrestrial and aquatic sources.
Food and sentinel species	Bivalves	Bivalve molluscan shellfish (BMS) may bioaccumulate pathogens of interest and analysis of BMS samples may be useful for biosurveillance in a qualitative or semi-quantitive way. Wild populations of BMS may provide convenient samples for such monitoring*. Invasive species such as zebra mussels may also be a useful

General matrix	Sub-type	Description
		matrix. Artificial populations of BMS (e.g. bagged mussels) may also be useful. Consideration must be given to the potential environmental impact from any increased activities in the harvesting area.

**It should be noted that where BMS are harvested for food, their use as sentinels for pathogens may be problematic due to the perception that the presence of that pathogen in foodstuffs may present a risk to health.*

Identification of Target Pathogens

It is essential to clearly define whether the focus will be on one or multiple specific pathogens or if the approach will be partially agnostic, examining the entire microbial community rather than a specific subgroup or species. For example, semi-targeted metagenomic sequencing can provide insights into the overall microbial community but is unlikely to be able to accurately quantify individual species. Truly agnostic metagenomic methods often face significant challenges in detecting pathogens at actionable levels due to the overwhelming presence of nucleic acids from species that are not of interest to the surveillance programme. The choice of targets also raises the question of whether the programme will prioritise the detection of viable or infectious pathogens, or if identification of nucleic acids is sufficient for the programme's objectives. In some cases, such as norovirus and *Cryptosporidium parvum* oocysts, methods to routinely assess viability in environmental samples do not currently exist.

Taxonomic Considerations

Determining the appropriate taxonomic level for surveillance is another critical consideration. The programme should decide whether to focus on specific variants or adopt a broader classification of pathogens, such as "DNA respiratory viruses" or "vector-borne pathogens". This decision will have implications for the design of sampling protocols, laboratory analyses, and data interpretation. For instance, focusing on specific serotypes may require more sophisticated detection methods and a deeper understanding of pathogen ecology. Conversely, broader classifications may allow for more extensive surveillance but could dilute the specificity needed for effective outbreak response.

Programme Duration

The duration of the programme should be carefully considered based on the epidemiological context and the pathogens of interest. Long-term surveillance is often necessary to capture seasonal variations and emerging trends in pathogen prevalence (Weidhaas *et al.*, 2021). A minimum commitment of several years may be needed to establish baseline data and assess the effectiveness of interventions. This extended timeframe allows for adaptive management strategies that can respond to evolving epidemiological data.

Spatial Resolution

The spatial extent of surveillance should consider regions identified as high-risk for pathogen emergence, including both urban and rural areas. This approach allows for a comprehensive understanding of pathogen dynamics across different environments. Surveillance may also extend to critical points of entry, such as ports and airports, where pathogens may be introduced from international sources. This border surveillance is essential for the early detection of pathogens that could lead to outbreaks, as it enables monitoring of both human and environmental samples at these high-traffic locations. Depending on the requirements of the programme, the spatial resolution must be sufficiently fine to allow for localised assessments. For instance, monitoring at the level of individual buildings or community clusters can provide insights into localised outbreaks and transmission dynamics. Identifying "hot spots" within urban settings, such as parks or playgrounds, can help target public health interventions more effectively.

Temporal Resolution

Regular sampling intervals, such as monthly or quarterly, are required for monitoring fluctuations in pathogen levels effectively. However, in certain contexts, more frequent sampling may be necessary to capture rapid changes in pathogen dynamics, particularly in environments where conditions can shift quickly, such as during seasonal outbreaks or following environmental changes. Establishing the appropriate temporal resolution also depends on the specific objectives of the surveillance programme. If the goal is to detect trends or emerging threats at a national level, a broader temporal resolution may suffice. However, if the focus is on near-source detection (such as monitoring pathogen levels in specific buildings or localised areas), higher temporal resolution may be required to capture critical data points that inform immediate public health responses.

Population Coverage

The population coverage of surveillance must be carefully considered across both human and animal populations, depending on the surveillance targets.

For human populations, this includes assessing total population size and demographic distributions, with urban areas typically offering high population density coverage with relatively few sampling points, while rural surveillance may require more extensive networks to achieve representative coverage. Temporal variations in human populations, such as seasonal tourism or academic terms, must be factored into coverage planning. For animal populations, coverage must account for both domesticated animals (e.g., livestock, aquaculture) and wildlife populations, including their seasonal movements and behavioural patterns. In agricultural settings, this might involve monitoring specific farm populations or entire production regions. Wildlife surveillance may need to consider migration patterns, breeding seasons, and habitat use. Marine environments present unique challenges, requiring consideration of both wild and farmed aquatic populations. The relationship between different population types must also be considered - for instance, the interface between wildlife and domestic animals, or between aquaculture and wild fish populations. Coverage decisions should balance the need for representative data across these diverse populations against practical constraints like laboratory capacity and transport logistics. Regular assessment of population coverage ensures that surveillance remains aligned with programme objectives and adapts to changes in both human and animal population dynamics.

Statistical Power and Sample Size Requirements

Determining the appropriate sample size is a critical step in designing a robust surveillance programme. Sample size calculations should be based on the specific surveillance objectives, expected effect sizes, and desired statistical power. For programmes aiming to detect the presence of pathogens, sample size should be sufficient to achieve the desired sensitivity and specificity at the population level. For quantitative monitoring programmes focusing on concentration trends, power calculations should determine the number of samples needed to detect meaningful temporal and/or spatial variations with sufficient statistical confidence.

Key considerations for sample size determination include:

- Minimum detectable effect size relevant to public health decision-making.
- Expected variability in the environmental matrix being sampled.
- Desired confidence level and statistical power.
- Spatial and temporal clustering effects that may require adjustment for non-independence.
- Resource constraints that might limit sampling capacity.
- The need for stratification across different populations or geographic areas.

The sample size should be calculated for each major analytical objective of the programme, and the most demanding requirement should be used for planning purposes. Where resources are limited, prioritisation should focus on achieving adequate power for the primary surveillance questions.

Integration of Data Sources

To enhance the spatial and temporal resolution of surveillance, integrating multiple data sources can be beneficial. For instance, combining environmental surveillance data with clinical case reports, animal health records, or meteorological data can provide a more comprehensive understanding of disease dynamics. Wastewater surveillance data might be integrated with hospital admission rates or pharmaceutical sales data to validate trends. For animal pathogens, environmental surveillance could be combined with veterinary reports and livestock movement records. This integration allows for the identification of patterns and trends that may not be evident from a single data source, thereby improving the overall effectiveness of the surveillance programme.

9.7. Data storage and access(P07)

Analytical data and associated metadata should be stored in a secure, centralised platform and/or linked to from accessible sources, to enable easy access and sharing among stakeholders. This central platform should support data visualisation and reporting tools to facilitate interpretation.

The data should have licensing applied (respecting national jurisdictions, international conventions, and legislation) preferably all under a minimally restrictive and voluntary common-use licence. This should be well documented for each dataset in supplementary discovery metadata, to grant permission, ensure proper attribution (including, citable using a persistent identifier) with preference to allow any programme analyst to copy, distribute and make use of the data. Data sharing agreements should be established to clarify conditions for more sensitive data (e.g. personal), or else key data sharing elements need to be covered for the programme under contractual arrangements. Where third party data are obtained, the licensing and conditions should be received along with the data from the provider for clarification on any reuse conditions, data limitations or restrictions. This is to ensure that all stakeholders have access to relevant data and are clear on any conditions for reuse or publishing, while maintaining confidentiality and compliance with regulations.

The data storage capacity and functional requirements must be estimated from the outset to ensure that appropriate levels of resources are available for the programme. This will be affected by the types of data that will be collected in the programme. For example, nucleic acid sequence data is likely to require greater storage capacity than pathogen load data.

At this stage, it is important to determine which stakeholders will need access to the database. This will ensure that the correct data access arrangements are established as early as possible and will minimise confusion at a later stage.

It is recommended that data access is managed by assuming access is allowed by all stakeholders within a programme by default, and that restricting access to data is only applied where the sensitive nature of data require restrictions.

However, some stakeholders will always need access to certain types of data to enable them to perform their roles within the programme efficiently. Below is a list of minimum recommended access requirements for each of the roles lists in P05. In the context of this list “analysed data” means all analytical data that has been through any initial analytical processes (for example conversion of qPCR data to pathogen loads).

- **Programme Director:** Access to all data.
- **Programme manager:** Access to analysed data.
- **Project managers:** Access to analysed data.
- **Technical Lead:** Access to all data.
- **Field Coordinators:** Access to all metadata and analysed data.
- **Laboratory Analysts:** Access to all metadata and analysed data.
- **Quality Control Officer:** Access to all data.
- **Data manager:** Access to all data
- **Data Analysts**: Access to all data.
- **Community Outreach Coordinators:** Access to analysed data.
- **Epidemiologists:** Access to all metadata and analysed data.
- **Public Health Officials:** Access to analysed data.
- **Regulatory Compliance Officer:** Access to analysed data.
- **Communication Specialists:** Access to analysed data.
- **Research Collaborators:** Access to analysed data (further access depending on nature of collaboration).
- **Logistics and Supply Chain Manager:** Access to all metadata and analysed data.

9.8. Define data and quality needs (P08)

It is important to consider the type of data, the accompanying metadata, measurement uncertainty and data quality required for each programme as outlined below.

Data Type

The data collected for pathogens from environmental samples can be broadly split into three categories: quantitative, semi-quantitative and qualitative.

Quantitative data involves measuring pathogen concentrations or loads in samples. For instance, in monitoring SARS-CoV-2 levels, quantitative data provides specific viral load measurements (e.g. copies per litre) or loads normalised by population (e.g. copies per person). This enables statistical analysis and trend identification over time, which is vital for public health decision-making. The importance of quantitative assessments in evaluating risks associated with pathogens is underscored by studies that emphasise that such data can elucidate the relationship between risk factors and pathogen prevalence.

Semi-quantitative data categorises pathogen levels into ranges (e.g. low, medium, high), which can be particularly useful in initial screenings or when resources for precise quantification are limited. For example, a surveillance programme might classify wastewater samples based on pathogen presence into categories that indicate relative risk levels, guiding further investigation or targeted interventions.

Qualitative or presence/absence data can also play a significant role in environmental biosurveillance. For instance, qualitative assessments can be employed for rapid screening of non-endemic pathogens at ports of entry, where the presence or absence of specific pathogens in wastewater or air samples can inform immediate public health actions. Similarly, monitoring air quality for airborne pathogens can use qualitative data to assess the presence of pathogens responsible for respiratory illnesses, guiding public health officials in outbreak preparedness.

Metadata Requirements

Each sample should be accompanied by specific metadata to provide context for the analytical data. Examples of relevant metadata may include the date and time of sample collection, geographical location (GPS coordinates), weather conditions at the time of sampling, sample volume, method of collection, and personnel involved in collection. These metadata are crucial for interpreting the analytical results and ensuring the reliability of the data

collected and is covered in greater detail in D01, D02 and D03. Additionally, each dataset should be accompanied by the appropriate discovery metadata (D04) to ensure it is findable when uploaded to a data repository.

Measurement Uncertainty and Precision

The required level of measurement uncertainty should be defined based on the specific pathogens of interest and the intended use of the data. The required level of measurement uncertainty should be such that it allows the programme to answer the primary questions effectively. However, it is important to consider what can be feasibly achieved within the confines of a laboratory (noting the highly variable nature of environmental matrices). Where possible and available, use existing validation criteria and method validation reports as a guide. At the time of writing, there were no standardised criteria for these measures, but general criteria were under development within ISO working groups. The requirements for measurement uncertainty and precision may vary depending on the nature of the surveillance questions being answered. For example, when higher throughput is desired, a reduction in precision may be acceptable (European Commission *et al.*, 2024). This should be assessed on a case-by-case basis.

Limits of Detection and Quantification

The requirements for the maximum limit of detection (LOD) and limit of quantification (LOQ) should be established based on the analytical needs. For example, if a pathogen must be detected above a threshold value to trigger an action, then the LOQ in combination with the measurement uncertainty must be lower than that threshold level. These limits are critical for ensuring that the surveillance system can accurately detect and quantify pathogens of concern appropriately.

Quality Checking of Metadata and Analytical Data

Quality of metadata can be checked through routine audits and validation checks, verifying that all required metadata fields are completed and consistent with established protocols. Additionally, analytical data quality should be monitored through various mechanisms, including the use of control samples during laboratory analyses, participation in external proficiency testing programs, and regular audits of laboratory processes. These quality assurance measures are vital for maintaining the integrity of the surveillance system and ensuring that the data collected is reliable and actionable.

9.9. Search for existing data (P09)

Before developing any new data collection programme, it is vital to assess existing data sources and engage relevant stakeholders. This process should adopt a One Health approach, integrating relevant human, animal, and environmental data to provide an understanding of disease epidemiology.

Existing Surveillance Data

One of the primary considerations is the availability of existing surveillance data for the target pathogen. If there is an existing programme in which the target pathogen is being monitored using environmental matrices, there will need to be integration between the existing programme and any new programme to ensure that there is no duplication of effort. Indeed, if there is an existing programme of this nature, then it is possible that an entirely new programme may be unnecessary, and that linking resources may result in better outcomes for both programmes. However, it is also important to determine whether the data from such an existing programme can be used to answer the questions for the new programme.

Other types of surveillance data for the target pathogen should also be considered such as reported clinical case data and data from other environmental matrices. Additionally, indirect data that may indicate prevalence of a disease include syndromic data, digital and social media data (or other novel digital data streams) and pharmaceutical data.

Prevalence of Other Pathogens

In addition to the target pathogen, understanding the prevalence of other pathogens and indicators in the environmental matrix can be helpful. This information can inform risk assessments and guide public health interventions, particularly in areas where multiple pathogens may co-circulate.

Environmental Data

Environmental factors such as weather conditions and water temperatures play a significant role in pathogen transmission dynamics. For example, fluctuations in environmental conditions such as temperature and precipitation can influence pathogen survival and transmission (Demeter *et al.*, 2021; Balta *et al.*, 2024). Integrating these environmental data into the surveillance programme will enhance predictive modelling and risk assessment capabilities.

Population Data

Understanding the demographics of human, animal, and plant populations is also critical. Population data can provide insights into potential reservoirs and

transmission pathways for the pathogen of interest. Additionally, when using wastewater as a surveillance matrix, population data can be used to normalise the concentration of the pathogen of interest to the population size, which can give more reliable insights into the prevalence of the disease in the population relative to pathogen concentration alone (Wade *et al.*, 2022; Walker, Witt, *et al.*, 2024). Where possible, dynamic population data should be acquired, whereby changes in population size are considered at a fine temporal scale rather than population sizes based solely on census data. This is particularly helpful where a population is likely to change on a seasonal basis. For human populations, this might include areas with high levels of seasonal tourism, or cities with high numbers of students that may leave the area during holiday periods. In the context of animal populations, this may include migratory behaviour or seasonal livestock movements.

Data Sources

For UK EBH programmes, the NBN Data Catalogue (E03) should be consulted in the first instance (if available). The NBN Data Catalogue aims to provide a comprehensive list of data and information sources that are relevant to biosurveillance. However, in some cases, it will not be possible to find all the data and information through the Data Catalogue. It is also important to consult relevant stakeholders that may hold or be aware of other sources. Other potential data catalogues with relevant data sources are listed in Table 5 (page 42).

9.10. Acquire and assess existing data (P10)

Once existing data sets have been identified, they should be acquired and assessed for their suitability for use in the new EBH programme. Following this review process, a decision will need to be made whether the current surveillance can go ahead without the need to collect new data, or whether new data must be collected.

The process by which data will be acquired will vary depending on the source of the data (country and nature of organisation) and the type of data. For example, publicly available data will often be freely available and in the UK for example, government data are provided under the open government licence with regulations that give rights of public access. Other licensing such as a freely available license under Creative Commons (CC) may otherwise be provided (in the case of non-government data). In this case the license will need to be checked, to ensure they provide the relevant permission to use the data for purpose of the surveillance programme.

For government data that are not publicly available, data sharing arrangements between government departments may already be in place to clarify conditions for use (for example via an existing data sharing agreement (DSA) or memorandum of understanding (MOU)). Public authorities in the UK also have obligations under legislation to make data and information available proactively, using easily accessible electronic means whenever possible (*Freedom of Information Act*, 2000; *The Environmental Information Regulations*, 2004; *The Re-use of Public Sector Information Regulations*, 2015). Formal requests for data can be made when the data are known about, and legislation states a set turnaround time for engagement and provision. If the data source refuses, they must provide a clear reason for the refusal based on the legislation, following a 'public interest test'.

Similarly, non-government data may be available through a licensing arrangement such as CC, data contract or a DSA, or may need to be purchased under a commercial licence. Each provider may differ in their approach towards handling each request, so using standardized CC or OGL licensing is increasingly preferred. This approach provides a consistent and streamlined route towards enabling clarity. Information about the specific requirements for data acquisition will in some cases be detailed in the NBN data catalogue (E03). However, where this information is not available, it will need to be discussed with the relevant stakeholders.

