Hydro Nation International Centre

Utility of wastewater surveillance for detecting and monitoring emerging and re-emerging pathogens and endemic infections

Eulyn Pagaling[1], Urmi Ghosh[1], Clare Harkins[1], Claus-Dieter Mayer[2] and Lisa Avery[1]

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Introduction

Wastewater-based epidemiology (WBE) was successfully used to monitor infectious disease markers at population level, exemplified by SARS-CoV-2, obtaining near real-time population level data. To merit further investment, policy teams need assurance that improved surveillance benefits health protection in Scotland. The main objective of Scotland's Wastewater Monitoring Programme Strategic Plan 2025-2028 is to deliver a quality-assured WBE program that fulfils the following needs:

- Delivers on the One Health agenda
- Is at the forefront of developments in WBE applied to public health practice
- Contributes to Scotland's future pandemic preparedness

Aims & Objectives and Approach

The aim of this project was to review the utility of wastewater surveillance for detecting and monitoring emerging and re-emerging pathogens and endemic infections, including blood-borne viruses and enteric viruses. The project aim was addressed through four specific objectives as follows:

O1. The effectiveness of wastewater surveillance compared to traditional surveillance methods, key factors influencing detection sensitivity and specificity, particularly in environments with multiple pathogens.

O2. The cost-effectiveness of wastewater surveillance compared to traditional surveillance methods (e.g., clinical testing, active case finding).

O3. Ethical/legal considerations.

O4. Gaps in the research, recommendations including additional studies needed to improve global health monitoring.

Objectives were addressed through systematic literature review, evidence synthesis, and engagement of key stakeholders and experts through informal discussion.

Key Findings

Pathogen detection methods

- Pathogen detection methods were categorised as culture-based, culture-independent detection of whole organisms, PCR-based, isothermal amplification, sequencing, spectrometry, microfluidics, biosensors and emerging and integrated methods.
- Culture-based methods cannot detect viable but non-culturable organisms (VBNC). Nucleic acid-based methods are affected by DNA extraction bias. PCR-based methods are affected by inhibition, competitive binding and primer bias. Spectrometry-based methods bypass issues with VBNC and inhibition but are affected by lack of reference standards. Both PCR-based and spectrometry-based approaches may be less effective in wastewater where multiple pathogens are present.
- The more established technologies have already been validated for use in wastewater, while some emerging technologies still require validation in wastewater.
- Other factors should also be considered to assess the effectiveness of WBE, including spatio-temporal trends in pathogen load, potential degradation during sample transport and storage and methods for the primary concentration step.
- Correlations between WBE data and the population have been demonstrated for SARS-CoV-2, *Salmonella* spp., Influenza virus and MPOX.

WBE Costs

- Costs for WBE need to account for personnel salaries (e.g. sampling team, laboratory technicians, bioinformaticians and specialists), sampling equipment, sample transport, and technology costs (e.g. laboratory equipment and laboratory consumables and reagents).
- WBE for SARS-CoV-2 estimated $USD0.07 – $USD0.10/person in Malawi and $USD0.07 – $USD0.13/person in Nepal. WBE for SARS-CoV-2 estimated $USD0.10/person in rural areas and $USD0.005/person in urban areas of the USA.
- Costs for clinical testing or active case finding in the UK could not be found. Given international experience, it is anticipated that WBE would incur a lower cost per person, however other surveillance is unlikely to be applied at population level, therefore comparisons are difficult to evaluate.

Ethical and legal considerations

- Ethical considerations concerning WBE include i) Privacy and the protection of personal data where results could potentially be traced back to an individual or groups of individuals, ii) Data analytics, big data and decision making where WBE data may make it possible for third parties to target specific groups of individuals, iii) Public health ethics where WBE may offer a more equitable solution to disease surveillance, iv) Research ethics where WBE results may lead to disproportionate measures on groups of individuals, and v) Environmental and water ethics where decisions over wastewater treatment may differ according to

risks to human health versus risks to aquatic animals or ecological health.

- Legal issues arise when enforcement of measures (e.g. stay at home orders, sanitary cordons or quarantines) violate an individual's protection against searches, seizures and discrimination.

Research gaps

- Costs comparisons between WBE and traditional methods (e.g. clinical testing) will need to be conducted by relevant stakeholders to assess the cost-effectiveness of WBE.
- Spatial (i.e. between catchments, wastewater treatment plants) and temporal (e.g. diurnal, seasonal) fluctuations in pathogen loads is still not known for Scotland.
- The effects of sewer sample transport and sample storage on pathogen degradation are unclear.
- The effect of primary concentration methods on pathogen detection is still unclear.
- Reference standards are still required for some advanced technologies (e.g. MALDI-TOF MS).
- Validation is still required for emerging technologies.
- Even if the technology has been found to work in wastewater, validation is still required for performance on specific pathogens.

Recommendations

Recommendations pertinent to Scotland

Towards furthering the application of WBE for wastewater monitoring in Scotland we make the following recommendations:

- Determine priority pathogens based on likelihood, prevalence and severity of health risk.
- Define populations of interest for priority pathogens.
- Develop and implement protocols to assess ethical aspects of inclusion/exclusion, anonymity and human genomic by-catch.
- Identify best available methodology for prioritised pathogens and determine technical capability.
- Determine monitoring requirements (e.g. sampling intensity and numbers of sites required to be representative of populations of interest).
- Determine cost of applying WBE for prioritised pathogens.
- Establish External Quality Assessment Schemes (i.e. interlaboratory trials) to ensure comparability across institutions.