It should be noted that datasets not currently available openly, publicly, or freely by digital means may take a significant amount of time to request and

acquire. Therefore, when making any data request, it is advisable to clarify the purpose (including any proposed onward sharing and potential outputs) and specify storage and handling arrangements to the data source early and in context. Requesting only what is necessary for the purpose can drive efficiencies and avoid burdensome requests that may be refused.

Once data are successfully acquired (or directly linked to from provider) and added to the PDIS, they must be assessed both for their quality and their ability to address the questions that the EBH programme is asking. The data provider may also be able to advise on their suitability for the intended purpose. The principles of data quality assessment were outlined by Grimsley et al. (personal communication, 10/09/2024) and focus on evaluating datasets across six key dimensions:

- **Uniqueness:** Ensuring that there are no duplicate records in the dataset. Each entity should be represented only once to maintain data integrity.
- **Completeness:** Verifying that all necessary records and essential values are present in the dataset. A complete dataset includes all required information without significant gaps. This includes both analytical data and associated metadata, such as date, location, weather conditions, and collection methods.
- **Consistency:** Checking that data values do not contradict each other within the dataset or across different datasets. Consistent data ensure reliability and coherence.
- **Timeliness:** Assessing whether the data are up-to-date and accurately reflects the period it represents. Timely data are crucial for making relevant and current decisions.
- **Validity:** Confirming that the data are within the expected range and format. Valid data adheres to predefined standards and formats, ensuring they are appropriate for their intended use.
- **Accuracy:** Measuring how closely the data match reality. Accurate data are free from errors and biases, providing a true representation of the information they are meant to convey.

Evaluating datasets against these dimensions, will help to ensure that the data are reliable and accurate. In addition to data quality, datasets must be evaluated for their relevance to the programme, and the implications for including them:

- **Relevance to Surveillance Purpose:** The data must be relevant to the key questions the programme is designed to answer. This includes:
    - Spatial and Temporal Resolution: The spatial and temporal coverage of the data should be detailed enough to detect trends over the time period and geographic extent required by the programme.

    - Sample Integrity and Collection Methods: The data should come from samples collected using standardised and reliable methods. Sample collection conditions and the logistics of sample transport must also preserve the integrity of the data.
    - Pathogen Detection Limits: The data should meet the required limits of detection (LOD), and quantification (LOQ) based on the specific pathogens of interest. If detection thresholds are too high, the data may not be sensitive enough for meaningful surveillance.
- **Cost and Feasibility:** The financial and logistical feasibility of continually acquiring, processing, and integrating the data into the new programme should be considered. As well as direct costs for acquiring data, this must also consider costs for integrating the data into the surveillance system (for example cleaning poorly structured datasets).
- **Ethical and Legal Considerations:** The data must adhere to ethical standards, particularly concerning privacy, consent, and the potential to stigmatise certain populations. Legal and regulatory compliance regarding data sharing and pathogen handling must also be reviewed (Hrudey *et al.*, 2021; Bowes *et al.*, 2023).

Decision to collect new data

The programme will likely need multiple datasets, and by acquiring the existing, available data and assessing their suitability, some of these data may not need to be collected anew. However, following the evaluation of all the existing and available dataset, a decision must be made specifically about whether new pathogen monitoring data must be generated using environmental samples using laboratory analyses. Using the knowledge gather to this point, the decision tree in K02 can be used to make a decision about whether new data collection is required.

Where new data are to be collected, plans need to be outlined to cover both the collection and data management across the entire data lifecycle (Figure 4), from preparation, use and maintenance, to archival and closeout. Adopting a Data Management Planning (DMP) approach is advantageous for any programme collecting and managing data. It helps identify needs, pre-empt challenges, clarify roles and responsibilities, and drive efficiencies. This approach also helps define the requirements to meet FAIR data principles. Increasingly, funders may stipulate a DMP approach, and there are standard templates available to support such activities, including those from the Digital Curation Centre (2024).

The DMP should plan for the completion and publication of discovery metadata to supplement the collected raw data, data products, and outputs. Discovery metadata for collected data should be documented in either the PDIS or listed in a register or data catalogue with links provided. Discovery metadata should

be created early during data collection and maintained and updated by the Data Manager as activities progress. It should be accessible to programme partners and shared during any transfer of the data or data products. Subsequently, it should be archived alongside the data and published openly, even if the data itself may not be.

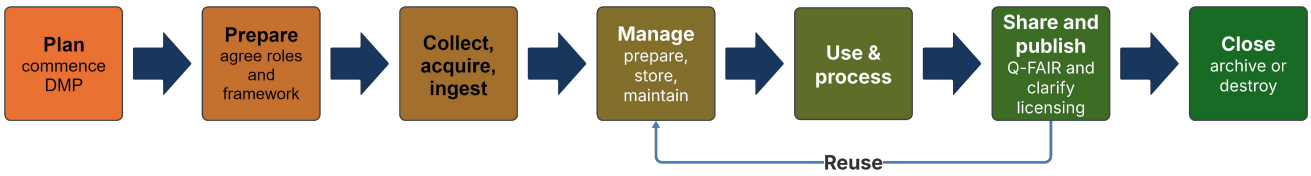


Figure 4: The stages of the data lifecycle adapted from the Government Data Quality Framework (Government Data Quality Hub, 2020).

9.11. Assess sampling requirements (P11)

Using the information gathered to this point, an assessment must be made to define the nature of the samples needed. For this guidance, a sample is defined as a portion of environmental material collected for analysis following the criteria below.

Sample collection type

Determine whether the samples should be sampled as grabs, composites or passive samples:

Grab sampling is where simple samples whereby a defined amount (volume or mass) of sample is taken at a single time point. Grab sampling provides a snapshot of the prevalence of a pathogen at the time of collection. Where the presence of the target is variable, grab samples may not be representative of target prevalence over time.

Composite sampling is where multiple grab samples are taken over a defined time-period and then pooled into a single sample. Composite sampling can be more representative of prevalence over a defined time period compared with grab sampling. However, composite samples can be more technically challenging and resource intensive to collect. This is because they usually require dedicated automated sampling equipment. Due to the reliance on automated equipment, there is also a possibility of equipment failure which could impact the results.

Passive sampling is where samples are taken continuously over a defined period using a sampling device that accumulates the target over time. This can improve the probability of detection especially where the target is not expected to be found at high concentrations. It should be noted that the ability of a passive sampler to accumulate a target over time can change substantially, and so the potential for uncertainties that this introduces must be considered.

Level of replication

In most cases, taking multiple samples from a single location at a defined point in time is not necessary in EBH. There may be instances, however, where taking duplicate or triplicate samples is required to provide a measure of uncertainty. If these samples were pooled prior to further processing they would be considered composite.

Sample amount (volume/mass)

The amount (volume/mass) of sample needed will be dictated by the laboratory analysis protocol and the level of technical replication required in

the laboratory. For sample matrices that are made up of discrete units, a minimum number of units may be needed to reliably reach the required sample mass. For example, in the case of BMS, each sample may require ≥ 50 g of flesh from a minimum of 10 animals.

It is recommended that excess sample is taken where possible to allow for wastage in laboratory processes. However, this must be balanced with the practicalities of transport and waste disposal. For example, if 100 ml of wastewater is required for analysis, it may be useful to collect 150 to 200 ml of sample, whereas a 1000 ml sample will ultimately lead to unnecessary resource expenditure on transport and waste disposal. There is also increased health risk from larger than necessary volumes from and increased likelihood of spillages occurring.

Sample transport requirements

Requirements for transporting the samples to a laboratory will depend on the nature of the material being used for analysis, the target of surveillance and the distance to the testing facility (e.g. laboratory). Most biological materials will need to be kept cool in transit. This is especially true if the target must remain alive (or viable) and transport times are longer than a few hours. Freezing samples in transport is an option that should be considered on a case-by-case basis as it may negatively impact the reliability of results. It is recommended that until the transport protocol is developed, samples should be kept >0°C and <10°C for the duration of transport.

Biological safety

Establishing what the biological safety requirements are for samples is vital to ensure the safety of all those who will handle the samples and to comply with relevant hazardous materials regulations. For some matrices, it may be possible to apply an assumed biological safety level. For example, raw influent wastewater will always be hazardous and by default should be handled as biohazard level 2. Food samples and many other environmental samples will typically be handled as biohazard level 1. However, if there is reason to believe that samples of these matrices contain pathogens from a higher biohazard group, then the handling requirements must be upgraded to match that hazard. For this reason, the hazards associated with sample matrices must be considered on a case-by-case basis and reviewed periodically. If there is doubt over the biological safety of samples, it is recommended the Health and Safety Executive or the Advisory Committee on Dangerous Pathogens are consulted.

A knowledge of the biohazard group that samples will fall under will allow appropriate planning for sample handling and transport. These requirements

can have significant impacts on the skills, facilities and shipping requirements, and therefore the overall cost of a programme.

Availability of suitable samples

Once the sample requirements have been established, determine whether there are samples being taken in another ongoing programme, which can be further analysed for the purposes of this programme. These samples must not only fit the physical sample requirements as discussed above, but they must also be capable of providing data that is relevant to the programme according to the surveillance parameters (P06).

If it is determined that there are samples being collected for ongoing programmes that fit the requirements of the new programme, then it is vital to have discussions with the leads for the existing programme as early as possible. It will need to be determined whether the samples shared and can be transported to the laboratory in a cost effective and timely way. A material transfer agreement (MTA) may be required to establish the acceptable use of the samples. Such agreements can take several weeks to put in place depending on prioritisation of workload and the ability for the organisations involved to come to an agreement.

A decision must then be made whether to use those samples instead of collecting new samples using decision tree K03.

9.12. Engage with stakeholders (P12)

Engage with stakeholders as early as possible once the initial needs of the programme are known. The operation of an EBH programme requires the involvement of a range of stakeholders, each contributing unique expertise and resources to ensure effective implementation and sustainability. The decision made in K03 will affect the stakeholders that will need to be engaged. This is noted below.

Public health officials

Public health officials are crucial stakeholders regardless of the source of any data or samples used. They use surveillance data to inform public health interventions and policies. Their role involves interpreting the data to assess community health risks and to guide responses to emerging pathogens, as demonstrated during the COVID-19 pandemic. Furthermore, public health officials must collaborate with other stakeholders to integrate wastewater surveillance findings with traditional epidemiological data, enhancing the overall understanding of disease dynamics.

Matrix facility operators

If new samples are required, then it is important to engage with facility operators. In the context of wastewater surveillance, facility operators will be responsible for the collection and processing of wastewater samples, which requires specialised knowledge of wastewater management systems. For other matrices, such as soil or air, operators may include agricultural managers or environmental monitoring organisations that can help with the collection of samples from these environments. In the case of BMS-based surveillance, collaborations with shellfish harvesters are also essential, as they can offer practical knowledge regarding harvesting practices and the socio-economic implications of pathogen detection in shellfish. Likewise, in the potential case of using abattoir wastes to monitor animal diseases and AMR, detailed knowledge of the animal processing chain is vital to ensure that samples are collected from the most relevant locations. In both the BMS and abattoir use cases, the use of these matrices may be viewed with some scepticism from the facility operators due to the perceived risk to reputation and income if pathogens are detected. Indeed, previous attempts to develop an abattoir wastewater project within the PATH-SAFE programme, were largely unsuccessful due to the inability to resolve these issues. This collaboration is critical for ensuring that surveillance efforts are relevant to both public health and local economies. The logistical challenges faced by these operators, such as supply chain issues highlight the importance of their involvement in the planning and execution phases of the programme.

Logistics companies

If new samples are required, or if existing samples will be transported to a new laboratory (K04), then logistics companies will need to be consulted. In EBH programmes, logistics companies are essential for effective sample transport. Early collaboration with public health authorities, wastewater treatment facilities, and laboratories is needed to facilitate an understanding of the programme needs and ensure the logistics system supports surveillance objectives. Designing efficient transport routes is crucial for timely sample collection from multiple sites, particularly in large-scale programmes. Minimising delays ensures prompt delivery and sample integrity. In some cases, such as where large numbers of samples are sent in a single consignment, it may be more efficient to use vehicles equipped with temperature control rather than using temperature-controlled packaging fir each sample. Coordination with laboratories is vital. Real-time tracking systems monitor sample location and status, facilitating seamless coordination. Contingency plans for delays or breakdowns minimise disruptions. Adhering to health and safety standards, including biohazard protocols and PPE, is essential when handling hazardous wastewater. Logistics companies should maintain detailed records for regulatory compliance and auditing. Involvement in early planning helps offer advice on sampling site feasibility, define transport timelines, and establish emergency protocols. Determining equipment and storage needs ensures sample integrity. A scalable logistics operation can manage programme growth without compromising quality or speed. Planning for potential expansion from the outset and developing scalable strategies aligned with public health objectives are key.

Laboratory facilities

Laboratory facilities will need to be engaged regardless of whether new samples are required or not. Though the precise methodology may not be defined at this stage, it is crucial to start collaborating with laboratory facilities as early as possible. Laboratories need to evaluate their ability to detect pathogens and pertinent biomarkers in the target matrix. If established methods exist for identifying or quantifying the target pathogens and related markers within the matrix of interest, laboratories will be best positioned to determine the feasibility of these analyses. Additionally, laboratory facilities require time to assess whether they have the resources to manage additional sample testing as needed. They must also address any health and safety concerns related to sample testing and consider how the proposed samples might impact existing activities (e.g., food microbiology laboratories may face challenges working with wastewater samples due to potential cross-contamination). Engaging laboratories early in the planning process will ensure that appropriate levels of quality assurance and control can be established. For

new methods laboratories will require lead-in time to adopt and verify new methods or scale up existing methods. It is advised that the National Laboratory Alliance framework is used when procuring laboratory services whenever possible.

Academic institutions

Academic researchers contribute significantly to the development and refinement of methodologies used in environmental surveillance and so should be included in any EBH programme where new techniques are required that are beyond the capacity of other facilities to develop. Expertise in microbiology, epidemiology, and environmental science is vital for designing studies that accurately assess pathogen prevalence and for developing innovative techniques for simultaneous detection of multiple pathogens. Additionally, it is possible that some academic institutions will already be working on methods for the target of interest, and so any previous research insights will be valuable for further development of the programme.

Collaborations between researchers and public health officials can enhance the effectiveness of surveillance programmes by ensuring that findings are translated into actionable public health strategies.

Competent Authorities

Competent authorities responsible for environmental protection and food safety play a crucial role in establishing guidelines and regulations for environmental monitoring. Their involvement ensures compliance with safety standards and facilitates the integration of surveillance data into public health frameworks in all cases.

9.13. Design sampling programme (P13)

Designing an effective sampling programme requires coordinating with asset owners and sampling contractors to transform the established requirements from previous processes into practical sampling schedules and procedures.

Meet with asset owners to establish site access protocols and identify any operational constraints that might affect sampling. For wastewater facilities, this includes understanding maintenance schedules, flow management operations, and safety requirements specific to each site. For other matrices, work with relevant facility operators to understand their operational patterns and constraints.

Create a detailed sampling schedule that accounts for both the surveillance requirements and operational realities. Map out exactly which sites will be sampled on which days, accounting for travel time between locations, hours of available daylight, site access restrictions, and laboratory processing capacity.

Develop site-specific sampling plans (S07) that detail the exact sampling points (including grid reference), methods, and procedures for each location. These should include photographs or diagrams of sampling points, specific access instructions, and any site-specific safety considerations. Each protocol should also specify the equipment needed for that location, including personal safety equipment.

Establish communication protocols between sampling teams, asset owners, and laboratories. Define how sampling teams will notify facilities of their arrival, how they will report any issues encountered during sampling, and how they will communicate with laboratories about sample delivery.

Create contingency plans for common scenarios that might disrupt sampling, such as site access issues, equipment failures, or extreme weather events. Include backup sampling locations where appropriate and establish clear decision-making procedures for when sampling plans need to be modified.

Develop a master schedule that coordinates sampling activities with laboratory capacity. This schedule should account for sample transport times, laboratory processing windows, and any specific handling requirements that might affect timing.

Contingencies should be developed for scenarios where sampling delays create mismatches between sample delivery and laboratory capacity. This is particularly important for time-sensitive analyses where sample integrity could be compromised by delays. The contingency plans may include:

- Alternative delivery schedules that can be activated when delays occur
- Procedures for notifying laboratories of delayed deliveries or unexpected sample volumes
- Backup laboratory capacity options for managing unexpected sample loads and/or inability of laboratory to process samples due to staff absences (e.g. mass illnesses in the event of an epidemic).
- Decision-making protocols for prioritising samples when capacity is constrained
- Procedures for documenting any deviations from standard sampling schedules
- Communication protocols between sampling teams, logistics providers, and laboratories

10. Operational processes

This section deals with the planning of the practical delivery of an EBH programme, including defining the change protocol (P14), assessing readiness metrics (P15), research and development (R&D, P16) sample collection (P17), sample transport (P18), sample analysis (P19) and data analysis (P20).

10.1. Define change protocol (P14)

10.1.1. Importance of change protocols

Change control is the process through which all requests to change the approved baseline of a programme are captured, evaluated and then approved, rejected or deferred. A change protocol in EBH programmes is essential to enable continued effectiveness, reliability, and alignment with changing needs. EBH programmes use several interconnected processes, and uncoordinated changes in one process may lead to inconsistencies and disruptions to operations. A clear change protocol provides a structured approach to implementing required operational changes, ensuring they are properly integrated across the programme.

Maintaining data integrity is particularly crucial. Modifications to operational methods can introduce variability that compromises the accuracy and comparability of data over time. Whether it is adopting more sensitive laboratory methods or complying with new data management conventions, a properly defined change protocol provides a systematic way to evaluate and integrate these changes while minimising disruption to operations.

Poorly planned changes can lead to delays, errors, or even data loss. By requiring pilot testing, staff training, and phased implementation, a change protocol ensures that updates are rolled out methodically. Clear documentation and communication throughout the change process enhance transparency and accountability, building trust among stakeholders.

Risk mitigation is another critical aspect of a change protocol. Changes can introduce unintended consequences, such as reduced efficiency or increased costs. A structured process ensures thorough risk assessment and enables appropriate mitigations to be used, allowing changes to occur only when their benefits outweigh drawbacks. Additionally, a well-defined change protocol enables a culture of continuous methodological improvement and so allows the programme to adapt to emerging challenges, such as new pathogens or obsoletion of analytical equipment.

A change protocol also helps to optimise the use of resources, prioritising changes with the greatest potential impact. This ensures that investments in new methods, training, or equipment are strategic and cost-effective. A well-defined change protocol ensures that programmes remain robust, adaptable, and capable of delivering reliable data to inform public health decisions.

10.1.2. Defining a change protocol

Below is an example of how an effective change protocol could be developed. However, there are other established principles for managing change within a programme. The choice of which principles to use will depend greatly on the needs of the programme and the experience and preferences of the programme and project managers and the management model under which the programme is managed (e.g. Agile, APM etc.).

- **Establish Governance and Oversight**
  - Assign a dedicated team responsible for overseeing changes, ensuring alignment with programme goals, and approving updates.
- **Develop a Framework for Change Management**
  - Outline the steps for proposing, evaluating, approving, implementing, and reviewing changes.
  - Specify criteria for initiating changes, such as the identification of new research findings, regulatory updates, or operational challenges.
- **Set Criteria for Evaluating Proposed Changes**
  - Develop a standardised checklist or evaluation framework to assess the feasibility, impact, and risks associated with proposed changes.
  - Include considerations such as technical performance, resource requirements, regulatory implications, and potential risks to data integrity or operational reliability.
- **Incorporate Pilot Testing and Validation**
  - Require all proposed changes to undergo pilot testing in a controlled environment before full implementation.
  - Define validation procedures to confirm that changes meet predefined performance standards and align with programme objectives.
- **Create a Communication and Training Plan**
  - Develop clear protocols for communicating proposed and approved changes to all stakeholders, ensuring transparency.
  - Provide training for staff to ensure they understand and can implement updated processes effectively.
- **Establish Monitoring and Feedback Mechanisms**
  - Implement systems to monitor the performance of updated processes and collect feedback from staff and stakeholders.

    - Use this feedback to identify areas for improvement and make iterative adjustments to the protocol.
- **Document and Maintain the Protocol (S04)**
    - Create detailed documentation of the change protocol, including procedures, evaluation criteria, and governance structures.
    - Regularly review and update the protocol to reflect new insights, technological advancements, or regulatory requirements.

10.2. Metrics for assessing readiness (P15)

10.2.1. Introduction to assessing readiness

Each operational process requires one or more methods that routinely produce tangible outcomes (e.g. physical samples or data). Each of these processes should follow robust and validated protocols for which the performance limitations are known. This means that there may be a requirement for research and development (R&D) for these processes. To facilitate the decisions of whether R&D is required, and which elements of R&D should be prioritised, we propose the use of Method Readiness Levels (MRL) and Operational Readiness Indices (ORI). However, it should be noted that organisations may have established systems for assessing the maturity and readiness of an operational programme. In this case, programmes may prefer to use these established systems.

10.2.2. Method Readiness Level

MRL is a scale developed for the purposes of this guidance, by which the readiness of a method can be assessed and is based loosely on the existing frameworks for Technology Readiness Levels (Mankins, 1995). This scale should be used to assess how mature methods are for EBH and so help to enable the choice of methods to use and where resources should be focussed for further development.

It should be noted that while the MRL scale outlines an ideal series of stages of method development, some methods may already be in use for which the MRL is uncertain. For example, a method must not be assumed to be MRL 8 just because it is in use; if it has not been robustly validated it is likely to be at an actual MRL of between 3 and 5.

The MRL should be assessed for each of the proposed sample collection, sample transport, sample analysis and data analysis methods for use in calculating the ORI.

MRL 0: Conceptualisation

Basic ideas or theoretical designs exist but lack any empirical evidence.

MRL 1: Early Feasibility Studies

Preliminary tests show that the method or system is plausible under idealised conditions.

MRL 2: Method Design and Development

Initial protocols are drafted, and basic functionality is tested.

MRL 3: Analytical Feasibility

Methods are tested under controlled conditions to assess performance metrics (e.g., reliability, robustness).

MRL 4: Optimisation in Environmental Matrices

The method is adapted to address real-world variables, such as environmental inhibitors or logistical challenges.

MRL 5: Single-Site Validation

The method is validated at a single site under operational conditions.

MRL 6: Multi-Site Validation

Methods are independently tested and validated across multiple sites, ensuring reproducibility.

MRL 7: Integration Readiness

The method is ready to be integrated with other stages (e.g., combining sample transport and analysis with downstream data processing or analysed data can be integrated with public health systems).

MRL 8: Routine Implementation

Methods are used routinely with ongoing monitoring and performance evaluation.

MRL 9: Standardisation and Global Adoption

The method is internationally recognised and widely adopted as a standard.

10.2.3. Operational Readiness Index

For the purposes of this guidance, a means to assess readiness was developed. This is termed the Operational Readiness Index (ORI). The ORI is a weighted average of the MRLs for Sample Collection (cMRL), Sample Transport (tMRL), Sample Analysis (aMRL), and Data Analysis (dMRL). It is used to evaluate the readiness of a surveillance operational programme to highlight any bottlenecks that may need to be addressed by further R&D. It recognises that the processes in biosurveillance are sequential (i.e. a sample cannot be transported if it has not been collected and cannot be analysed in a laboratory if it has not been transported) and that some of the processes require more effort and time to develop than others.

To calculate the ORI (Equation 1), all four MRLs must be determined. Weights must also be determined for at least two factors, importance and effort:

1) Importance weight: The need for the process to be defined before another can be developed. By default, it is suggested to use the following weights for this factor:
   cMRL: 0.4
   tMRL: 0.3
   aMRL: 0.2
   dMRL: 0.1
2) Effort weight: The relative effort required to develop the process. Processes that require high effort to develop will require higher effort weighting.

Weights must be a value between 0 and 1 and the sum of all for weights for each factor must be equal to 1. If required, additional weighting factors can be included, but they must also have sums equalling 1.

Examples of importance weight adjustments

- For near-source surveillance (e.g., building level monitoring), sample collection might need higher weighting due to increased importance of precise sampling locations
- When using well-established collection and transport methods but novel analytical techniques, the analysis weight might be increased to reflect its critical role
- For programmes focusing on trend analysis, data analysis weight might be increased due to the importance of robust statistical approaches

Examples of effort weight adjustments

- When developing new sampling methods for difficult matrices (e.g., sampling from deep sewers), collection effort weight might be increased.
- If complex preservation methods are needed during transport, transport effort weight could be increased.
- When using established analytical methods that require minimal optimization, analysis effort weight might be decreased.

Equation 1: Operational Readiness Index

$$O = \sum_{i=1}^{4} \frac{M_i \sum_{i=1}^{n} W_i}{n}$$

where:

- O is ORI.
- M_i is the MRL of process i.
- W_i is the importance weight of process i.
- n is the number of weighting factors.

Worked example of ORI calculation

In this example, an EBH programme is proposed whereby a new sample analysis method is to be used for an existing programme. The sample collection and transport methods are internationally recognised, and the data analysis method has been validated for other target pathogens previously. The new sample analysis method has been shown to be effective in initial laboratory trials and has been optimised for use with real-world samples. No formal validation work has been carried out on the new method.

Table 7: Example metrics for calculating the ORI of a hypothetical EBH programme.

Process	MRL	Importance weight (Σ=1)	Effort weight (Σ=1)
Sample collection	9	0.4	0.1
Sample transport	8	0.3	0.1
Sample analysis	4	0.2	0.6
Data analysis	6	0.1	0.2

For each process, the sum of weights is calculated as:

- Sample collection: 0.4+0.1 = 0.5
- Sample transport: 0.3+0.1 = 0.4
- Sample analysis: 0.2+0.6 = 0.8
- Data analysis: 0.1+0.2 = 0.3

These are multiplied by the MRL for each process and divided by 2 (two weighting factors used):

- Sample collection: (0.5 x 9) / 2 = 2.25
- Sample transport: (0.4 x 8) / 2 = 1.6
- Sample analysis: (0.8 x 4) / 2 = 1.6
- Data analysis: (0.3 x 6) / 2 = 0.9

The sum of these values is then calculated:

- O = 2.25 +1.6 + 1.6 + 0.9
- O = 6.35

Interpretation of ORI

The ORI should be interpreted holistically, considering both the aggregate score and the individual MRLs that contribute to it. The following broad interpretation framework is recommended:

Low Readiness (0-3) Significant development is needed before operational deployment. Multiple processes likely require substantial improvement, particularly those with high importance or effort weightings. The programme may function in limited scenarios but lacks the reliability needed for routine surveillance operations.

Moderate Readiness (3-6) The programme can operate with limitations, and continued development is required in specific areas. While some processes may be well-developed, others likely need refinement to ensure consistent performance. Review the individual MRLs to identify which components are limiting overall readiness and prioritise these for further development.

High Readiness (6-9) The programme is generally ready for full implementation with ongoing refinement. Most processes are sufficiently reliable for routine operations, but specific improvements may still enhance performance. At the upper end of this range, the programme approaches optimal readiness across all processes.

Pattern Recognition in MRL Profiles

When interpreting the ORI, look for specific patterns in the MRL distribution that provide deeper insights into programme readiness:

Balanced Profile: Similar MRLs across all processes indicate consistent development but may mask the need for specialised expertise in certain areas.

Front-End Weighted: High MRLs in sample collection and transport but lower in analysis and data processing suggest field operations are more mature than laboratory capabilities.

Back-End Weighted: Advanced data analysis and laboratory methods paired with less developed sample collection processes indicate a programme that may produce high-quality results but struggle with sample acquisition.

Critical Path Limitations: Low MRLs in processes with high importance weights represent bottlenecks that may severely hinder the entire programme regardless of strengths elsewhere.

It is essential to recognise that two programmes with identical ORI scores may have very different operational profiles. For example, one programme might have moderate readiness across all processes, while another might have highly developed sample collection methods but significant limitations in sample analysis. Always examine the individual MRLs alongside the ORI to gain a complete understanding of programme readiness and to identify specific areas for targeted improvement.

For planning purposes, consider both the importance and effort weightings when prioritising development activities. Processes with high importance weights that show low MRLs should typically receive priority, as they represent critical bottlenecks in the surveillance system.

10.3. Undertake research & development (P16)

Initiating R&D Activities

The decision to begin R&D activities should be based on the MRL and ORI calculations and change protocol requirements outlined in P14 and P15 as outline in K05. Once the need for R&D has been established, the technical lead should develop a detailed project plan that includes specific objectives, resource requirements, timelines, and quality control measures.

When planning R&D activities, consideration should be given to whether the work can be conducted within existing programme resources or whether external support is needed. External support might include academic partnerships, government agencies, specialist laboratories, or consultancy services. The decision should be based on factors including available expertise, equipment requirements, time constraints, and cost implications.

Quality Assurance in R&D

All R&D activities must maintain appropriate quality standards. While research work may not initially fall under formal quality management systems like ISO/IEC 17025, the Joint Code of Practice for Research (Defra, 2015) should still be followed. This includes:

- **Method Development Documentation:** Comprehensive records must be maintained throughout the R&D process. These should include experimental designs, raw data, analysis methods, and results, including negative outcomes. Documentation should be sufficiently detailed to allow work to be reproduced by other competent scientists.
- **Validation Requirements:** Method validation work should follow relevant international standards where they exist. For novel applications where no specific standards exist, validation should follow the principles outlined in the most relevant available guidance. The technical lead should document the rationale for choosing specific validation approaches.

Staff resources

Staff assigned to R&D should have appropriate technical expertise and sufficient time to dedicate to the work. It is important that routine surveillance activities are not compromised by R&D commitments. Where necessary, temporary staff may need to be recruited to maintain programme capacity.

Equipment and Material

Any specialist equipment or materials required for R&D should be identified during the planning phase. Consideration should be given to whether items can be borrowed or shared with partner organisations to reduce costs. Where purchases are necessary, appropriate procurement procedures should be followed. For government led surveillance programmes, where expensive equipment that is classified as capital is required, long-term plans must be in place to ensure that capital depreciation costs can be recovered for the lifetime of the equipment.

Progress Monitoring and Review

Regular reviews of progress against R&D objectives should be carried out at predetermined intervals. These reviews should assess technical progress, resource use, and alignment with programme needs. Plans should be adjusted if necessary following reviews to ensure efficient use of resources.

Reporting Requirements

R&D activities should be regularly reported using the format outlined in S10, with the frequency determined by programme management requirements.

10.4. Sample collection (P17)

10.4.1. Standardisation and key considerations

While no comprehensive international standards currently exist specifically for EBH sampling, several existing standards can be adapted for specific matrices. The ISO 5667 series provides guidance for water and sediment sampling, while the ISO 18400 series covers soil sampling, and the ISO 16000 series addresses air sampling.

Where the programme aims to detect pathogens present at very low concentrations, and where rapid degradation can occur, particular attention to several critical factors is needed: The sample volumes required for detection, prevention of cross-contamination, temperature control, preservation methods, and time constraints between collection and analysis.

All sampling programmes should adhere to the following core principles:

- **Protocol Adherence:** All sampling activities must follow established, documented procedures to provide the foundation for consistent, reliable sample collection.
- **Documentation Requirements:** Comprehensive records must be maintained at every step, including field notes, chain of custody forms, and quality control data.
- **Quality Assurance:** Systematic quality checks must be implemented throughout the collection process, from equipment preparation through sample handling.
- **Safety Considerations:** Appropriate safety measures must be implemented based on site-specific hazards and sample characteristics.

10.4.2. Factors affecting sample collection

- **Sample Matrix Considerations:** Different environmental matrices present unique challenges that must be carefully considered when developing sampling protocols. Each matrix type requires specific approaches and considerations to ensure effective sample collection.
    - **Wastewater** sampling requires attention to access point safety, particularly regarding confined spaces. Flow variations can significantly impact sample representativeness, while solids content may affect sample processing requirements. Both biological and chemical hazards must be managed through appropriate safety measures. Sample temperature is crucial for sample preservation and so samples such as composites should be kept at an appropriate temperature to minimise degradation during the sampling period.

    - **Surface water** sampling presents challenges related to flow rate variations and depth requirements that may change seasonally and in response to recent raifnall. Access challenges often require specialised equipment or approaches, while turbidity variations and background contamination can impact sample quality and processing requirements. Seasonal changes in water bodies may require different sampling strategies throughout the year.
    - **Air** sampling must account for both indoor and outdoor conditions, with particular attention to particle size distribution and flow rates. Temperature and humidity can significantly impact collection efficiency, while collection duration and equipment placement must be optimised for representative sampling. Indoor air sampling may require consideration of ventilation systems and building occupancy patterns.
    - **Soil and sediment** sampling requires consideration of depth profiles and moisture content variations. Particle size distribution and organic content can affect both sampling methods and subsequent analysis, while contamination distribution may require specific sampling patterns. There may also be considerable heterogeneity in target distribution within soils and sediments. Seasonal variations can impact both access and sample characteristics, particularly in areas with extreme weather conditions.
- **Site Access and Safety:** Site access and safety considerations form a fundamental component of sampling operations. Permit and license requirements must be in place before sampling begins, with particular attention to confined space procedures where applicable. Personal protective equipment (PPE) requirements should be clearly defined based on site- and sample-specific hazards. Emergency procedures must be established and communicated to all team members, supported by robust communication protocols. Weather considerations can significantly impact both safety and sample collection, requiring flexible planning and clear criteria for postponing sampling activities.
- **Equipment Requirements:** Equipment selection and management requires consideration of matrix compatibility and decontamination capabilities. Transport considerations must account for equipment size, weight, and fragility, while backup equipment needs should be evaluated based on critical failure points. Regular calibration and maintenance schedules should be established and documented to ensure reliable operation. Each piece of equipment should be evaluated for its suitability to the specific sampling environment and analytical requirements.
- **Personnel Considerations:** Successful sampling operations rely heavily on properly trained and qualified personnel. Training requirements must be clearly defined and documented, including both technical competencies and safety certifications. Physical capabilities should be

matched to sampling tasks, particularly for challenging environments or heavy equipment operation. Time constraints must be realistically assessed to prevent rushed sampling or documentation. Team composition should be planned to ensure appropriate supervision and support for all sampling activities.