Methodological recommendations

- Follow up of this study to determine for which priority pathogens WBE has been applied successfully in a research or applied context worldwide.
- Research is required to fully understand the effect of the methodological biases and limitations on the accuracy of WBE to capture infectious disease circulation in the population.
- Further research into the relative shedding of different pathogens by the human population into the wastewater systems, and the methods that link pathogen signals back to human numbers in a catchment.
- Rigorous testing and validation of emerging technologies, especially those not yet used in wastewater is required.
- Standardised reporting of technological specifications should be established to allow comparison.
- Establish External Quality Assessment Schemes (i.e. interlaboratory trials) to ensure comparability across institutions and countries.

Recommendations for pathogen reporting

- Constant re-assessment or 'horizon scanning' of emerging pathogens with pandemic potential should be conducted at regular intervals (e.g. annually).
- Further research is required to elucidate and minimise methodological biases and limitations on accuracy of WBE to represent infectious disease circulation in populations, including better understanding of rates of pathogen shedding to wastewater, impact of sewer transit/sample storage and optimal statistical and data analysis pipelines.
- Implementation of WBE trials for monitoring of prioritised pathogens should be undertaken to provide evidence of utility, efficacy and integration with clinical testing and contact tracing.
- Agreement on a standard detection method for WBE that could be implemented globally (especially in resource-limited areas) is recommended to allow direct comparisons.
- Standardised reporting of WBE results is recommended to allow direct global comparison.
- Establish External Quality Assessment Schemes (i.e. interlaboratory trials) to ensure comparability across institutions and countries.

Recommendations for coordinated monitoring

- Following generation of evidence of cost-effectiveness, significant investment in global infrastructure for sampling, transport and testing laboratories would be required to facilitate coordinated WBE at an international level.
- Development of a global open access database where WBE data and clinical data can be deposited is recommended for constant pathogen surveillance and vigilance. Database must be accessible and fit for purpose.
- Analysis of deposited data should take place in a timely manner and lead to action. Therefore, development of modelling or a decision support tool to translate data into resource planning is recommended.
- Formulation of pandemic action plans should include potential use of WBE to aid disease monitoring.

1 Introduction

1.1 Background

Wastewater-based epidemiology (WBE) was successfully used to monitor infectious disease markers at population level, exemplified by SARS-CoV-2, obtaining near real-time population level data. The main objective of Scotland's Wastewater Monitoring Programme Strategic Plan 2025-2028 is to deliver a quality-assured WBE program that fulfils the following needs:

- Delivers on the One Health agenda
- Is at the forefront of developments in WBE applied to public health practice
- Contributes to Scotland's future pandemic preparedness

This project underpins these aims and also relates to Pathogens Genomics in Scotland's Strategic Plan (Public Health Scotland, 2024) and current UKHSA Science Strategy (UK Health Security Agency, 2023). To merit further investment, policy teams need assurance that improved surveillance benefits health protection in Scotland. To make recommendations, we require evidence on:

- which emerging or re-emerging pathogens are currently observed within the Scottish (or UK) population and whether there are emerging threats globally that will likely spread to the UK;
- how pathogens are shed, what concentrations enter wastewater, how their biology/behaviour during transport (e.g. die-off, cell/nucleic acid degradation, viability) influences detection;
- established and reliable detection techniques that are applicable to wastewater, and whether they are widely used within Scottish laboratories (or the UK).

It is critical to develop an ethical approach to surveillance, ensuring individuals are anonymised and populations do not become stigmatised.

The aim of this project was to conduct a rapid evidence review and synthesis on the utility of wastewater surveillance for detecting and monitoring emerging and re-emerging pathogens and endemic infections, including blood-borne viruses and enteric viruses.

1.2 Aims and Objectives

The project aim was addressed through four specific objectives as follows:

O1. The effectiveness of wastewater surveillance compared to traditional surveillance methods, key factors influencing detection sensitivity and specificity, particularly in environments with multiple pathogens.

O2. The cost-effectiveness of wastewater surveillance compared to traditional surveillance methods (e.g., clinical testing, active case finding).

O3. Ethical/legal considerations.

O4. Gaps in the research, recommendations including additional studies needed to improve global health monitoring.

These objectives were addressed through systematic literature review and evidence mapping exercise, engagement with key stakeholders and virus experts through informal discussion, and close interaction with the Project Steering Group (PSG).

1.3 Approach

To achieve the objectives, three tasks were undertaken.

Task 1. Stakeholder Interviews

Information to feed into all objectives was sought from stakeholders on i) emerging and re-emerging pathogens and endemic infections in Scotland, including blood-borne and enteric viruses; ii) costs of clinical testing and active case finding; iii) ethical/ legal considerations for surveillance; iv) costs for establishing regular wastewater surveillance. Information on wastewater sampling infrastructure had already been gathered in a previous CREW project: "Review of psychoactive substances wastewater monitoring approaches and recommendations for the feasibility of applying different approaches in Scotland" (Avery et al., 2025).

Stakeholders were identified primarily through discussion with the steering group and also through existing contacts of the research team and included representation from Scottish Water, NHS Highland, Public Health Scotland, SEPA and academics. Stakeholders were engaged through email (Appendix 4) and/or videoconferencing. Questions were tailored to different stakeholder sectors.

Task 2. Rapid Evidence Review

A rapid evidence review was undertaken to underpin objectives O1 and O2. Criteria for the rapid evidence review were devised by the research team and search terms and inclusion/exclusion criteria were reviewed by the PSG and further optimised and trialled by the project team. Searches were initially carried out in both Web of Science and Google Scholar and later restricted to Web of Science only. Information was gathered to i) confirm the identity of emerging and re-emerging pathogens in Scotland; ii) review

methods for detecting these pathogens in wastewater, including method sensitivity and specificity, especially in cases where multiple pathogens may be present; iii) assess the costs associated with these detection methods. Details of search methods are provided in Appendix 1.

Task 3. Rapid Evidence Synthesis

Evidence from Tasks 1 and 2 from the last 5 years was synthesised to summarise the main findings, identify knowledge gaps, and develop recommendations, including proposals for further studies needed to enhance global health monitoring.