10.4.3. Quality control and assurance

- **Documentation Requirements:** Quality assurance in sample collection relies on comprehensive documentation at every stage. Standard Operating Procedures (SOPs) form the foundation of this documentation, providing detailed guidance for all aspects of the sampling process. These procedures should include step-by-step collection methods, equipment preparation protocols, safety procedures, quality control measures, documentation requirements, and contingency procedures for potential issues that may arise during sampling.
  Field documentation serves as both a legal record and a quality control measure. Chain of custody forms track sample handling from collection through analysis, while field logs capture environmental conditions and sampling observations. Equipment maintenance records ensure proper function and calibration status, while sample tracking forms document preservation and transport conditions. Incident reports should be maintained to record any deviations from standard procedures or unexpected events that could impact sample quality.
- **Training Requirements:** A robust training programme ensures consistent and high-quality sample collection across all team members. Initial training should thoroughly cover SOP familiarisation, equipment operation, safety procedures, documentation requirements, quality control measures, and emergency procedures. This foundation should be supplemented by ongoing training including refresher courses, updates on new procedures, and regular competency assessments. Best practice sharing and lessons learned sessions help maintain and improve sampling quality over time.
- **Field Quality Controls:** Field quality control measures provide essential validation of sampling procedures and sample integrity. The use of control samples including field blanks, equipment blanks, and duplicate samples should be considered. Temperature monitoring throughout the sampling and transport process ensures maintenance of appropriate preservation conditions.
  Equipment controls form another crucial aspect of quality assurance. Pre-use checks verify proper function and cleanliness, while calibration verification ensures accurate measurements. Cleaning verification confirms decontamination effectiveness, and maintenance checks help prevent equipment failure. Performance monitoring throughout sampling events allows early detection of potential issues, while post-use checks help maintain equipment reliability.

10.4.4. Generic collection workflows

The sample collection process follows a systematic workflow that ensures consistency and quality across sampling events. While specific procedures vary based on matrix and analytical requirements, the core workflow consists of five key stages.

- **Pre-sampling Preparation:** Pre-sampling preparation begins with a thorough review of site-specific requirements and safety considerations. Equipment preparation includes cleaning, calibration verification, and function testing of all required tools and instruments. Documentation must be prepared in advance, including chain of custody forms, field logs, and site-specific procedures. To improve efficiency, electronic versions of these forms can aid efficient input of this information into the PDIS. Weather conditions should be reviewed to ensure they meet sampling requirements, and coordination with site contacts should be completed to ensure access and support. The sampling team should receive a comprehensive briefing covering safety requirements, sampling objectives, and specific procedural considerations.
- **On-site Assessment:** Upon arrival at the sampling location, the team must first verify site safety conditions and confirm the specific sampling points match the sampling plan. Environmental conditions should be documented, including any unusual circumstances that might affect sample quality. When setting up equipment, particular attention should be paid to preventing cross-contamination. Area security should be established where necessary, and communication systems tested to ensure team safety and coordination.
- **Sample Collection:** Sample collection begins with a final safety check and equipment preparation. Field measurements should be recorded immediately, and samples labelled. Quality control samples (such as field blanks) should be collected at predetermined intervals and properly documented.
- **Sample Handling:** Proper sample handling immediately after collection is crucial for maintaining sample integrity. Preservation methods must be implemented according to analytical requirements, with careful attention to temperature control and storage in dark conditions where needed. Contamination or leakage must be prevented by ensuring the samples are properly sealed. Documentation must be completed accurately and promptly, with particular attention to chain of custody requirements. Storage conditions should be prepared in advance to maintain sample integrity until transport.
- **Post-collection Procedures:** After sampling is complete, all equipment must be cleaned. Documentation should be finalised while still on site when possible, including any deviations from standard procedures. Transport arrangements should be confirmed to ensure samples will reach the laboratory within required timeframes. The laboratory should

be notified of incoming samples, particularly for time-sensitive analyses. A team debrief helps identify any issues or improvements needed for future sampling events. Any suggested improvements should be reported to the Technical Lead to allow for appropriate adjustments to subsequent transport operations according to the Change Protocol (P14).

10.4.5. Balancing critical parameters

The success of EBH sampling requires a balance of several critical parameters, each presenting trade-offs that must be considered in sampling design.

- **Speed versus Quality:** Sampling speed must be balanced against quality requirements, particularly in emergency response situations. While rapid collection may be necessary in some scenarios, it can compromise sample representativeness through rushed procedures or insufficient quality controls. Reduced sample volumes might be necessary for faster processing but could impact detection limits. Time pressure can also affect documentation quality, potentially compromising data reliability. Fast approaches may limit quality control measures, while rapid transport options often increase costs significantly.
- **Cost versus Comprehensiveness:** Sampling costs must be evaluated against programme requirements for comprehensiveness. Automated equipment can reduce labour costs over time but requires significant initial investment and ongoing maintenance. Quality control measures add expense but are essential for data reliability. Training programmes increase short-term costs but improve long-term efficiency and data quality.
- **Repeatability versus Flexibility:** Standardised methods improve consistency across sampling events but may limit adaptability to changing conditions. While rigid protocols ensure reproducibility, they can make it difficult to respond to unexpected situations or emerging requirements. Documentation improves repeatability but adds time and complexity to sampling operations. Training enhances consistency but must be balanced against the need for adaptive responses to field conditions.

10.4.6. Example collection methods

- **Wastewater Grab Sampling from a Manhole:** This method requires consideration of both safety and sample quality requirements and often needs a minimum of two personnel for safe execution. Sample collection should account for flow conditions and depth to ensure representative sampling. The sampling pole length must be appropriate for the specific manhole depth, and all equipment must be properly decontaminated between sampling sites.

- **Auto-sampler Collection from Wastewater Treatment Works:** Automated samplers require initial setup and regular maintenance to ensure reliable operation. Flow-proportional sampling may be achieved through integration with treatment works flow meters if available, providing more representative sampling than time-based collection. Temperature control and sample preservation must be managed, particularly in extreme weather conditions.
- **Surface Water Sampling Using Pole Sampler:** Surface water sampling requires consideration of sampling depth and location to ensure representative samples. Flow conditions must be documented and considered in sample collection strategy. Cross-contamination prevention is particularly important when sampling multiple sites, requiring decontamination procedures between sites.
- **Sediment Sampling Using Grab Sampler:** Sediment sampling requires careful positioning of the vessel and precise deployment of a grab sampler to ensure consistent sample collection depth and minimal disturbance of the sediment structure. Sample integrity must be assessed upon retrieval, with attention to potential loss of fine materials or surface layers. The selection of appropriate grab sampler type (e.g., Van Veen, Ekman, Ponar) depends on sediment characteristics and water depth.
- **Indoor Air Sampling Using Bioaerosol Samplers:** Indoor air sampling requires consideration of room layout, ventilation patterns, and occupancy patterns. Sampler placement should account for air flow patterns and potential source locations. Collection duration must balance detection sensitivity with practical constraints while maintaining sample integrity.
- **Bivalve Sentinel Organism Collection:** Bivalve collection timing must consider tidal cycles and seasonal variations in organism availability. Size selection criteria should be standardised to ensure comparable results across sampling events. Rapid cooling and appropriate preservation are important for maintaining sample integrity.

10.4.7. Sample collection research and development

In many cases, some degree of research and development for sample collection methods will be required. The nature and extent of this will depend on the MRL of existing methods and the specific requirements of the programme.

For established environmental matrices such as wastewater or surface water, standardised collection methods may already exist that can be adapted for EBH purposes. However, novel scenarios such as unusual environmental matrices may require development of new collection approaches, including new equipment and methodologies. Even when using established methods,

validation may be needed to confirm their suitability for specific EBH applications.

In an ideal situation, sample collection methods should be adopted for national surveillance programmes no earlier than MRL 6 or 7, at which stage the method has been fully validated. However, in situations where no validated methods exist (such as during emergency response to a novel pathogen), use of methods at a lower MRL may be necessary. In this case, the limitations of the method performance must be documented, and a plan should be put in place for further research and development if required.

Method validation should consider practical field conditions rather than just laboratory-based assessment. Field validation helps identify operational constraints and practical limitations that may not be apparent in controlled environments. This should include assessment of method robustness across different environmental conditions and evaluation of potential interference from matrix-specific factors.

Where possible, comparative studies between different collection methods should be conducted to understand the relative advantages and limitations of each approach. This information supports evidence-based selection of methods for specific surveillance scenarios and helps identify areas where further development may be beneficial.

10.5. Sample transport (P18)

10.5.1. Standardisation and practical considerations

While specific standards for EBH sample transport do not exist, several relevant standards can be adapted, including ISO 20387 for biobanking and ISO 17025 for laboratory competence. Transport of biological materials must comply with relevant dangerous goods regulations, particularly UN3373 Category B requirements for most environmental samples.

Sample transport procedures should be documented comprehensively (S06), consistently applied, regularly reviewed, and supported by appropriate training. Key considerations include maintaining sample integrity, preventing cross-contamination, ensuring proper temperature control, and meeting regulatory requirements while optimising logistics efficiency.

10.5.2. Factors affecting sample transport

- **Sample Stability Considerations:** Sample stability during transport varies by matrix type and target analytes. Environmental samples present unique challenges due to their complex composition and varying degradation rates. Temperature requirements range from ambient to frozen, depending on the analytes of interest. Maximum transport times must be based on the least stable target component, which may be less than 8 hours in some cases. Some matrices may require immediate preservation steps or immediate removal from light exposure at the sampling site. Understanding these stability factors is essential for designing appropriate transport protocols that maintain sample integrity.
- **Regulatory Requirements:** Most samples fall under UN3373 Category B classification, requiring specific packaging, labelling, and documentation. International transport involves additional customs requirements. Transport providers must be certified for biological materials, and staff must receive appropriate training.
- **Logistical Constraints:** Moving samples from collection points to laboratories requires balancing multiple practical considerations. Geographic distribution affects route planning and transport efficiency. For programmes covering large geographical areas, using an existing network of regional laboratories can significantly optimise transport logistics. This distributed laboratory approach allows samples to be transported to the nearest facility, reducing transport times and costs while maintaining sample integrity. However, this must be balanced with any potential increases in resource needs such as purchasing new equipment, and additional training. Available transport options may be limited by location (e.g. remote locations may be difficult to access in a timely manner), time constraints, or service availability. Factors such as

increased seasonal traffic may also cause unexpected delays to transport. Access restrictions and weather conditions can impact scheduling, particularly outside normal working hours.

- **Equipment and Materials:** Effective sample transport relies on appropriate equipment and materials throughout the process. Temperature control options range from passive systems with ice packs to active cooling with monitoring capabilities. Packaging must meet UN specifications for biological substances. Documentation ensures chain of custody and regulatory compliance. Regular quality checks and backup supplies are essential for critical components. All materials should be validated for their intended use and storage conditions.

10.5.3. Quality control and assurance

Quality control and assurance in sample transport integrates documentation, training, and monitoring systems to maintain sample integrity throughout the transport chain.

Documentation provides the evidence base for transport quality. Chain of custody forms track sample transfers, while temperature logs and incident reports capture environmental conditions and deviations. Transport providers must maintain current certifications demonstrating their capability to handle biological materials. SOPs standardise all transport processes and guide documentation requirements.

Training ensures personnel competency in sample handling, packaging, documentation, and emergency response. Staff must demonstrate proficiency in using preservation materials and monitoring equipment while maintaining regulatory compliance.

Consider using transport controls to verify process effectiveness. Temperature logging and time tracking confirm preservation conditions are maintained within stability limits. Package integrity checks at transfer points help to identify potential issues before they impact sample quality. These controls provide real-time verification of transport system performance while enabling continuous process improvement. Recommendations for process improvements should be fed back to the technical lead for consideration in accordance with the Change Protocol (P14).

10.5.4. Generic transport workflows

- **Pre-transport Preparation:** All packaging materials must be verified to meet regulatory requirements while preservation materials and monitoring equipment are prepared according to sample specifications. Required documentation is completed and transport providers are

coordinated with to ensure proper handling. Personnel involved in the transport chain receive briefings on specific requirements for the shipment, including any special handling or monitoring needs.

- **Sample Handoff:** Each sample's labelling and packaging is verified against requirements, with chain of custody documentation completed to record the transfer. Temperature monitoring devices are activated and configured according to preservation requirements. Transport personnel receive briefings on handling requirements and delivery timelines, with clear communication of any special considerations.
- **Laboratory Receipt:** Laboratory staff check package integrity on receipt of the shipment. Sample receipt documentation captures final condition and any notable observations. Any issues are reported to the Technical Lead to allow for appropriate adjustments to subsequent transport operations according to the Change Protocol (P14). Sample transfer to appropriate storage conditions completes the transport chain.

10.5.5. Transport research and development

Research and development in sample transport typically focuses on improving preservation methods, monitoring systems, and logistics efficiency. Examples of ongoing development include:

- New preservation technologies that extend sample stability
- Smart monitoring systems for real-time condition tracking
- Sustainable packaging solutions
- Optimisation algorithms for transport routing
- Integration of transport data with LIMS

Transport method selection should consider the MRL of available options. While established methods are be preferred, novel approaches may be necessary for emerging challenges or specific programme requirements. Any limitations in method performance should be documented, and plans for further development should be established where needed.

10.6. Sample analysis (P19)

10.6.1. Standardisation and accreditation

Once the samples have been received by the laboratory, they must be analysed using methods capable of generating data that conform to the requirements of the data and quality needs (P08). At the time of writing, there were no standards at the international (ISO), European (CEN) or national (BSI) levels for methods specifically used for EBH. However, it is possible that other nations or national organisations have developed relevant methods that could be adopted. At the time of writing, the first version of ISO 7014 (General requirements for the determination of SARS-CoV-2 and its variants in wastewater) is under development and will be the first ISO standard published that is specifically intended to be used for EBH. However, there may be existing standardised methods that can be adapted to be of use for EBH.

Regardless of the method used, in most circumstances, laboratories should work according to the requirements of ISO/IEC 17025 (General requirements for the competence of testing and calibration laboratories). Where possible, laboratories in the UK should also be accredited by United Kingdom Accreditation Service (UKAS) to carry out the sample analysis for the target analyte. This will ensure that the data generated can be relied upon to support policy decisions and to inform public health actions. However, where there is an emerging or unknown threat, it is likely that no laboratories will be accredited for the analysis, and new methods may be required. In this case, it is still important that laboratories put in place quality control and assurance measures and conform as closely as possible to ISO/IEC 17025. If surveillance is to continue long-term, then accreditation should be sought as soon as possible.

10.6.2. Factors affecting choice of methods

The nature of the analyses that will be undertaken will depend largely on the following factors. These factors are not listed in order of priority, because the variable priorities of individual programmes preclude this.

- **Sample matrix:** The type of sample will have a major impact on which analytical techniques are chosen. For example, methods that are appropriate for wastewater, may not be appropriate for river water. The nature of the sampling matrix may also affect the required containment level (CL) for laboratories. For example, wastewater should always be handled in at least a CL2 laboratory, whereas river water may be handled in a CL1 laboratory assuming the samples are not known or likely to contain live pathogens. Biological safety requirements for sample

handling must be assessed locally and in line with relevant Health and Safety Executive (HSE) guidance.

- **Analytical target:** Some methods that are appropriate for capturing bacterial or other cellular organisms may not be suitable for capturing viral targets and vice versa. The specific targets may also affect the fraction of a sample analysed. For example, some viruses are associated more with suspended solids in wastewater, while others are associated more with the liquid fraction. It is therefore important to ensure that the appropriate fraction of the sample is analysed. Additionally, the biological hazard group of the target organism(s) will determine some of the requirements for sample handling. Biological safety requirements for sample handling must be assessed locally and in line with relevant Health and Safety Executive (HSE) guidance.
- **Participant laboratories:** If multiple laboratories will be included within a programme, it is important to ensure that the methods used are standardised across those laboratories by developing generic protocols and carrying out interlaboratory trials. It is therefore important to ensure that the methods selected can be applied in a reproducible way across multiple sites. This may exclude the use of some specialist equipment.
- **Expected analyte concentrations:** The expected concentration of the target analyte(s) will affect how much sample must be analysed (e.g. the volume) to ensure that the method can achieve the required detection and/or quantification limits. In many cases, if a large amount of sample must be analysed to detect an analyte, the method will need to include some form of target concentration. It should be noted that in some cases, intentional concentration of pathogens will impact the CL required for laboratories.
- **Matrix interference:** Environmental samples by their nature are typically highly variable and often contain substances that can interfere with analytical processes. The methods chosen for analysis must therefore ensure that this is considered and controlled where necessary to minimise uncertainty in data.
- **Data type:** The type of data (i.e. quantitative, semi-quantitative, qualitative) that is required from each sample may affect the way in which samples are processed and data are collected. For example, quantitative results may demand a greater degree of precision over qualitative results. The choice of data collection method such as conventional PCR vs. quantitative PCR may also be impacted by required data types.
- **Method readiness level:** The degree to which a method has been developed and validated should play an important role in the selection of analytical methods. Typically, cutting edge techniques that do not have a substantial evidence base supporting them will have a high degree of uncertainty. Fully validated and standardised methods should be used

wherever possible. However, this may not always be possible, especially in the case of emerging threats.