2 Results - O1. The effectiveness of wastewater surveillance compared to traditional surveillance methods, key factors influencing detection sensitivity and specificity, particularly in environments with multiple pathogens.

2.1 Pathogens search results

To address Objective 1, it was important to firstly identify pathogens of importance in the Scottish context. Grey literature was reviewed for diseases and pathogens of interest. These sources were then reviewed manually for relevance to the UK (Appendix 2). A further search in Web of Science resulted in 40 pathogens of interest. A few weeks into the project, the UK Health Security Agency published their list of 24 priority pathogens, many of which were already included in our results. (UK Health Security Agency, 2025)

2.2 Priority pathogens

The UK Health Agency list is not ranked because any one of the pathogens could pose significant threat to human health based on criteria defined in the UKHSA tool. Table 1 is information extracted from the report on viral pathogens, and details the pandemic and epidemic potential, the notable pathogens within each viral family and the disease they cause, transmission (human-to-human or zoonotic), geographical spread, and availability of diagnostics. Table 3 is information extracted from the report on bacterial pathogens, and details the disease they cause, transmission, AMR concern, geographic spread and availability of diagnostics.

Further review of the grey literature (Appendix 2) and stakeholder interviews (Appendix 4) identified an additional 31 pathogens that are of concern to Scotland, and are detailed in Table 2 (viruses), Table 4 (bacteria) and Table 5 (protozoa). The pathogens identified may already be present in Scotland, but also include some of global concern with potential for transmission to Scotland.

Table A: Priority viral pathogens and supporting information published by UK Health Security Agency, with additional information on detection in wastewater or application of WBE from this current literature review. Blank cells indicate that data are not available. Diagnostics availability relates to tests specific for the notable pathogen listed. P = PCR, S = serology, L = LFD, HT = high throughput, LT = low throughput, LDT = laboratory developed test, RUO = research use only, ADT = academic developed test, Ag = antigen, Ag RUO = antigen research use only, Ab = antibody, Ab RUO = antibody research use only, POC = point of care.

Viral family	Overall pandemic potential	Overall epidemic potential	Notable pathogen	Disease	Human-to-human transmission	Zoonosis	Geographical spread	Diagnostics availability (based on market search and expert opinion)	Detected in WW or WBE applied
Adenoviridae	Medium	High	Human adenovirus (B, C, E, F)	Adenovirus infection	Food or water. Respiratory. Contact.	No	Worldwide	LABORATORY P: HT, LT, LDT S: RUO NEAR PATIENT P: POC; L: Ag	Y
Arenaviridae	Low	Medium	Lassa virus	Lassa fever	Contact	Yes	Endemic in sub-Saharan Africa	LABORATORY P: LT, LDT S: LDT, RUO NEAR PATIENT L: Ag RUO	
Calciviridae	Medium	High	Norovirus	Norovirus infection	Food or water	No	Worldwide	LABORATORY P: HT, LT, LDT S: ADT NEAR PATIENT P: POC; L: Ag	Y
Coronaviridae	High	High	MERS-CoV	MERS	Respiratory (uncommon; typically zoonotic)	Yes	Middle East, notably Saudi Arabia.	LABORATORY P: LT, LDT S: RUO NEAR PATIENT P: POC	
Filoviridae	Low	High	Ebola virus (EBOV)	Ebola virus disease	Contact	Yes	Outbreaks in sub-Saharan Africa	LABORATORY P: HT, LT, LDT S: RUO NEAR PATIENT L: Ag	
Filoviridiae	Low	High	Sudan virus (SUDV)	Sudan virus disease	Contact	Yes	Outbreaks in sub-Saharan Africa	LABORATORY P: LDT, RUO S: RUO NEAR PATIENT None	
Filoviridae	Low	High	Marburg virus	Marburg virus disease	Contact	Yes	Outbreaks in sub-Saharan Africa	LABORATORY P: LT, LDT S: RUO NEAR PATIENT L: Ag RUO	
Flaviviridae	Low	High	Dengue virus	Dengue	VBD	No	Common globally. Increasing in Europe	LABORATORY P: LT, LDT S: LT, LDT, RUO NEAR PATIENT P: POC; L: Ag, Ab	
Flaviviridae	Low	High	Zika virus	Zika	VBD. Contact	Yes	Americas, Pacific islands, Africa and Asia	LABORATORY P: LT, LDT S: HT, LT, LDT, RUO NEAR PATIENT P: POC; L: Ag, Ab	Y

Flaviviridae	Low	High	Hepatitis C virus	Hepatitis C	Contact	No	Worldwide	LABORATORY P: HT, LT, LDT S: HT, LT, LDT, RUO NEAR PATIENT P: POC; L:Ag RUO, Ab	Y
Hantaviridae	Low	Low	Hantaan virus	Haemorrhagic fever with renal syndrome (HFRS)	Respiratory (rare; typically zoonotic)	Yes	Asia and Northern and Western Europe	LABORATORY P: LT, LDT, RUO S: LT, LDT, RUO NEAR PATIENT L: Ab RUO	
Nairoviridae	Low	Medium	CCHF virus	Crimean-Congo Haemorrhagic fever	VBD Contact	Yes	Endemic in all of Africa, the Middle East and in Asia	LABORATORY P: LT, LDT S: LDT, RUO NEAR PATIENT None	
Orthomyxoviridae	High	High	Non-seasonal influenza	Flu	Respiratory	Yes	Worldwide	LABORATORY P: LT, LDT S: LDT, RUO NEAR PATIENT L: Ag	Y
Paramyxoviridae	High	High	Nipah virus	Nipah virus infection	Contact	Yes	Asia (incl Malaysia, Bangladesh, India)	LABORATORY P: LT, LDT, RUO S: ADT NEAR PATIENT P: POC	Y
Peribunyaviridae	Low	Medium	Oropouche virus	Oropouche fever	VBD	Yes	Caribbean and Latin America	LABORATORY P: LDT, RUO S: ADT NEAR PATIENT None	
Phenuiviridae	Low	Medium	Rift Valley fever virus	Rift Valley fever	VBD Contact	Yes	Mostly occurs in Africa	LABORATORY P: LT, LDT S: LDT, RUO NEAR PATIENT None	
Phenuviridae	Low	Medium	SFTS virus	Severe fever with thrombocytopenia virus syndrome	VBD Contact	No	Sporadic cases in Asia	LABORATORY P: LDT, RUO S: ADT NEAR PATIENT None	
Picornaviridae	High	High	Enterovirus D68, A71	Acute flaccid myelitis	Food and water. Respiratory	No	Worldwide	LABORATORY P: LT, LDT S: RUO NEAR PATIENT P: POC; L: Ag	Y (Enteroviruses, not specific)
Pneumoviridae	Medium	High	Human metapneumo-virus	hMPV infection	Respiratory. Contact	No	Worldwide	LABORATORY P: HT, LT, LDT NEAR PATIENT P: POC; L: Ag	Y
Poxviridae	Medium	High	MPOX virus (clade I)	MPOX	Contact	Yes	Central Africa with sporadic cases elsewhere	LABORATORY P: LT, LDT, ADT NEAR PATIENT None	Y
Togaviridae	Low	Medium	Chikungunya virus	Chikungunya	VBD	No	Outbreaks in Africa, Asia and the Americas	LABORATORY P: LT, LDT S: LT, LDT, RUO NEAR PATIENT P: POC; L: Ab	

Table B: Viral pathogens identified through literature review. Specific information on transmission, geographic spread and diagnostics availability garnered from the WHO website with additional information on detection in wastewater or application of WBE from this current literature review. Blank cells indicate that data are not available.

Viral family	Overall pandemic potential	Overall epidemic potential	Notable pathogen	Disease	Human-to-human transmission	Zoonosis	Geographical spread	Diagnostics availability	Detected in WW or WBE applied
Paramyxoviridae	Low	High	Measles virus	Measles	Airborne, droplets	No	Global	Widely available	Y
Matonaviridae	Low	High	Rubella virus	Rubella	Airborne, droplets	No	Global	Widely available	Y
Herpesviridae	Low	High	Varicella-zoster virus	Varicella (Chickenpox)	Airborne, droplets, contact with blisters	No	Global	Widely available	Y
Orthomyxoviridae	High	High	Influenza A/B	Influenza	Airborne, droplets, surfaces	Yes	Global	Widely available	Y
Orthomyxoviridae	Medium	Medium	H5N1, H7N9	Avian Influenza	Airborne, animal contact	Yes	Global, mainly Asia	Limited	Y
Poxviridae	Low	Medium	MPOX virus	MPOX	Contact, respiratory droplets	Yes	Africa, global spread in outbreaks	Available	Y
Picornaviridae	Low	Medium	Poliovirus	Poliomyelitis	Faecal-oral	No	Endemic in some regions	Widely available	Y
Reoviridae	Low	High	Rotavirus	Rotavirus	Faecal-oral	No	Global	Widely available	Y
Caliciviridae	Low	High	Norovirus	Norovirus	Faecal-oral, surfaces	No	Global	Widely available	Y
Adenoviridae	Low	Medium	Adenovirus	Adenovirus	Airborne, contact	No	Global	Widely available	Y
Retroviridae	Medium	High	Human Immunodeficiency Virus	HIV	Bloodborne, sexual contact, vertical transmission	No	Global	Widely available	Y
Hepadnaviridae	Low	High	Hepatitis B virus	Hepatitis B	Bloodborne, sexual contact	No	Global	Widely available	Y
Flaviviridae	Low	High	Hepatitis C virus	Hepatitis C	Bloodborne	No	Global	Widely available	Y
Papillomaviridae	Low	High	HPV	Human Papillomavirus	Sexual contact	No	Global	Widely available	Y
Picornaviridae	Low	High	Rhinovirus	Rhinovirus	Airborne, droplets, surfaces	No	Global	Widely available	Y
Picornaviridae	Low	Medium	Coxsackievirus	Coxsackievirus	Faecal-oral, respiratory droplets	No	Global	Limited	Y
Astroviridae	Low	Medium	Astrovirus	Astrovirus	Faecal-oral	No	Global	Limited	Y
Picornaviridae	Low	Medium	Hepatitis A virus	Hepatitis A	Faecal-oral	No	Global	Widely available	Y
Hepeviridae	Low	Medium	Hepatitis E virus	Hepatitis E	Faecal-oral, zoonotic (pigs)	Yes	Global	Limited	Y
Flaviviridae	Low	Medium	West Nile virus	West Nile Virus	Mosquito-borne	Yes	Americas, Africa, Europe	Limited	Conflicting Reports
Paramyxoviridae	Low	Medium	Human parainfluenza viruses	Parainfluenza	Airborne, droplets	No	Global	Widely available	Y
Pneumoviridae	Low	Medium	Human metapneumovirus	Human Metapneumovirus	Airborne, droplets	No	Global	Limited	Y
Caliciviridae	Low	Medium	Sapovirus	Sapovirus	Faecal-oral	No	Global	Limited	Y
Coronaviridae	High	High	SARS-CoV-2	COVID	Airborne, droplets	Yes	Global	Widely available	Y

Table C: Priority bacterial pathogens published by UK Health Security Agency with additional information on detection in wastewater or application of WBE from this current literature review. Blank cells indicate that data are not available. Diagnostics availability relates to tests specific for the notable pathogen listed. P = PCR, S = serology, L = LFD, HT = high throughput, LT = low throughput, LDT = laboratory developed test, RUO = research use only, ADT = academic developed test, Ag = antigen, Ag RUO = antigen research use only, Ab = antibody, Ab RUO = antibody research use only, POC = point of care.