- **Throughput:** Depending on the number of samples that must be processed, and targets analysed on a given day by a single laboratory, it may be necessary to make use of automated or semi-automated systems to increase sample throughput. Some methods are more suited to high throughput techniques than others.
- **Time constraints:** The timeframe in which the results must be available will have an impact on the analytical methods chosen and the operation of the laboratory. For example, if results are required on the same day that samples are received, the methods chosen must be able to reliably and consistently achieve that.
- **Availability of materials:** Some methods rely on materials (such as laboratory consumables) that are commonly available, while others may use materials that are exclusively supplied by a single source. Methods that rely either on a large volume of consumables or on a single source/supplier of materials have an inherent risk associated with them if there are supply shortages. This was seen during the COVID-19 pandemic, where shortages of plasticware and reagents had negative impacts on the ability of laboratories to analyse samples.
- **Availability of expertise:** Most laboratory analysis requires a degree of specialist knowledge. The ability to use novel or specialist techniques may be limited by a lack of available expertise. This is not only restricted to experts working within the testing laboratories, but also includes support resources such as manufacturer technical support and data analysis.
- **Environmental impact:** Analytical methods typically use a range of different consumable materials which contribute to the overall impact that the methods have on the environment. For example, some methods rely heavily on disposable plastics. Other, methods may use substances that are detrimental if released into the environment. Additionally, methods relying on consumables that are shipped in relatively small quantities by air may have a greater carbon footprint than those shipped in bulk by land or sea.
- **Ethical considerations:** Ethical factors should be considered when determining which methods to use. Working with suppliers that use exploitative working conditions should be avoided to minimise the contribution of the programme to modern slavery. Other ethical considerations include, but are not limited to, the use of animal-based assays (e.g. mouse bioassay), and the unnecessary collection of sensitive data.
- **Cost:** The key contributors to the cost of laboratory analysis are staff time, consumables, facility maintenance and other overheads. In the UK, staff time is often (but not always) the greatest cost associated with laboratory analyses. Therefore, methods that minimise the time spent by

analysts at the bench will often cost less per sample than methods that use expensive consumables but require less bench time.

It is not possible to outline every scenario within this document for how samples should be analysed. Indeed, there are often several valid methodologies for any given scenario.

10.6.3. Note on dilution, flow and population normalisation

One of the key differences between EBH surveillance and other types of surveillance, such as those for food safety pathogens or environmental pollution, is the requirement to normalise the data to the affected population (in the case of quantitative data). This is necessary because while the absolute concentration of a pathogen in a food sample or in bathing waters is directly relevant to safety, the absolute concentration of a pathogen in a matrix such as wastewater may not be directly indicative of the disease burden in a community. Factors such as the dilution of sewage by surface water infiltration or changing population sizes, may greatly alter the correlation between pathogen concentrations in an environmental sample and the level of disease in a community. For this reason, it is recommended that in addition to analysing target pathogens in samples for EBH, at least one (but preferably two) relevant normalisation factors are also measured. The specifics of approaches to use these normalisation factors are discussed in Data analysis (P20). However, these variables must be measurable and represent changes in the factor that needs to be normalised. Examples used for normalising for populations or flow in wastewater surveillance include faecal indicator organisms (such as *Escherichia coli*, pepper mild mottle virus and crAssphage), nutrients (such as ammonium and orthophosphate) and other anthropogenic chemicals (such as caffeine, and sertraline). The laboratory that is carrying out the analysis of the samples for EBH must be able to measure the analytes used for normalisation. It cannot be assumed that laboratories equipped to carry out molecular biological analyses are able to carry out chemical assays. It therefore may not be reasonable to select a pharmaceutical indicator for normalisation when the primary target is viral.

10.6.4. Generic analysis workflows

While the specifics of sample analysis procedures vary greatly, the stages of a generic workflow are relatively consistent:

- **Sample receipt:** When samples are received into the laboratory, they must be booked into the laboratory's records in a consistent and traceable way. Additionally, at this stage, it must be decided whether a sample should be tested or rejected. It is therefore vital to ensure that

sample rejection criteria are established as part of the analytical protocol. Some of these factors will be controlled for in the sample transport protocols. Examples of rejection criteria include:

  - Samples arriving later than the maximum time since sampling.
  - The sample temperature being outside of the acceptable range.
  - Samples unlabelled or missing identification information
  - No paperwork or other forms of metadata being supplied.
  - Damaged sample containers.
  - Unsafe packaging used (e.g. wastewater not shipped according to the requirements for Biological B material).

- **Sample inactivation:** In the case of samples that carry a high level of risk (such as wastewater), some laboratory facilities may require that pathogens within the samples are inactivated prior to sample handling. This is typically done using heat, because this can be applied without opening the sample vessel and has the least impact on downstream analysis. It should be noted however that heat inactivation can add a significant amount of time to overall sample analysis due to the time required to heat and then cool the sample prior to analysis. Sample inactivation also precludes the use of culture dependent assays.
- **Addition of external process control:** It is highly recommended that a suitable process control is added to samples at the earliest stage of sample analysis possible. This should be an organism that is expected to behave similarly to the target organism during sample analysis and serves as an indicator of the success of the sample analysis process.
- **Initial sample processing:** This step is present in most workflows but may not be present in some cases. This step may consist of processes such as clarification and virus concentration in the case of water samples, or homogenisation and dilution in the case of bivalve shellfish samples. In some cases, such as the detection of viruses occurring at high concentrations in wastewater, this step might be skipped.
- **Target enrichment:** In some cases, it may be necessary to enrich the target organism prior to nucleic acid extraction. This is particularly important where the concentration of the target is expected to be below the detection limits of the downstream analytical process, or where the matrix may interfere with downstream analysis. Examples include pre-enrichment culture of *Salmonella* spp. in food samples (ISO, 2017), and bead-based capture of viruses from water samples (Oh *et al.*, 2022). It should be noted that enrichment steps can often complicate quantification of targets.
- **Nucleic acid extraction and purification:** Prior to detection or quantification of target analyte specific genetic markers, the nucleic acids must be liberated from cells or viral capsids (lysis), and then purified to remove substances that could degrade the nucleic acids or have a detrimental effect on the analytical techniques.

- **Data generation:** Examples of data generation techniques include (but are not limited to) conventional PCR followed by gel electrophoresis, quantitative PCR (qPCR), digital PCR (dPCR) and sequencing. The choice of methods will depend greatly on the type of data required.

10.6.5. One sample, many analyses (OSMA) and biobanking

One of the most expensive and time-consuming elements in any biosurveillance programme is often the collection and transport of samples. Where there is a strong likelihood that other analytes of interest can be measured in samples, consideration should be given towards biobanking, to allow for one sample, many analyses (OSMA) approaches. For example, during the English wastewater surveillance programme for SARS-CoV-2, RNA extracts were archived in -80°C freezers. These samples were later used for several projects including the analysis of norovirus at a national level (Walker, Witt, *et al.*, 2024). Samples collected for other purposes such as for food safety surveillance may be of use for detecting pathogens for EBH. Sample analysis is also not only restricted to microbiology; chemical or other analyses may also yield useful data from a single sample. Figure 5 outlines a possible OSMA workflow for wastewater samples.

When considering biobanking, it is important to realise that samples must be stored in an appropriate way that will allow high quality data to be obtained from them later. Typically, this will include the use of deep-freezers (<-70°C) or preservatives. However, it may not be suitable or feasible to store whole samples in this way. For example, if storing wastewater samples for later extraction of other viruses, the freezing process may disrupt the ability to concentrate those viruses from thawed samples. Additionally, storage of large volumes of wastewater will require a large storage volume (along with the associated costs and environmental impacts) and may present a major biological hazard if the freezers fail. In this case, it may be better to partially process the sample prior to biobanking. The nature of the partial processing will depend on the part of the sample that needs to be preserved and its intended use.

It should also be noted that some methods are more suited to OSMA workflows than others. For example, for wastewater analysis, virus concentration techniques that require the addition of high concentrations of chemicals (such as PEG and sodium chloride) will limit the use of the remaining liquid fraction for chemical analyses. In this case, a filtration method may be more suitable because the solutes within the filtrate will remain relatively unchanged, making them more representative. Additionally, the use of filtration in this case will

remove most of the pathogens (depending on filter size), which may make the samples safe enough to use in a chemistry laboratory that is not equipped to work with pathogens. However, a local risk assessment is vital to ensure that such samples can be handled safely.

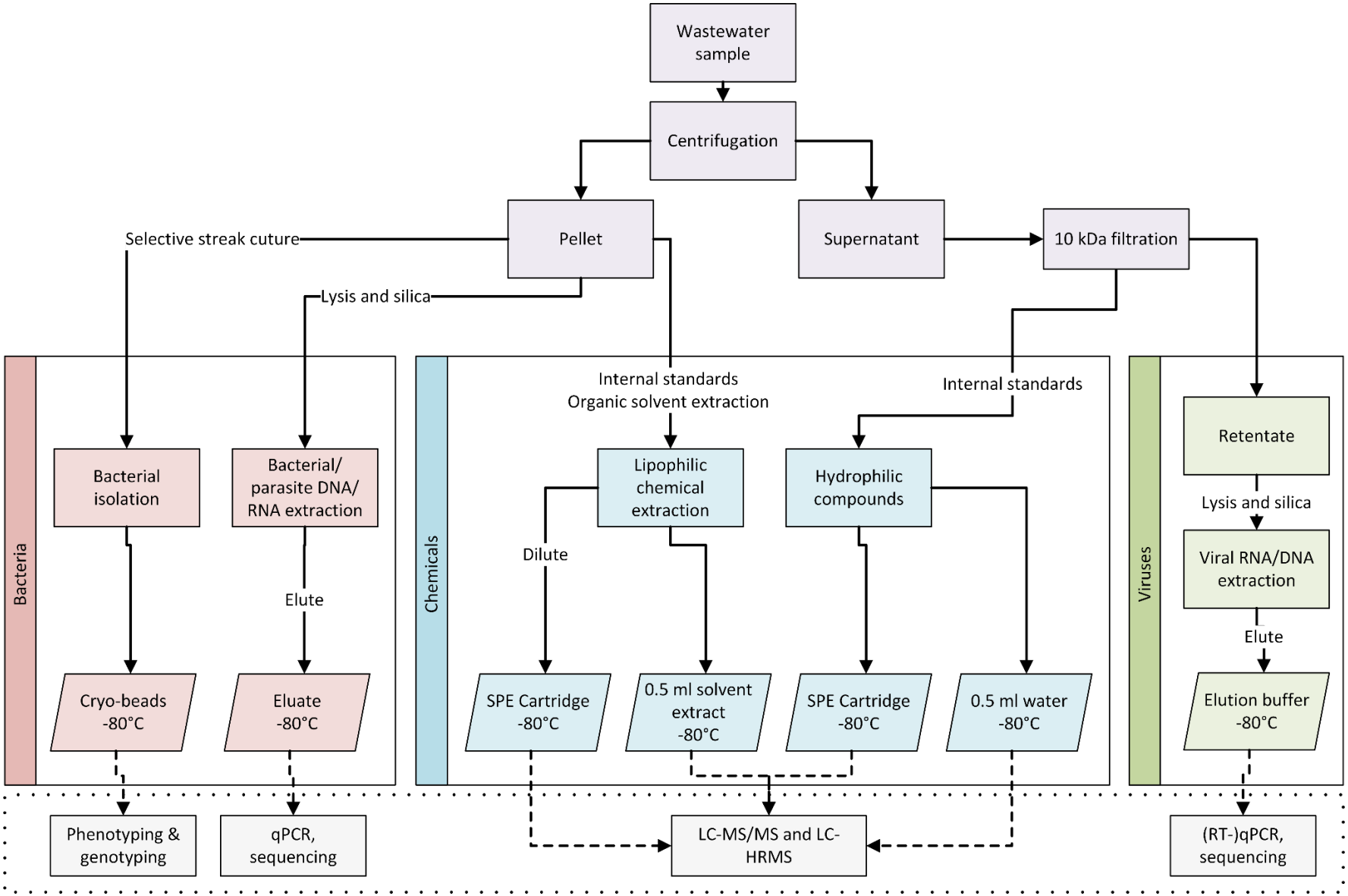


Figure 5: An example of a one sample, many analyses (OSMA) workflow for wastewater samples in which a single wastewater sample can generate many types of data.

10.6.6. Quality control and assurance

To ensure that the results obtained remain reliable, it is important to put in place quality control and assurance measures. Many of these measures are outlined in ISO/IEC 17025. Method specific measures may include the use of appropriate controls such as process control and negative controls. Process controls are used to show whether the method is performing as expected either on a batch-wise or sample-wise basis. Negative controls are used to indicate a lack of contamination which might result in false positive results.

Method validation is an important part of the adoption of methods for a biosurveillance programme. For some matrices, ISO standards exist that outline the requirements for method validation. However, at the time of writing the validation of molecular biology-based methods for environmental samples is relatively under-represented. In the case where no validation standards exist that are suitable for the matrix and target analyte of interest, validation should be performed using the most relevant guidance available. In the case of wastewater, guidance on method validation is not currently available from ISO but has been prepared by Walker (2022).

It should also be noted that adoption of validated methods by a new laboratory requires the new laboratory to test the performance of the method within the laboratory against the expected performance of the method validation study. This process is known as verification and is distinct from but related to validation. It ensures that the measurement uncertainty can be estimated and included as part of the sample results.

10.6.7. Methods for wastewater-based surveillance of viruses

The analysis of pathogens in environmental samples involves multiple stages, each with various methodological options. Table 8, Table 9 and Table 10 present some of the methodological options for analysing viruses in wastewater, their relative performance characteristics, and important considerations for method selection.

Table 8: Initial processing and concentration methods for wastewater samples and their key features (Ahmed *et al.*, 2020; Lu *et al.*, 2020; Farkas *et al.*, 2021)

Method	Processing Time	Sample Volume (ml)	Recovery Efficiency	Complexity	Consumables Cost	Key Applications	Key Limitations
Direct Analysis	0.5 hours	0.1-1	Low	Very Low	Very Low	High concentration targets	Low sensitivity
Centrifugation	1 hour	50-200	Moderate	Low	Low	Solid-associated viruses	Target limitations
Filtration	1-2 hours	100-1000	Moderate-High	Moderate	Moderate	Clean samples	Membrane fouling, target limitations
PEG Precipitation	12-24 hours	100-1000	High	Moderate	Low	Large volume processing	Time required
Ultracentrifugation	3-4 hours	200-500	Very High	High	Low	Research applications	Equipment needs
Ultrafiltration	2-3 hours	50-100	High	Moderate	High	Routine surveillance	Membrane fouling and consumables availability
Bead-based Capture	2-3 hours	5-50	Moderate-High	Moderate	High	Automated processing	Cost and specificity limitations

Table 9: Nucleic acid extraction methods for environmental samples and their key features (Ahmed *et al.*, 2021; Pecson *et al.*, 2021; Zheng *et al.*, 2022)**.**

Method	Processing Time	Purity	Yield	Automation Potential	Cost per Sample	Key Applications
Manual columns and magnetic beads	1-2 hours	Very High	High	Low	Moderate	Small sample numbers
Automated columns & magnetic beads	1-2 hours	High	Moderate-High	Very High	High	High throughput
Direct lysis	0.5-1 hour	Low	Variable	Low	Very Low	Rapid screening
Organic extraction	2-3 hours	Very High	High	Low	Low	Maximum recovery
Heat treatment	0.5 hours	Very Low	Low	Moderate	Very Low	Emergency response

Table 10: Data generation methods for environmental samples and their key features (Pecson *et al.*, 2021; Ahmed *et al.*, 2022; Stefan *et al.*, 2022; Tshiabuila *et al.*, 2022; Bejaoui *et al.*, 2025)**.**

Method	Analysis Time	Sensitivity	Information Gained	Complexity	Cost per Sample	Primary Use
Direct PCR	2-4 hours	Moderate	Presence/Absence	Low	Low	Screening
qPCR	3-5 hours	Moderate-High	Quantification	Moderate	Moderate	Routine monitoring
Digital PCR	4-6 hours	Very High	Precise Quantification	Moderate to High	High	Research/validation
Illumina Sequencing	24-72 hours	Variable	Complete Genetic Profile	High	Very High	Variant detection
Oxford Nanopore Sequencing	12-48 hours	Variable	Rapid Genetic Profile, long sequence reads	High	High	Rapid genomics, variant detection

10.6.8. Sample analysis research and development

In many cases, some degree of research and development for the sample analysis methods will be required. The nature and extent of this will depend on the MRL and the requirements of the programme.

In an ideal situation, methods should be adopted for national surveillance programmes no earlier than MRL 6 or 7, at which stage the method has been fully validated. However, in situations where no validated methods exist (such as during emergency response to a novel pathogen), use of methods at a lower MRL may be necessary. In this case the limitations of the understanding of method performance must be documented and a plan should be put into place for further research and development if required.