Notable pathogen	Bacterial family	Disease	Human-to-human transmission	Zoonosis	AMR concern	Geographical spread	Diagnostics availability	Detected in WW or WBE applied
Bacillus anthracis	Bacillaceae	Anthrax	Contact. Respiratory.	Yes	No major concern	Worldwide	LAB P: LDT, RUO S: RUO NEAR PT P: POC RUO L: Ag RUO	Y
Coxiella burnetii	Coxiellaceae	Q-fever	VBD	Yes	No major concern	Worldwide (exception of New Zealand)	LAB P: LT, LDT S: LT, LDT, RUO NEAR PT None	
Klebsiella pneumoniae	Enterobacteriaceae	Pneumonia, Bloodstream and wound infections	Contact	No	Critical	Worldwide	LAB P: HT, LT, LDT S: RUO NEAR PT P: POC	Y
Escherichia coli (ETEC, STEC, EPEC)	Enterobacteriaceae	Gastro-intestinal disease, HUS	Food or water	Yes	Critical	Worldwide	LAB P: HT, LT, LDT S: LDT, RUO NEAR PT P: POC; L: Ag	Y E. coli as a species
Escherichia coli (UPEC)	Enterobacteriaceae	Cystitis, Pyelonephritis,	Food or water. Contact	Yes	Critical	Worldwide	LAB P: ADT NEAR PT None	Y E. coli as a species
Yersinia pestis	Enterobacteriaceae*	Plague	VBD. Respiratory	Yes	No major concern	USA, South America, Africa and Asia	LAB P: LDT, RUO S: RUO NEAR PT None	Y
Francisella tularensis	Francisellacae	Tularaemia	VBD. Food or water	Yes	No major concern	Northern hemisphere	LAB P: LT, LDT, RUO S: LT NEAR PT P: POC L: Ab	
Acinetobacter baumannii	Moraxellaceae	Pneumonia, Bloodstream infections, UTI	Contact	No	Critical	Worldwide	LAB P: HT, LT, LDT S: RUO NEAR PT P: POC	
Neisseria gonorrhoeae	Neisseriaceae	Gonorrhoea	Contact	No	High	Worldwide	LAB P: HT, LT, LDT NEAR PT P: POC L: Ag	
Staphylococcus aureus	Staphylococcaceae	Cellulitis, Endocarditis, Pneumonia	Contact	No	High	Worldwide	LAB P: HT, LT, LDT S: RUO NEAR PT P: POC	Y
Group A Strep	Streptococcaceae	Pharyngitis, Impetigo, Scarlet fever, Septicaemia	Respiratory. Contact.	No	Medium	Worldwide	LAB P: HT, LT, LDT S: LDT, RUO NEAR PT. P: POC; L: Ag	Y
Group B Strep	Streptococcaceae	Chorioamnionitis, Pneumonia, Meningitis, Septicaemia	Contact. Intra-partum	No (typically)	Medium	Worldwide	LAB P: HT, LT S: LDT, RUO NEAR PT. P: POC; L: Ag	
Streptococcus pneumoniae	Streptococcaceae	Pneumonia, Meningitis	Respiratory	No	Medium	Worldwide	LAB P: LT, LDT S: LDT, RUO NEAR PT P: POC; L: Ag	

*reclassified as *Yersiniaceae*

Table D: Bacterial diseases identified through literature review. Specific information on transmission, geographic spread and diagnostics availability garnered from the WHO website with additional information on detection in wastewater or application of WBE from this current literature review. Blank cells indicate that data are not available.

Notable pathogen	Bacterial family	Disease	Overall pandemic potential	Overall epidemic potential	Human-to-human transmission	Zoonosis	Geographical spread	Diagnostics availability	Detected in WW or WBE applied
Bordetella pertussis	Alcaligenaceae	Pertussis	Low	High	Airborne, droplets	No	Global	Widely available	Y
Mycobacterium tuberculosis	Mycobacteriaceae	Tuberculosis	Medium	High	Airborne, droplets	No	Global	Widely available	Y
Campylobacter spp.	Campylobacteriaceae	Campylobacteriosis	Low	Medium	Foodborne	Yes	Global	Widely available	Y
Salmonella spp.	Enterobacteriaceae	Salmonellosis	Low	Medium	Foodborne	Yes	Global	Widely available	Y
Shigella spp.	Enterobacteriaceae	Shigella	Low	Medium	Faecal-oral	No	Global	Widely available	Y

Table E: Protozoal diseases identified through literature review. Specific information on transmission, geographic spread and diagnostics availability garnered from the WHO website with additional information on detection in wastewater or application of WBE from this current literature review. Blank cells indicate that data are not available.

Notable pathogen	Protozoal family	Disease	Overall pandemic potential	Overall epidemic potential	Human-to-human transmission	Zoonosis	Geographical spread	Diagnostics availability	Detected in WW or WBE applied
Cryptosporidium spp.	Cryptosporidiidae	Cryptosporidiosis	Low	Medium	Waterborne, faecal-oral	Yes	Global	Limited	Y
Entamoeba histolytica	Endamoebidae	Entamoeba histolytica	Low	Medium	Faecal-oral, waterborne	No	Global	Limited	Y
Giardia lamblia	Hexamitidae	Giardiasis	Low	Medium	Faecal-oral, waterborne	Yes	Global	Limited	Y

2.3 Detection methods and technologies search results

Optimisation of the search terms in Web of Science (Feb 2025) returned 36 review articles, which were manually screened for relevance and narrowed down to 30 review articles. Since detection methods do not have geographical boundaries, we did not set any geographical limits on the literature search, so in most cases, the technologies have been implemented in international contexts. Methods used to detect SARS-CoV-2 were the most dominant due to the recent COVID-19 pandemic, however, detection methods for other pathogens were also captured e.g. MPOX (Appendix 3). Each identified pathogen was subsequently reviewed in the literature to determine if it had been detected using WBE. The detection methods used are detailed in Appendix 2. The majority of these methods were based on a polymerase chain reaction (PCR) approach, and therefore already covered in our initial search.

2.4 Advantages of WBE for infectious disease monitoring

Real-time monitoring can complement existing clinical and contact tracing approaches to enhance timely action against infectious disease outbreaks. For example, clinical testing may not be rapid for some pathogens and does not always capture individuals who do not seek medical intervention for their illness, are asymptomatic or only have mild symptoms.

Post hoc analysis for recent pandemics showed a lag between initial disease circulation and detection. SARS peaked in February 2003 but was first detected in November 2002. MERS peaked in September 2012, but initial cases were detected as early as April 2012. Influenza H1N1 is now suspected to have been spreading for 4 months prior to detection. Some studies indicated that SARS-CoV-2 was potentially circulating in Europe weeks prior to the first confirmed cases (e.g. La Rosa et al., 2021), and earlier warning of these pandemics could have provided opportunity for better preparedness.

WBE has long been demonstrated to capture disease outbreaks. In the 1930s, poliovirus was detected in sewage samples (Trask and Paul, 1942) and in the 1940s, wastewater samples were used to find carriers of typhoid (Moore, 1951, Moore et al., 1952). However, the recent COVID-19 pandemic saw WBE gain traction as a useful tool for monitoring infectious diseases amongst large populations. Given the occurrence of asymptomatic infection (He et al., 2021), estimated to affect between 0 and <40% of individuals (Shi et al., 2024; Wang et al., 2025), WBE provides a more accurate indication of disease prevalence. The approach served as an early warning system for tracking. For example, Kumar et al. (2021) demonstrated that in an Indian study, SARS-CoV-2 RNA in influent wastewater spiked 1 to 2 weeks before clinically confirmed cases. A similar lead time between wastewater measurements and general hospital admissions and ICU admissions (8-11.6 days and 14.8-17.7 days respectively) was reported by Schenk et al. (2023) who monitored SARS-CoV-2 RNA at wastewater treatment plants across Austria (lead time was shorter during the Omicron wave compared to Delta wave). Transmission as well as outbreaks were captured in near real time (Mao et al., 2020). The resolution gained from WBE was even found to capture outbreaks from new SAR-CoV-2 variants (Amman et al., 2022). This allowed timely decision-making regarding reopening efforts and directing resources.

Box 1. Benefits of WBE

- Early warning system
- Near real time surveillance
- Captures asymptomatic individuals and poor reporting
- Captures transmission as well as outbreaks
- Captures emerging and re-emerging infectious diseases (e.g. new variant strains)
- Allows timely decision making and resource planning

2.5 Detection methods and technologies

More established methods such as cultivation, nucleic acid detection (e.g., PCR) and immunological assays (e.g., Enzyme-Linked Immunosorbent Assay (ELISA)), while effective, often suffer from limitations such as being time-consuming, costly, and impractical for rapid detection in resource-limited settings. Some advanced techniques, such as spectroscopy-based approaches, offer more rapid results, but further research and development are necessary to optimise and validate their performance for large-scale wastewater monitoring applications. These methods are advanced for detecting certain targets but are still at the experimental stage for other targets. Emerging technologies offer promising alternatives due to their simplicity, rapid response, portability, low cost, and potential for real-time monitoring. However, they are still in the experimental phase, and some require validation in wastewater.

Table 6 gives an overview of the technologies that were returned from the literature review. Where the technologies have detected pathogens from our priority lists (Tables 1-5), it has been included in the table as 'notable pathogens', but they can also detect other pathogens not on our lists. For more detailed information on specific technologies and the range of pathogens they can detect, refer to Appendix 3.

The Technology Readiness Levels (TRLs) are scored from 1 (basic principles) to 9 (operational) (Box 2) and therefore give an indication of the technologies already established or most likely to be implemented in the near future. Technologies at higher TRLs should be considered for use in the Scottish context.

2.5.1 Culture-based

Culture-based methods involve the growth of organisms on selective media (for bacteria) or cell culture (for viruses). This is usually followed by a biochemical identification method, for example, latex agglutination for *S. aureus*, however, molecular methods could also be used, for example, whole genome sequencing. Such approaches have been used to detect *Enterobacteriaceae* and parasites from wastewater. One advantage of isolating pathogens is that further phenotypic testing can be done such as antibiotic resistance testing. However, major disadvantages are that is it labour-intensive and, depending on the organisms, incubation times can be long. As such, Chen et al, (2024), state that this is not a viable approach for large-scale sewage monitoring (Chen et al., 2024).

2.5.2 Culture-independent detection of whole organisms

Whole cell/organism detection methods do not require growth for detection. Microscopy has been used to detect parasites either directly or after treatment and staining, while electron microscopy has been used to detect viruses. Fluorescence *in situ* hybridisation (FISH) locates nucleic acids in cells or sample matrices and counts specific microbial populations. Similarly, immunoassays (e.g. ELISA) can detect whole cells through the use of antibodies. Pathogens detected by these approaches include *Enterobacteriaceae* and *Salmonella*. Such methods are not susceptible to inhibitors like molecular methods (see below) but are labour intensive and time-consuming.