10.7. Data analysis (P20)

10.7.1. Standardisation and practical considerations

While specific analytical approaches must be tailored to individual programme objectives, standardisation of certain elements ensures consistency and reliability across analyses:

- **Data Validation Protocols:** Standard procedures for checking data quality, completeness, and consistency.
- **Statistical Methods:** Documented and validated approaches for common analytical tasks.
- **Reporting Templates:** Standardised formats for different types of outputs and audiences.
- **Quality Control Measures:** Consistent approaches to assessing and documenting analysis quality.
- **Documentation Requirements:** Standard formats for recording analytical decisions and methods.

The flexibility to adapt analyses for specific hypotheses must be balanced against the need for reproducibility and consistency in public health decision-making.

10.7.2. Factors affecting data analysis

- **Data Quality Considerations:** Data must be evaluated for completeness and accuracy, considering any gaps or inconsistencies that might affect interpretation. The reliability of normalisation factors, particularly those used for population or environmental adjustments, plays a crucial role in generating comparable results across different locations or time periods. Confounding variables must be identified and accounted for in the analysis, whilst ensuring that temporal and spatial data structure is taken into account to support the intended analytical objectives. Understanding and properly accounting for measurement uncertainty throughout the analytical process is essential for producing reliable and actionable results.
- **Sequencing Method Choice:** The sequencing method used, such as shotgun metagenomics versus amplicon sequencing, significantly impacts data analysis. Metagenomics provides a comprehensive overview of all genetic material in a sample, allowing for the detection of a wide range of organisms and functional genes. However, it generates large, complex datasets that require substantial computational resources and sophisticated bioinformatics tools for analysis. In contrast, amplicon sequencing targets specific genetic regions, making it more cost-effective and easier to analyse, but it may miss less abundant or unexpected

organisms. The choice between these methods should be guided by the specific research questions, available resources, and desired resolution of the data.

- **Quality Assessments and Optimisation:** Quality assessments are crucial in sequencing to ensure data reliability and accuracy. This includes evaluating read quality, coverage depth, and contamination levels. Optimisation involves refining protocols to maximise data quality and minimise errors. For metagenomics, this might mean optimising DNA extraction methods to ensure representative sampling of all organisms. For amplicon sequencing, it could involve selecting the most appropriate primers to target the desired genetic regions effectively. Continuous quality control and optimisation help in producing high-quality, reliable data that supports robust analysis.
- **Statistical Requirements:** The selection of appropriate statistical methods requires consideration of several key factors. Sample size should be verified to ensure that the analyses meet the statistical power requirements established during planning. The underlying distributions and levels of the data often influence method selection, so that assumptions of statistical models must be explicitly tested and documented. When conducting multiple tests, appropriate corrections must be applied to control error rates.
- **Operational Factors:** Time pressures may influence method selection, particularly in programmes surveillance requiring rapid turnaround of results. The availability of resources, including specialised software and computing capabilities, may limit the complexity of analyses that can be routinely performed. Technical expertise requirements must be matched with available staff capabilities, and any new analytical approaches must be successfully integrated with existing systems and workflows. These operational considerations often require careful balancing of analytical sophistication against practical constraints.

10.7.3. Quality Control and Assurance

Quality assurance in data analysis requires robust documentation, validation, and systematic quality control measures. Detailed records of methods, assumptions, and analytical decisions provide a log which is valuable if data need to be re-analysed or audited. Robust version control of scripts and comprehensive archiving ensures reproducibility, repeatability and support ongoing improvement.

Novel analytical approaches must be peer-reviewed and thoroughly tested, while routine analyses need regular performance monitoring and threshold reviews. Automated processes must be sufficiently validated to ensure reliability under varying conditions.

Quality control measures range from systematic error checking to comparisons between analysts. External expert review can also provide independent validation, particularly for novel or critical analyses.

10.7.4. Ethical Considerations

The analysis of environmental surveillance data carries substantive ethical responsibilities that must be considered throughout the analytical process. Privacy protection is particularly important when dealing with data that could potentially identify individuals or communities. While environmental samples are typically considered aggregate data, the analysis of certain markers or patterns could reveal sensitive information about specific populations or geographical areas. Analysts must therefore implement appropriate data aggregation and anonymisation techniques to protect community privacy while maintaining scientific validity.

The selection of analytical methods and interpretation of results must account for potential biases that could disproportionately affect certain communities. This includes careful consideration of how normalisation factors and population adjustments might impact different demographic groups and ensuring that action thresholds are evaluated for their potential to create or reinforce existing inequalities in public health response.

Analysts must clearly communicate assumptions, uncertainties, and limitations in their analyses, to ensure that decision-makers understand the strength of evidence supporting any conclusions. This transparency extends to the documentation of analytical choices and their potential impacts on different stakeholder groups.

Whilst open science principles generally support broad data sharing, this must be balanced against the potential for misuse or misinterpretation of results. Particular care must be taken when reporting results that could stigmatise specific communities or locations, or that might cause unwarranted public concern.

10.7.5. Detection of non-targets of high importance

In some cases, particularly those in which metagenomics approaches are used, non-target organisms of high importance may be detected inadvertently. This may include pathogens that cause notifiable diseases or invasive species. In such cases it may not be the implications of such an observation may be unclear. Any such observations should be reported to the relevant competent authority, who will be able to advise whether further action is required.

10.7.6. Data analysis research and development

The scope and extent of required research and development activities depend primarily on the MRL of existing analytical approaches and the specific requirements of the programme.

For national surveillance programmes, analytical methods should ideally have achieved MRL 6 or 7, indicating full validation and documented performance characteristics. These mature methods provide the reliability and reproducibility needed for consistent decision-making. However, emerging public health threats may require the use of methods at lower MRLs, particularly during emergency response situations. In these cases, the limitations of method performance must be thoroughly documented, and a plan should be made for further validation and development.

The research and development process should focus on several key areas. Method validation studies need to assess performance under real-world conditions, accounting for variations in data quality and environmental factors. Comparative analyses between different analytical approaches help identify the most suitable methods for specific surveillance scenarios. Integration of new statistical techniques or automated analysis tools requires evaluation of their impact on existing workflows and decision-making processes.

11. Documents

11.1. Initial Decision Report (S01)

Following the initial processes (P01 to P04) the decision whether to continue to the next phase (K01), should be documented in a brief report including an overview of the decision-making process, key findings, recommendations, and the rationale for continuing the development of the EBH programme. This Initial Decision Report should contain the following minimum information:

- **Decision to Proceed to Planning Phase (K01):** State the decision whether to proceed to the next phase of planning the programme (P05) or to end the process (T02). This includes the rationale behind the decision and any key factors that influenced it.
- **Questions, Objectives and Actions:** State the primary (and secondary if relevant) questions and objectives that the programme aims to address. Describe actions that will be taken if the target is detected or quantified above action thresholds. Include any alternative criteria for actions when immediate response is not warranted.
- **Value and feasibility:** Include a description of key factors that impact the distribution of the target within the matrix of interest (e.g. shedding dynamics, environmental stability etc.). Include a basic assessment of the estimated costs of a programme and any initial findings on logistical considerations.
- **Ethical and policy considerations:** Include an assessment of the ethical implications of the programme along with any mitigations that need to be put in place. Describe policy considerations including stakeholder support and public perception.
- **Continuous Review and Adjustment:** Note the need for continuous review and adjustment of the programme to ensure it remains aligned with the primary questions and objectives. This includes any mechanisms for regular review and updates.

11.2. Statement of Intent (S02)

The Statement of Intent outlines the overall purpose and objectives of the EBH programme. It serves as a guiding document that brings together key information about the intent of the programme and ensures that all stakeholders have a unified understanding of the programme's goals. The information included in this section is derived from the processes (P05 to P08) following the Decision to Proceed to the Planning Phase (K01). This document should contain the following minimum information:

- **Intended Purpose of the EBH Programme:** Clearly state the primary (and secondary if relevant) objectives and intended outcomes of the programme. This should include the specific health threats the programme aims to monitor and address.
- **Operating Parameters:** Define the targets, spatial, and temporal factors that will guide the surveillance activities. This includes the geographic areas to be covered, the frequency and timing of data collection, and any specific conditions or thresholds that will trigger actions.
- **Key Success Criteria:** Outline the criteria that will be used to measure the success of the programme. This may include specific performance indicators, milestones, and benchmarks that will be monitored throughout the programme's implementation.
- **Stakeholder Engagement:** Identify and list the key stakeholders who need to be engaged in the programme. Specify the roles and responsibilities of each stakeholder.
- **Roles and Responsibilities:** Provide a detailed list of roles within the programme and the individuals or teams who will fulfil those roles.
- **Data and Data Quality Requirements:** Record the data requirements for the programme, including the types of data to be collected, the methods of data collection, and the standards for data quality. This should also include any specific metadata that needs to be recorded, such as sampling methods, geographic locations, and temporal details.

11.3. Statement of Sample Requirements (S03)

The Statement of Sample Requirements outlines the specific samples needed for the programme and their sources. This section consolidates key information from the decision to generate new data (K02), the decision on whether new samples are needed (K03), and the assessment of sample requirements (P11). It serves as a guiding document to ensure that all necessary samples are acquired and that the sampling programme is designed effectively. This document should contain the following minimum information:

- **Decision to collect new samples:** State the outcome of the decision whether to collect new samples or to reuse exiting samples. Record the exploration of other programmes that may have relevant samples. This includes documenting who was contacted, the reasons why their samples are or are not useful, and any agreements or collaborations established.
- **Description of Samples Required:** Clearly state the types of samples needed for the programme. This includes specifying the sample matrix (e.g., water, soil, air), sample collection type (e.g., composite, grab, passive), the level of replication required, the (estimated) sample amount (mass/volume), any specific sample transport requirements and the biological hazard level of the samples.
- **Modifications to Surveillance Parameters:** Note any modifications to the surveillance parameters and their agreement by all relevant stakeholders required to accommodate the OSMA approach and the impact these modifications will have on the programme outcomes. This includes changes to sampling locations, frequency, or methods, and the rationale behind these adjustments.
- **Agreement to Modify Parameters:** Record any agreements to modify surveillance parameters if relevant. This includes documenting the stakeholders involved in the decision-making process, the specific modifications agreed upon, and the expected impact on the programme.

11.4. Change Protocol (S04)

A Change Protocol must be documented that is compatible with section 10.1.2 (Defining a Change Protocol). It should be referred to when operational changes are required in the programme. This document must be accessible to all relevant stakeholders to ensure transparency and consistency.

11.5. R&D needs (S05)

Following a decision to carry out R&D (K05), an assessment of the R&D needs for a programme should be documented. This will enable efficient use of resources towards the most important developments for improving the programme. Ensuring that this document is accessible to all relevant stakeholders will allow the broadest range of input into these developments. However, any work towards the R&D needs must be managed so that work is not replicated unnecessarily.

It is recommended that this is treated as a live document that can be updated as new R&D needs are identified and as R&D goals are realised. Any changes to this live document must be captured in a version control history. Permissions to make changes to the document must also be restricted, and so it is recommended that the document is curated by one or a few individuals.

11.6. Sampling and Transport Protocol (S06)

The Sample Collection and Transport Protocol outlines the standardised procedures and requirements for collecting and transporting environmental samples within the EBH programme. This protocol ensures consistency, quality, and compliance across all sampling activities. The information included in this section is derived from the Sample Collection (P17) and Sample Transport (P18) processes. The minimum information to include in the protocol includes:

Protocol Documentation Control

- Protocol version number
- Clear indication of the protocol's status – draft or accepted version

Introduction

- Brief statement of protocol purpose and context
- Statement defining what the protocol covers and excludes
- Summary of the analytical approach and scientific basis

Safety Precautions

- Overview of key safety requirements and references to safety documentation

Training Requirements

- Summary of required qualifications and competencies

Collection Requirements

- Detailed sampling procedures
- Required sample volumes and collection depths (where relevant)
- Required competencies and training of sampling staff
- Sample preservation methods and timelines
- Quality control measures
- Acceptable sampling conditions and criteria for postponing collection

Health and Safety Requirements

- Personal protective equipment (PPE) specifications for each sampling scenario
- Site access procedures and required permits/licenses
- Confined space entry protocols where applicable
- Emergency response procedures and contact information
- Communication protocols for field operations
- Weather-related safety criteria and limitations
- Decontamination procedures for personnel and equipment

Equipment and Materials Specifications

- Complete list of required sampling equipment
- Specifications for preservation materials and storage containers

- Calibration requirements and schedules
- Maintenance procedures and documentation
- Backup equipment requirements
- Equipment cleaning and decontamination protocols
- Transport container specifications meeting UN3373 Category B requirements

Quality Control Measures

- Types and frequency of field quality control samples
- Temperature monitoring protocols
- Cross-contamination prevention measures
- Documentation requirements for quality control samples
- Corrective action procedures for quality control failures

Transport and Preservation Requirements

- Temperature requirements for each sample type
- Maximum allowable transport times
- Preservation method specifications
- Packaging requirements for biological materials
- Sample hand off and chain of custody procedures
- Temperature monitoring requirements during transport
- Regulatory compliance requirements for biological material transport

Documentation and Records

- Required field documentation forms and logs
- Chain of custody documentation requirements
- Equipment maintenance and calibration records
- Sample tracking and labelling requirements
- Temperature monitoring records
- Incident reporting procedures
- Documentation storage and retention requirements
- Required metadata for each sample type

11.7. Sampling Plan (S07)

A comprehensive sampling plan is crucial for a successful an EBH programme. This section outlines the essential information that must be included in a sampling plan to ensure systematic and reliable sample collection.

11.7.1. Programme-level information

- **Sampling Schedule:** Specify the general frequency, timing of sample collection and level of replication (e.g., weekly, monthly). This should include the overall period during which samples will be collected, such as the start and end dates (or review timelines for continuous programmes) of the sampling campaign.
- **Programme-Level Personnel:** Identify the key personnel responsible for the coordination of logistics and supply chains. This includes roles such as the Programme Director, Logistics Coordinator, and Supply Chain Manager. These individuals will ensure that all logistical aspects of the sampling process are managed efficiently.
- **Quality Control Measures:** Include any quality control measures that will be implemented to ensure the integrity of the samples. This might involve duplicate samples, field blanks, or other controls.
- **Risk Management:** Identify potential risks associated with the sampling process at a programme level and provide corresponding mitigation strategies. This ensures that common risks are addressed consistently across all sampling sites.
- **Contingency Plans:** Outline any contingency plans for dealing with unexpected issues, such as equipment failure, adverse weather conditions, unavailability of scheduled sampling personnel, mismatches between sample delivery and laboratory capacity or other disruptions.

11.7.2. Site-specific plans

- **Sampling Locations:** List all the locations where samples will be collected. This should include specific details such as geolocation and site descriptions. If necessary, include photograph or map for greater clarity.
- **Site-Specific Schedule:** Define the specific days on which samples will be collected at each location. This should account for any variations in sampling frequency or timing based on the needs of the programme.
- **Personnel:** Identify the teams or organisations responsible for collecting samples at each site. This should include their roles, contact information, and any relevant qualifications or training.
- **Site-Specific Risks and Mitigations:** List potential risks specific to each sampling site and provide corresponding mitigation strategies.

- **Additional Information:** Include any other relevant information specific to each sampling site, such as unique environmental conditions, access requirements, or special considerations.