2.5.3 Polymerase Chain Reaction (PCR)

Molecular methods rely on extraction of genomic material. In methods based on Polymerase Chain Reaction (PCR), including quantitative PCR (qPCR), multiplex PCR, digital PCR (dPCR), real-time PCR (RT-PCR), multiplex PCR and reverse transcription quantitative PCR (RT-qPCR), specific gene markers are amplified, and for some methods, quantified to allow concentrations of genome equivalents to be determined. It is the most commonly used method for WBE for various pathogens (Table 6). During the COVID-19 pandemic, RT-qPCR was predominantly used in WBE in Scotland (Fang et al., 2022). Other notable pathogens detected by this approach include *E. coli*, *Klebsiella* spp., *Campylobacter* spp., *Salmonella* spp., *Cryptosporidium* spp., *Mycobacterium* spp., Group A *Streptococcus* measles virus, mumps virus, rubella virus, influenza, hepatitis A, E, adenovirus, astrovirus, zika virus, MPOX, polio virus, rotavirus, norovirus, human papillomavirus, rhinovirus, seasonal coronaviruses, coxsackievirus, parainfluenza and metapneumovirus.

2.5.4 Isothermal amplification

Isothermal amplification is similar to PCR, except that it occurs at a constant temperature, and in some cases, can occur at room temperature. Techniques include Loop mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), rolling circle amplification (RCA), recombinase polymerase amplification (RPA) and helicase-dependent amplification (HDA). Specific markers can be amplified (e.g. LAMP) or the entire genome (e.g. RCA). Variations in the technique arise depending on whether DNA or RNA is being amplified. Notable pathogens detected by this technique include SARS-CoV-2, Hepatitis C, avian influenza, Zika virus, *Cryptosporidium* spp.

2.5.6 Sequencing

Sequencing methods determine sequences of specific markers (e.g. Sanger sequencing, meta-taxonomic sequencing) or the entire genome (e.g. whole genome sequencing, metagenomics) to identify pathogens. With sequencing costs reducing, this is becoming a viable option for end users. However, the wealth of data this can generate (particularly the case for metagenomics) means that high performance computing and specialist training to deal with this data is required. Notable pathogens detected by this approach include *E. coli*, Coronavirus, *Giardia intestinalis*, *Entamoeba histolytica*, SARS-CoV-2, Astrovirus, Hepatitis C, Adenoviridae, Caliciviridae, Coronaviridae, Flaviviridae, Hepeviridae, Herpesviridae, Matonaviridae, Papillomaviridae, Picornaviridae, Poxviridae, Retroviridae, Togaviridae, Eastern equine encephalitis virus.

2.5.7 Spectrometry

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be used to identify microorganisms through the analysis of positively charged proteins as the sample is vaporized with laser irradiation. It has been used to detect *Salmonella* Typhi in wastewater. While this method is faster than biochemical examination, it lacks sufficient reference spectra in the database, leading to challenges in distinguishing between closely related species (Rychert, 2019).

Raman spectroscopy has also been used to detect pathogens. Single-cell Raman takes cellular spectral

fingerprints unique to the chemical composition of different microbial species. Raman-stable isotope probing (SIP) utilises the microbial uptake and integration of stable isotopes into cellular components to target microbes with specific metabolic features. Surface-enhanced Raman (SERS) utilises electromagnetic enhancement generated by excitation of surface resonance of nanometals. Raman is rapid and allows phenotypic characterisation at single-cell levels (Cui et al., 2021), however, it remains challenging to distinguish between pathogenic and non-pathogenic strains of the same species (Sil et al., 2020).

In China, Liquid chromatography–mass spectrometry (LC-MS/MS) was used to measure concentrations of a retroviral drug (lamivudine) to track Hepatitis B because it is predominantly used in the treatment of chronic Hepatitis B (Hou et al., 2020).

2.5.8 Microfluidics

Microfluidic technologies have been used to miniaturise laboratory processes for rapid detection of pathogens, often offering point-of-care testing. They have mostly been used to detect SARS-CoV-2. They include paper-based devices, lateral flow devices and Smartphone-based detection (links biosensors to smartphones for real-time monitoring). Paper-based devices are low cost, low-tech and disposable, making them suitable for use in limited-resource areas (Pan et al., 2022). However, the Smartphone-based technologies suffer from balancing the number of detection components attached to the smartphone and obtaining target accuracy and sensing (Jagannath et al., 2022).

2.5.9 Biosensors

A number of biosensors have been developed for pathogen detection to allow rapid and portable solutions and therefore have great potential for real-time monitoring. They can even offer point of care testing. They include electrochemical biosensors (e.g. field-effect transistor (FET)-based sensors, electrochemical impedance spectroscopy (EIS)), optical biosensors, phage-based biosensors using engineered bacteriophages, nanoparticle-based biosensors, and colorimetric biosensors. Some biosensors are still in the research phase and may require specialised reagents (e.g. nanoparticles) or equipment. Notable pathogens detected by these technologies include HIV, SARS-CoV-2, *E. coli*, *Salmonella* spp., enterovirus, hepatitis virus, parasites and *Bacillus anthracis*.

2.5.10 Emerging and Integrated Methods

Emerging and integrated technologies utilise the techniques detailed above in a higher throughput and portable way. They include 'lab-on-a-chip' solutions, integrated RT-LAMP microfluidic chip, CRISPR-based detection, and aptamer-based electrochemical chips. These also offer point-of-care testing and can give rapid results withing minutes to hours. However, these technologies are still in the experimental phase. Notable pathogens detected by these technologies include SARS-CoV-2, Zika virus, human papillomavirus, Dengue virus, Ebola virus, *Salmonella* spp., Avian influenza and *E. coli*.

Table F: Overview of detection technologies and methods. Data derived from multiple literature sources therefore both bacterial families and members of those families may be given.