11.8. Sample Analysis Protocol (S08)

The Sample Analysis Protocol outlines the standardised procedures and requirements for analysing environmental samples within the EBH programme. This protocol ensures consistency, quality, and reliability. The information included in this section is derived from the Sample Analysis (P19) process. The minimum information to include in the protocol includes:

Protocol Metainformation

- Protocol version number
- Clear indication of the protocol's status – draft or accepted version

Introduction

- Brief statement of protocol purpose and context
- Statement defining what the protocol covers and excludes
- Summary of the analytical approach and scientific basis

Safety Precautions

- Overview of key safety requirements and references to safety documentation

Training Requirements

- Summary of required qualifications and competencies

Laboratory Requirements and Accreditation

- Laboratory accreditation status and requirements (ISO/IEC 17025)
- Containment level specifications for different sample types
- Quality management system requirements
- Documentation of non-accredited methods where applicable
- Requirements for method validation and verification
- Specifications for laboratory facilities and equipment

Detailed Analytical Workflow Requirements

- Sample receipt and rejection criteria
- Sample inactivation protocols where required
- Initial sample processing procedures
- External process control requirements
- Nucleic acid extraction and purification methods
- Chemical and biochemical analysis methods (if relevant)
- Data generation techniques and requirements
- Quality control measures for each workflow stage
- Documentation requirements for each step

Normalisation Requirements

- Required normalisation factors for data analysis
- Methods for measuring normalisation markers
- Quality control requirements for normalisation data

Quality Control and Assurance

- Required control samples and their frequency
- Acceptance criteria for quality control measures
- Performance monitoring requirements
- Quality control documentation requirements

Sample Storage and Biobanking

- Requirements for sample retention and archiving
- Storage conditions and duration
- Chain of custody requirements for stored samples
- Documentation requirements for stored samples
- Access control procedures
- Sample disposal procedures
- OSMA considerations

Data Management and Reporting

- Required data formats and units
- Result calculation procedures
- Uncertainty reporting requirements
- Quality control data reporting
- Required metadata and documentation
- Data verification procedures
- Timeframes for result reporting
- Communication protocols for critical results

11.9. Data Analysis Protocol (S09)

The Data Analysis Protocol outlines the standardised procedures and requirements for analysing environmental surveillance data within the EBH programme. This protocol ensures consistency, quality, and reliability in the analysis of surveillance data. The information included in this section is derived from the Data Analysis (P20) process. The minimum information to include in the protocol includes:

Protocol Documentation Control

- Protocol version number
- Clear indication of the protocol's status – draft or accepted version

Introduction

- Brief statement of protocol purpose and context
- Statement defining what the protocol covers and excludes
- Summary of the analytical approach and scientific basis

Safety Precautions

- Overview of key safety requirements and references to safety documentation

Training Requirements

- Summary of required qualifications and competencies

Data Input Requirements

- Data format specifications
- Required metadata fields
- Data quality acceptance criteria
- Required normalisation factors
- Input validation procedures
- File naming conventions
- Data handling procedures

Analysis Procedures

- Data preparation steps
- Molecular sequence
- Data analysis methods
- Mathematical modelling methods
- Coding and programming standards
- Statistical analysis methods
- Required quality control checks
- Acceptance criteria for results
- Analysis workflow steps
- Analytical software requirements
- Code and analysis accessibility and provenance

Quality Control Measures

- Required control measures
- Quality check frequency
- Performance criteria

Results Processing

- Calculation procedures
- Uncertainty determination
- Data transformation steps
- Result validation checks
- Output format requirements

Reporting Specifications

- Report format requirements
- Data presentation standards
- Distribution procedures
- Review requirements
- Reporting timelines
- Critical result protocols

11.10. R&D reports (S10)

Research and Development (R&D) reports are essential for documenting the progress and outcomes of R&D activities within an EBH programme. In most cases, these reports should provide a comprehensive overview of the R&D processes and their alignment with the programme's objectives. This will most effectively be achieved by reporting in the traditional standard research report format (Introduction, Methods, Results & Discussion). However, there are scenarios where this is not appropriate, and alternatives are outlined below. The following minimum information should be included in most R&D reports:

11.10.1. Standard report format

Introduction

- **Objective:** State the objective of the R&D activity. This should include the specific research question or problem being addressed and its relevance to the programme.
- **Background:** Provide a brief background on the R&D activity, including any previous work or studies that have informed the current research.

Methodology

- **Description of Methods:** Detail the methods used for sample collection, sample transport, sample analysis, and data analysis. This should include any protocols followed, equipment used, and specific procedures undertaken.
- **Rationale for Methods:** Explain the rationale behind the chosen methods, including any alternatives considered and why they were not selected.

Results

- **Findings:** Present the findings of the R&D activity. This should include any data collected, analyses performed, and key results obtained.
- **Data Presentation:** Use tables, graphs, and other visual aids to present the data clearly and effectively.

Discussion

- **Interpretation of Results:** Discuss the implications of the findings, including how they satisfy (or do not satisfy) the needs identified in the R&D needs document.
- **Readiness Metrics:** Evaluate the readiness metrics, such as the MRL, and discuss how the R&D impacts these metrics. This should include any improvements or limitations identified during the research.

Conclusion

- **Summary:** Summarise the key findings and their significance to the programme.

- **Recommendations:** Provide recommendations for future R&D activities or changes to the current methods based on the findings.

Appendices

- **Supporting Documents:** Include any supporting documents, such as raw data, detailed protocols, or additional analyses, as appendices to the report.

11.10.2. Alternative Formats

While the research paper format is often appropriate, there may be instances where an alternative format is more suitable, for example:

- **Executive Summaries:** For high-level stakeholders who need a concise overview of the R&D activity without detailed technical information.
- **Technical Briefs:** For internal teams who require specific technical details and actionable insights without the broader context provided in a full research paper.
- **Presentation Slides:** For meetings or workshops where visual summaries and key points are more effective than detailed written reports.
- **Short Form Reports:** For rapid reporting during a crisis or for interim R&D progress reports, where timely information is critical. These reports should focus on the most essential information, such as key findings, immediate implications, and urgent recommendations, to facilitate quick decision-making.

11.11. Surveillance Report (S11)

The Surveillance Report is the primary output of the EBH programme, translating analytical findings into actionable information. This document communicates surveillance results and their implications, supporting evidence-based decision-making for public health interventions. The specific content and structure of surveillance reports should be tailored to the programme's objectives, stakeholder needs, and reporting frequency.

11.11.1. Examples of report contents

Executive Summary

The executive summary should provide a concise overview of the most significant surveillance results, trends, and any detected anomalies that require attention. This section should briefly outline the public health implications of the findings, including any recommended actions or interventions (if relevant). Contextual information that helps interpret the current results should also be included, such as comparisons to historical patterns or baseline data. The executive summary should be written in clear language that enables quick comprehension by lay people.

Surveillance Data

The surveillance data section should present the analytical results using appropriate visualisations to highlight key patterns and trends. Temporal analyses showing changes in pathogen levels over the reporting period should be included, with comparisons to previous periods where relevant. Where applicable, geographical distributions of findings should be presented, highlighting any regional variations or hotspots. Relevant statistical analyses that support the interpretation of the data should be incorporated, with appropriate measures of uncertainty and confidence. This section should focus on presenting the data objectively, without detailed interpretations.

Interpretation and Context

This section should provide interpretation of the surveillance results, explaining their significance in the context of the programme's objectives. Any environmental, seasonal, or demographic factors that may influence the interpretation of the results should be discussed thoroughly. The report should present any limitations in the data collection, analysis, or interpretation that stakeholders should consider when reviewing the findings. Where available and relevant, correlations with clinical data, other surveillance systems, or relevant environmental measurements should be included to provide additional context.

Implications and Recommendations

This section should outline the implications of the findings for public health decision-making, including any recommended interventions or responses. Any results that exceed predefined action thresholds should be presented, with specific recommendations for responses. Emerging patterns or trends that require monitoring in future should be highlighted. This section bridges the gap between scientific findings and practical actions, providing clear guidance on what steps should be considered based on the surveillance data.

Technical Information

A brief description of any changes to the sampling, laboratory, or analytical methods used during the reporting period should be included in this section. The quality control measures applied during the reporting period should be summarised, along with any quality-related issues that may affect data interpretation. The completeness of the data should be reported, including any sampling locations or time points where data collection was not possible. This technical information provides transparency about the surveillance process and allows readers to assess the reliability and robustness of the findings.

11.11.2. Report Types and Frequencies

The structure and content of surveillance reports should be adapted based on their frequency and purpose. For routine reporting, different frequencies require different approaches. For example, weekly reports may focus on the most recent data points, emerging trends, and any immediate actions required. These reports should be concise, emphasising changes from previous reports and new findings that require attention. Monthly reports may provide a more comprehensive analysis of temporal trends and spatial patterns, with greater context and interpretation. These reports may include more detailed statistical analyses and integration with other data sources. Annual reports may present comprehensive analyses of long-term trends, programme performance, and broader public health implications. These reports should include in-depth evaluations of the programme's effectiveness in meeting its objectives and recommendations for future improvements.

When surveillance data exceed predefined action thresholds, critical findings should be reported immediately, providing clear information on the nature of the exceedance, its public health implications, and recommended response actions. If the surveillance system identifies patterns indicative of a potential outbreak, specialised reports should be prepared for public health officials,

including detailed analyses supporting the outbreak hypothesis and recommendations for verification and response.

All surveillance reports should maintain consistent formatting and terminology to allow comparison across reports and ensure clarity for stakeholders.

12. Terminators

12.1. Detection challenge (T01)

Below are some potential scenarios under which the use of EBH might be considered. This should not be considered exhaustive, but rather just an aid to the thought process towards initiating an initial assessment for EBH.

Emerging Disease Outbreak: A new infectious disease has been identified in a neighbouring region, and there is a concern that it might spread to the local population. Public health authorities may initiate EBH to detect the presence of the pathogen in wastewater or other environmental samples to provide early warning and inform response efforts.
Public Health Surveillance Enhancement: Existing public health surveillance systems are not providing sufficient data to track the spread of a known pathogen. Authorities may decide to use EBH to complement traditional surveillance methods, such as clinical reporting, to gain a more comprehensive understanding of the pathogen's prevalence and distribution.
Environmental Contamination Incident: An industrial accident or natural disaster has led to the release of hazardous biological agents into the environment. Environmental biosurveillance can be initiated to monitor the spread and concentration of these agents in various environmental media, such as water, soil, and air, to assess the potential impact on public health.
Bioterrorism Threat: Intelligence reports indicate a potential bioterrorism threat involving the release of a biological agent. Environmental biosurveillance can be used to detect the presence of the agent in the environment, enabling rapid response and mitigation measures to protect public health.
Evaluation of Public Health Interventions: Authorities have implemented a new public health intervention, such as a vaccination campaign or sanitation improvement project, and want to evaluate its effectiveness. Environmental biosurveillance can be used to monitor changes in the prevalence of target pathogens in the environment, providing data to assess the impact of the intervention.
Baseline Data Collection: There is a need to establish baseline data on the prevalence of specific pathogens in the environment for future reference. Environmental biosurveillance can be initiated to collect this baseline data, which can be used to detect trends and inform future public health decisions.

12.2. End the process (T02)

Below is a list of the steps to conclude the programme, either because it is not deemed valuable following an initial assessment (K01) or because an established programme is no longer needed. This ensures that the programme is terminated systematically and that all relevant information is documented for future reference and learning.

12.2.1. Ending the Programme After Initial Assessment

- **Initial decision report:** Ensure that the initial decision report (S01) is completed.
- **Stakeholder feedback:** Share the initial decision report with relevant stakeholders to provide transparency about why no further development will be carried out.

12.2.2. Ending an Established Programme

- **Final Evaluation:** Conduct a final evaluation of the programme's outcomes, including an assessment of its impact on public health and any long-term benefits achieved.
- **Reporting:** Prepare a comprehensive final report that summarises the programme's activities, findings, and outcomes. This report should include an analysis of the data collected, the methods used, and any recommendations for future surveillance efforts.
- **Stakeholder Communication:** Communicate the decision to end the programme to all relevant stakeholders, including public health officials, programme staff, and community representatives. Ensure that all stakeholders are informed of the programme's conclusion and any next steps.
- **Archiving:** Archive all programme documentation, including data, reports, and communications, in a secure and accessible manner. This ensures that the information is available for future reference and can inform future surveillance efforts.

13. Process summaries

This section summarises each of the processes in sections 9 and 10 to be used as a quick reference guide. The roles that are responsible for those processes are also suggested.

13.1. Define surveillance purpose (P01)

Responsibility for this process

Lead: Programme Director

Support: Epidemiologists, Public Health Officials

Process summary

Set clear objectives.

- **Objective Setting:** Clearly define the overall objectives of the EBH programme to ensure all stakeholders have a unified understanding.

Develop Testable Hypotheses

- **Formulate Questions:** Develop specific, testable hypotheses that the surveillance programme aims to answer. This step will maintain focus and context throughout the programme.
- **Examples of Questions:**
    - Is the presence of a specific pathogen in the environment the causative agent of disease outbreaks in the population?
    - Do certain environmental conditions (e.g. temperature, rainfall) increase the prevalence of a pathogen?
    - Does a new sanitation policy or infrastructure project affect the concentration of a specific pathogen?
    - Is there a correlation between the genetic diversity of a pathogen in wastewater and the rate of new infections?
    - Do vaccination campaigns reduce the environmental load of vaccine-preventable diseases?

Prioritise Questions

- **Primary Questions:** Identify and prioritise the primary questions that the surveillance programme must answer. These are the most critical questions that will guide the design and implementation of the programme.
- **Secondary Questions:** Acknowledge secondary questions as supplementary.

Define Actions Based on Detection

- **Immediate Actions:** Determine if the detection or quantification of a pathogen should lead to immediate actions (specific actions to be defined in P02).
- **Baseline Data:** If no immediate action is required, explicitly define the purpose of the surveillance, such as establishing a baseline dataset for future reference.

Review and Adjust

- **Continuous Review:** Regularly review the surveillance programme to ensure it remains aligned with the primary questions and objectives.
- **Adjustments:** Make necessary adjustments based on new findings or changes in the environmental conditions or public health needs.

13.2. Define detection actions (P02)

Responsibility for this process

Lead: Public Health Officials

Support: Programme Director, Epidemiologists, Regulatory Compliance Officer

Process summary

- **Establish Action Criteria**
  - **Define Thresholds:** Determine specific thresholds for pathogen detection that will trigger actions.
  - **Consider policies:** Determine whether the ultimate aim is disease eradication or the control of further spread.
  - **Outline Actions:** Specify actions to be taken if a pathogen is detected or exceeds the threshold, such as:
    - Initiating enhanced surveillance
    - Deploying vaccination campaigns
    - Implementing public health education initiatives
- **Immediate Response**
  - **Enhanced Surveillance:** Increase monitoring efforts, such as increased clinical monitoring.
  - **Vaccination Campaigns:** Launch targeted vaccination efforts in areas with low vaccination rates.
  - **Public Health Education:** Disseminate information on hygiene practices, vaccination schedules, and the importance of reporting symptoms.
- **Alternative Criteria for Action**
  - **Monitor Trends:** Establish criteria for monitoring temporal or geospatial trends.

    - **Trigger Responses:** Use consistent increases in pathogen levels over time or in specific areas to trigger public health responses, such as increased clinical surveillance or targeted interventions.
- **Ongoing Monitoring and Analysis**
    - **Data Analysis:** Continuously analyse wastewater and environmental data to identify patterns.
    - **Identify Hotspots:** Use data to identify potential hotspots for pathogen transmission.
    - **Allocate Resources:** Direct resources and interventions to areas identified as high-risk.
- **Review and Adaptation**
    - **Evaluate Effectiveness:** Regularly review the effectiveness of the actions taken.
    - **Update Criteria:** Adjust thresholds and action plans based on new data and emerging threats.
    - **Community Feedback:** Incorporate feedback from the community and public health officials to refine strategies.

13.3. Assess technical value (P03)

Responsibility for this process

Lead: Technical Lead

Support: Laboratory Analysts, Data Analysts, Epidemiologists, Public Health Officials, Programme Manager.

Process summary

- **Determine Pathogen Shedding Dynamics**
  - **Assess Shedding Patterns:** Evaluate whether the target pathogen is shed in a manner detectable in wastewater or other environmental matrices.
  - **Characterise Shedding:** Improve characterisation of pathogen shedding to estimate concentrations accurately.
- **Interpret Detection Implications**
  - **Understand Environmental Behaviour:** Determine what the detection of the pathogen in wastewater or other matrices implies about its presence in the population.
  - **Correlate with Clinical Cases:** Ensure that the implications of detected pathogens are carefully interpreted in relation to relevant clinical data.
- **Ensure Data Feasibility**
  - **Answer Key Questions:** Confirm that the identified questions can be answered using data from wastewater or other environmental matrices.
  - **Timeliness of Data:** Ensure that data are collected and analysed promptly to allow for rapid responses.
- **Review Technical Value Intermittently**
  - **Regular Reviews:** Establish a frequency for reviewing the technical value of the surveillance programme to adapt to changing epidemiological landscapes and emerging threats.

13.4. Assess other values (P04)

Responsibility for this process

Lead: Programme Director

Support: Programme manager, Public Health Officials, Technical Lead, Logistics and Supply Chain Manager, Regulatory Compliance Officer.

Process summary

- **Evaluate Cost**

- **Initial Setup Costs:** Estimate expenses for equipment, laboratory facilities, and personnel training.
- **Operational Costs:** Calculate ongoing expenses for sample collection, analysis, and data interpretation.
- **Cost-Benefit Analysis:** Assess potential savings from environmental surveillance, such as early detection of outbreaks.

Assess Logistics

- **Sample Collection and Transportation:** Ensure efficient logistics for timely sample collection and transportation to laboratories.
- **Laboratory Capacity:** Verify that laboratories can process samples quickly and accurately.
- **Integration with Existing Systems:** Design the programme to integrate with existing surveillance systems where possible**.**

Consider Ethical Implications

- **Privacy Concerns:** Address concerns about individual privacy and ensure data protection.
- **Equity in Surveillance:** Ensure surveillance efforts do not disproportionately target specific populations.
- **Informed Consent:** Emphasise ethical considerations regarding informed consent and community engagement.

Evaluate Political Considerations

- **Support from Government and Stakeholders:** Secure political support for funding and resources.
- **Policy Alignment:** Align the programme with existing policies and frameworks.
- **Public Perception and Trust:** Build public trust through transparent communication about the programme's goals and benefits.

Review Frequency

- **Regular Reviews:** Establish a frequency for reviewing the value of the programme to adapt to changing cost effectiveness, political, ethical and logistical landscape.

13.5. Define roles and responsibilities (P05)

Responsibility for this process

Lead: Programme Director

Support: Programme Manager

Process Summary

- **Define to following roles:**
  - Programme Director
  - Programme Manager

- Project Manager(s)
- Technical Lead
- Field Coordinators
- Laboratory Analysts
- Quality Control Officer
- Data Manager
- Data Analysts
- Community Outreach Coordinators
- Epidemiologists
- Public Health Officials
- Regulatory Compliance Officer
- Communication Specialists
- Research Collaborators
- Logistics and Supply Chain Manager

13.6. Define surveillance parameters (P06)

Responsibility for this process

Lead: Programme Director

Support: Technical Lead, Data Analysts, Epidemiologists, Research Collaborators, Public Health Officials, Community Outreach Coordinators

Process summary

- **Define the sample matrix**
  - Ensure the specific type of sample matrix is defined to enable appropriate planning.
- **Identify Target Pathogens**
  - **Define Focus:** Decide whether the programme will target one or multiple pathogens or if it will take an agnostic approach, focusing on the entire microbial community.
  - **Method Selection:** Choose whether to use methods such as metagenomic sequencing for a broad view or focus on specific pathogens.
  - **Viability vs. Identification:** Determine if the goal is to detect viable/infectious pathogens or if identifying nucleic acids (e.g., DNA or RNA) is sufficient.
- **Set Taxonomic Level**
  - **Decide on Taxonomic Scope:** Establish whether the programme will focus on specific variants (e.g., serotypes), a particular pathogen species or broader pathogen classification such as "DNA respiratory viruses" or "vector-borne pathogens."

    - **Sampling and Detection:** Align this decision with the design of sampling protocols and laboratory analyses, as narrower focuses may require more precise methods.
- **Determine Programme Duration**
    - **Epidemiological Context:** Consider the epidemiological background and pathogens of interest to set the programme duration.
    - **Commitment:** Plan for a long-term duration (typically several years) to capture seasonal variations, establish baseline data, and enable adaptive management.
- **Define Spatial Resolution**
    - **Target Regions:** Identify regions at high risk of pathogen emergence, including urban, rural areas, and key points of entry (e.g., airports, ports).
    - **Hotspot Identification:** Define whether surveillance will be at a community level, monitoring specific locations like buildings or parks to better understand localised outbreaks.
    - **Cross-border Surveillance:** Consider cross-border monitoring to detect international pathogen introductions early.
- **Establish Temporal Resolution**
    - **Sampling Frequency:** Set regular sampling intervals (e.g., monthly or quarterly). Adjust frequency based on environmental changes or outbreak conditions.
    - **Resolution According to Objectives:** For national trends, broader intervals may be acceptable, but near-source detection (e.g., within buildings) requires more frequent sampling.
- **Assess Required Population Coverage**
    - Based on population size, demographics, seasonal variability, and laboratory capacity
- **Assess Statistical Power and Sample Size Requirements**
    - Based on specific surveillance objectives, expected effect sizes, and desired statistical power.
    - Key considerations include minimum detectable effect size, expected variability, desired confidence level, spatial and temporal effects, resource constraints, and the need for stratification.
- **Integrate Data Sources**
    - **Multi-Source Approach:** Enhance data collection by integrating ground-level sampling with remote sensing data for a broader understanding of environmental conditions.
    - **Pattern Identification:** Use the integration to detect trends that might not be visible through single data sources.

13.7. Data storage and access(P07)

Responsibility for this process

It is vital that this process is carried out by those stakeholders that have an in depth understanding of the data requirements and resources:

Lead: Data Manager

Support: Programme Manager, Project Managers, Technical Lead, Data Analysts, Regulatory Compliance Officer.

Process summary

- **Establish a Database**
  - **Analytical Data and Metadata Storage:** Secure and centralised allowing easy access and sharing among all relevant stakeholders.
  - **Data Visualisation and Reporting:** Enables data visualisation and reporting.
- **Define Data Sharing Agreements:**
  - Establish Agreements for Stakeholder Access Early on
  - **Default Open Access Approach:** Grant all stakeholders access to the database unless the data are sensitive and require restricted access.
- **Estimate Data Storage Capacity Requirements:**
  - Assess Type and Volume of Data
  - Ensure adequate resources and infrastructure are in place

13.8. Define data and quality needs (P08)

Responsibility for this process

Lead: Technical Lead

Support: Data Manager, Data Analysts, Epidemiologists, Laboratory Analysts

Process summary

- **Define the Data Type Requirements**
  - Quantitative Data
  - Semi-Quantitative Data
  - Qualitative Data

Identify Metadata Requirements

- Critical metadata to provide context and ensure data reliability.
- Examples of metadata:
  - Date and time of collection
  - GPS coordinates of sampling location

    - Weather conditions
    - Sample volume
    - Methods used

Set Measurement Uncertainty and Precision Requirements:

- Based on pathogen type and program goals.
- Balance precision with feasibility, especially in high-throughput scenarios.
- Consider existing validation criteria and method validation reports as a guide.

Establish Limits of Detection (LOD) and Quantification (LOQ):

- Define the LOD and LOQ based on the programme's analytical needs.
- Ensure that the LOQ, along with the measurement uncertainty, is below any action-triggering threshold for pathogens of concern.

Plan for Quality Checks of Data and Metadata:

- Perform routine audits and validation checks on metadata to ensure completion and protocol adherence.
- For analytical data:
    - Use control samples during laboratory testing.
    - Participate in external proficiency testing programmes.
    - Conduct regular audits of laboratory processes to maintain data integrity.

13.9. Search for existing data (P09)

Responsibility for this process

Lead: Data Manager

Support: Data Analysts, Research Collaborators

Process summary

1. **Assess Existing Data Sources:**
    - Engage Stakeholders:
    - Review Existing Surveillance Data:
2. **Identify Other Surveillance Data:**
    - Clinical case data
    - Data from other environmental matrices
    - Indirect data such as:
        - Syndromic data
        - Digital and social media data
        - Pharmaceutical data
3. **Prevalence of Co-circulating Pathogens:**
4. **Include Environmental Data**
5. **Include Population Data:**

- Use dynamic population data where possible, especially in areas with seasonal population changes

6. **Consult Relevant Data Sources:**
    - Use Established Resources
    - Engage Additional Stakeholders

13.10. Acquire and assess existing data (P10)

Responsibility for this process

Lead: Data Manager

Support: Data Analysts

Process summary

- **Assess data acquisition requirements**
- **Acquire existing datasets**
- **Evaluate datasets using the following criteria:**
    - Relevance to Surveillance Questions
    - Q-FAIR data
    - Data Accuracy and Quality
    - Timeliness
    - Spatial and Temporal Resolution
    - Sample Integrity and Collection Methods
    - Metadata Completeness
    - Pathogen Detection Limits
    - Cost and Feasibility
    - Ethical and Legal Considerations
- **Make a decision on new data collection**
    - Use the decision tree in K02.

13.11. Assess sampling requirements (P11)

Responsibility for this process

Lead: Technical Lead

Support: Laboratory Analysts, Quality Control Officer, Data Analysts, Epidemiologists, Regulatory Compliance Officer, Research Collaborators

Process summary

- **Define sample requirements**
    - Assess the nature of the samples required, considering the environmental material and purpose.
- **Determine sampling type**

    - Grab, composite or passive
- **Decide on replication**
- **Calculate likely sample amount**
    - Based on laboratory needs and replication and wastage.
- **Sample transport requirements:**
    - Plan for cool transport (0°C to 10°C) unless freezing is appropriate.
    - Transport conditions depend on sample type and analysis.
- **Determine biological safety level**
- **Check for existing samples**
    - Determine if samples from another programme fit the requirements of the programme and can be re-analysed.
- **Agree use the samples with sample owner**
- **Decide whether to use existing samples**
    - Use decision tree K03.

13.12. Engage with stakeholders (P12)

Responsibility for this process

Lead: Programme Director, Programme Manager

Support: Project managers, Communication Specialists

Process summary

- **Identify key stakeholders:** Determine the necessary stakeholders based on the decision made in K03.
- **Engage with stakeholders early:** Once the initial needs of the programme are known, engage stakeholders promptly.
- **Public health officials:** Use surveillance data to inform public health interventions and policies.
- **Matrix facility operators:** Responsible for sample collection and processing. Engage with operators of wastewater, soil, air, or shellfish harvesting facilities.
- **Logistics companies:** Arrange for sample transport, ensuring timely and temperature-controlled delivery. Collaborate to define transport timelines and establish emergency protocols.
- **Laboratory facilities:** Collaborate with labs early to evaluate their capability to detect pathogens, manage additional sample testing, and maintain quality assurance and control.
- **Academic institutions:** Include researchers with expertise in microbiology, epidemiology, and environmental science for developing methodologies and translating findings into public health strategies.
- **Competent Authorities:** Engage authorities responsible for environmental protection and food safety to establish guidelines, ensure

compliance, and integrate surveillance data into public health frameworks.

13.13. Design sampling programme (P13)

Responsibility for this process

Lead: Programme Director

Support: Programme manager, Project managers, Technical Leads, Field Coordinators, Quality Control Officer, Data Manager, Epidemiologists, Public Health Officials, Communication Specialists, Logistics and Supply Chain Manager

Process summary

- **Determine operational constraints and access requirements**
- **Map proposed sampling locations against operational and logistical constraints**
- **Create a master schedule accounting for:**
  - Required sampling frequencies
  - Site access windows
  - Travel times between locations
  - Laboratory processing capacity
  - Staff availability
- **Develop site-specific sampling plans with detailed access and safety instructions**
- **Establish communication protocols between sampling teams, facility operators, and laboratories**
- **Create contingency plans for sampling disruptions**
- **Document the complete programme with maps, schedules, and contact information**
- **Review the draft programme with stakeholders and revise based on feedback**

13.14. Define change protocol (P14)

Responsibility for this process

Lead: Programme Manager, Project Managers

Support: Programme Director, Technical Lead, Quality Control Officer, Data Manager, Regulatory Compliance Officer, Logistics and Supply Chain Manager

Process summary

- **Importance:** A change protocol ensures consistent, reliable operations, maintains data integrity, mitigates risks, and supports continuous improvement in response to evolving needs.
- **Governance:** Assign a team to oversee, approve, and align changes with programme goals (as defined in P01 and P02)
- **Change Framework:** Define steps for proposing, evaluating, implementing, and reviewing changes.
- **Evaluation:** Standardise criteria to assess feasibility, risks, and impacts of changes.
- **Testing and Validation:** Pilot and validate changes before full implementation.
- **Communication and Training:** Ensure clear communication and provide staff training for updates.
- **Monitoring and Documentation:** Track performance, gather feedback, and update the protocol regularly.

13.15. Metrics for assessing readiness (P15)

Responsibility for this process

Lead: Programme Director

Support: Technical Lead, Quality Control Officer, Data Manager, Logistics and Supply Chain Manager, Programme Manager, Project managers, Research Collaborators

Process summary

- **Assessing readiness**
  - **Purpose:** Assess readiness of methods and operational programmes to identify R&D priorities.
  - **Focus:** Use robust, validated protocols with known performance limitations.
- **Method Readiness Levels (MRL)**
  - A scale to evaluate the readiness of methods across four stages:
    - Sample Collection (cMRL)
    - Sample Transport (tMRL)
    - Sample Analysis (aMRL)
    - Data Analysis (dMRL)
  - Helps determine the development stage of methods and guide resource allocation.
- **Operational Readiness Index (ORI)**
  - **Definition:** A weighted average of the four MRLs to measure overall programme readiness.
  - **Factors:**

    - Importance weight: Reflects process dependencies.
    - Effort weight: Accounts for development effort required.
  - Identifies bottlenecks and prioritises processes needing further development.
- **Additional notes**
  - R&D should focus on areas with low readiness, particularly high-priority or effort-intensive processes.
  - Separate indices for importance and effort weights can provide additional insights for improving operations.

13.16. Undertake research & development (P16)

Responsibility for this process

Lead: Technical Lead

Support: Research Collaborators, Laboratory Analysts, Quality Control Officer, Data Manager, Programme Manager.

Process summary

- Initiate R&D activities based on MRL and ORI calculations and change protocol requirements.
- Develop a detailed project plan outlining objectives, resources, timelines, and quality control measures.
- Consider whether R&D can be conducted internally or if external support is needed.
- Maintain quality standards in R&D, including comprehensive documentation and method validation.
- Assign staff with appropriate technical expertise and ensure routine activities are not compromised.
- Identify and procure necessary equipment and materials, considering cost-sharing where possible.
- Regularly review progress and adjust plans as needed to ensure efficient resource use.
- Report R&D activities regularly, following the programme's reporting requirements.

13.17. Sample collection (P17)

Responsibility for this process

Lead: Technical Lead

Support: Field Coordinators, Quality Control Officer, Data Manager, Logistics and Supply Chain Manager

Process summary

- **Pre-Collection Planning:** Consider matrix-specific challenges, evaluate site access/safety, assess equipment/personnel needs, review documentation requirements.
- **Establish Quality Controls:** Develop standard operating procedures (SOPs) implement documentation system, set up training programme, define field quality controls, establish equipment procedures.
- **Prepare for Sampling:** Review site requirements, verify equipment, prepare documentation, check weather, brief team, coordinate access.
- **Field Collection:** Assess site conditions, set up equipment, collect samples as per SOPs, implement preservation, complete documentation, maintain chain of custody.
- **Post-Collection Tasks:** Clean equipment, finalise documentation, arrange transport, notify laboratory, conduct team debrief.
- **Consider Method Development:** Evaluate MRL, adapt existing methods if needed, validate under field conditions, document limitations, plan further development.

13.18. Sample transport (P18)

Responsibility for this process

Lead: Logistics and Supply Chain Manager

Support: Field Coordinators, Technical lead, Laboratory Analysts, Quality Control Officer, Data Analysts, Data Manager

Process summary

- **Assess Transport Requirements:** Review sample stability, preservation needs, regulatory requirements, and time constraints
- **Implement Preservation:** Select appropriate preservation methods, prepare necessary materials and equipment
- **Ensure Compliance:** Verify packaging meets safety regulations and maintain required conditions (e.g. cold temperatures) for entire transport duration, complete required documentation
- **Plan Transport Chain:** Map collection points to laboratories, establish routes, identify suitable transport providers
- **Maintain Chain of Custody:** Document all sample transfers and handling steps

13.19. Sample analysis (P19)

Responsibility for this process

Lead: Technical lead

Support: Laboratory Analysts, Quality Control Officer, Data Analysts, Data Manager, Research Collaborators

Process summary

- **Assess Key Considerations:**
  - Review factors such as sample matrix, analytical target, expected analyte concentrations, and time constraints.
  - Evaluate the MRL of potential methods and ensure they align with your objectives.
- **Plan the Workflow:**
  - Map out the key stages of the analysis workflow (e.g. sample receipt, processing, data generation).
  - Consider whether additional steps like sample inactivation or inclusion of process controls are required.
- **Decide on Biobanking:**
  - Evaluate whether to archive samples for future analysis.
  - Determine the storage method (e.g. deep-freezing, partial processing) to maintain sample integrity while minimising hazards and costs.
- **Ensure Quality Assurance:**
  - Implement quality control measures, including process and negative controls, to validate your results.
  - Adhere to quality standards such as ISO/IEC 17025 to ensure data reliability.
- **Evaluate Resource Needs:**
  - Consider availability of materials, equipment, and expertise required for the selected method.
  - Assess the environmental impact and ethical implications of your approach.
- **Conduct Research and Development (if needed):**
  - If no validated methods exist for your target analyte, adapt existing methods or develop new ones.
  - Document limitations of unvalidated methods and establish a plan for further validation or improvement.
- **Finalise the Method:**
  - Select a method that best balances your objectives, resources, and practical constraints.
  - Verify the chosen method to ensure it performs reliably within your specific laboratory context.

13.20. Data analysis (P20)

Responsibility for this process

Lead: Technical Lead

Support: Laboratory Analysts, Quality Control Officer, Data Manager, Data Analysts, Community Outreach Coordinators, Epidemiologists, Public Health Officials, Regulatory Compliance Officer, Communication Specialists, Research Collaborators

Process summary

- **Establish Standardisation Requirements:**
  - Review standard procedures for data validation and quality checks.
  - Confirm documentation formats for analytical methods and decisions.
  - Define reporting templates for different stakeholder groups.
- **Assess Data Quality:**
  - Evaluate data completeness and accuracy requirements.
  - Review reliability of normalisation factors.
  - Identify potential confounding variables.
  - Identify the temporal and spatial structure of the data to inform statistical approaches.
- **Define Statistical Framework:**
  - Verify sample sizes meet the statistical power requirements established during planning.
  - Select appropriate statistical methods based on data distributions.
  - Establish procedures for handling multiple comparisons.
  - Account for spatial and temporal correlations.
- **Address Quality Assurance:**
  - Implement documentation and validation procedures.
  - Establish version control for analytical scripts.
  - Define requirements for method validation.
  - Set up systematic quality control measures.
- **Consider Ethical Requirements:**
  - Implement privacy protection measures.
  - Establish data aggregation protocols.
  - Review potential community impacts.
  - Define responsible reporting procedures.
- **Plan Operational Delivery:**
  - Assess time constraints and reporting deadlines.
  - Review available computational resources.
  - Evaluate staff expertise requirements and training.
  - Consider integration with existing systems.

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