Technology or method type	Example technologies	Notable pathogens detected	Advantages	Disadvantages	TRL
Cultivation	Selective media, enrichment, multiple tube fermentation, biochemical tests (e.g. agglutination tests), high throughput phenotypic tests (e.g. Biolog MicroLog)	*Enterobacteriaceae, Salmonella* spp., *Acinetobacter* spp., *Campylobacter* spp., *E. coli*, parasites, measles virus, enterovirus	Captures live organisms, high sensitivity and specificity, allows further phenotypic testing,	Labour-intensive, time consuming (days to weeks), difficulty in culturing certain pathogens, quantification can be difficult	9
Whole cell/organism detection	Microscopy, electron microscopy, FISH, immunoassay (e.g. ELISA)	*Enterobacteriaceae, Salmonella* spp., enterovirus	Not susceptible to inhibitors	Labour-intensive, time-consuming	9
PCR	PCR, Quantitative PCR (qPCR), digital PCR (dPCR), real-time PCR (RT-PCR), multiplex PCR and reverse transcription quantitative PCR (RT-qPCR), multiplex PCR	SARS-CoV-2, *E. coli, Klebsiella* spp., *Campylobacter* spp., *Salmonella* spp., *Cryptosporidium* spp., *Mycobacterium* spp., Group A *Streptococcus* , measles virus, mumps virus, rubella virus, influenza, hepatitis A, E, adenovirus, astrovirus, zika virus, MPOX, polio virus, rotavirus, norovirus, human papillomavirus, rhinovirus, seasonal coronaviruses, coxsackievirus, parainfluenza, metapneumovirus	Rapid, detects unculturable organisms, can be quantitative	Susceptible to inhibition	8-9
Isothermal amplification	Loop mediated isothermal amplification (LAMP), nucleic acid sequence-based amplification (NASBA), rolling circle amplification (RCA), recombinase polymerase amplification (RPA) and helicase-dependent amplification (HDA)	SARS-CoV-2, Hepatitis C, Zika virus, Avian influenza, *Cryptosporidium* spp.	Rapid, detects unculturable organisms, can be quantitative	Susceptible to inhibition	8-9
Sequencing	Sanger sequencing, meta-taxonomics, metagenomics, whole genome sequencing	*E. coli*, Coronavirus, *Giardia intestinalis*, *Entamoeba histolytica*, SARS-CoV-2, Astrovirus, Hepatitis C, Adenoviridae, Caliciviridae, Coronaviridae, Flaviviridae, Hepeviridae, Herpesviridae, Matonaviridae, Papillomaviridae, Picornaviridae, Poxviridae, Retroviridae, Togaviridae, Eastern equine encephalitis virus	High throughput, large datasets, detects unculturable organisms, can be quantitative, metagenomics captures entire microbial populations	High performance computing and specialist training required	8-9
Spectrometry	Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS), Raman spectroscopy (single-cell, SIP, SERS), LC-MS/MS	*Salmonella* Typhi, *Bacillus* spp., Hepatitis B	Rapid	MALDI-TOF MS: Limited reference spectra Raman: low accuracy between pathogenic and non-pathogenic strains	8
Microfluidics	Paper-based devices, lateral flow devices, Smartphone-based detection	SARS-CoV-2	Low cost, low tech, rapid, offers point-of-care testing, potential use in resource-limited areas	Smartphone-based methods may suffer from low accuracy and sensitivity	3
Biosensors	Electrochemical biosensors, optical biosensors, phage-based biosensors, nanoparticle-based biosensors, colorimetric biosensors	HIV, SARS-CoV-2, *E. coli, Salmonella,* enterovirus, hepatitis virus, parasites, *Bacillus anthracis*	Rapid, portable, offers point-of-care testing. High sensitivity and specificity. High potential for real-time monitoring.	Some still in the research phase. May require specialised reagents or equipment.	3-4
Emerging and integrated methods	'Lab-on-a-chip' solutions, integrated RT-LAMP microfluidic chip. CRISPR-based detection, aptamer-based electrochemical chips	SARS-CoV-2, Zika virus, human papillomavirus, Dengue virus, Ebola virus, *Salmonella* spp., Avian influenza, *E. coli*	High throughout. Rapid (minutes to hours), portable, offers point-of-care testing. High sensitivity and specificity.	Still in experimental phase.	3

Box 2. Technology Readiness Levels

Level 1: Basic principles observed and reported

Level 2: Technology concept or application formulated

Level 3: Analytical and experimental critical function or characteristic proof-of-concept

Level 4: Technology basic validation in a laboratory environment

Level 5: Technology basic validation in a relevant environment

Level 6: Technology model or prototype demonstration in a relevant environment

Level 7: Technology prototype demonstration in an operational environment

Level 8: Actual technology completed and qualified through test and demonstration

Level 9: Actual technology qualified through successful mission operations

2.6 Sensitivity and specificity

Sensitivity and specificity data were not consistently reported (see Appendix 3). For some technologies, this was not reported at all. When reported, in some cases, they were described qualitatively (e.g. "excellent", "good" or "high") rather than with specific values. When limit of detection (LOD) was reported, they were presented in varying units (e.g. number of cells, specific gene markers, whole genomes, cellular components, etc), making direct comparisons challenging.

Technologies that rely on the growth of organisms (e.g. culture-based, whole genome sequencing) will miss viable but non culturable (VBNC) organisms. Specifically, bacteria can become dormant when under environmental stress, but come out of dormancy when conditions are favourable (Colwell, 2000). This will result in lower sensitivity and under-estimation of the pathogen burden. This could be circumvented by technologies that rely on detection of metabolites because VBNC bacteria maintain a low metabolic activity, but do not divide (Zhang et al., 2018). Moreover, the selective media used for isolating pathogens were developed for clinical applications and do not perform well on environmental samples (Milligan et al., 2023).

Technologies that detect genomic DNA or RNA (e.g. qPCR, metagenomics) will detect all organisms, whether they are viable or not. This is a particular issue in drinking water compliance where the treatment such as UV and chlorine will kill or inactivate organisms, but due to the persistence of nucleic acids, they are still detected (Girones et al., 2010). This may be less important for WBE given that detection, whether viable or not, gives an indication that it may be circulating in the population. In any case, the methods used to extract the nucleic acids will have biases. Typically, commercial kits are used, which utilise any combination of chemical, mechanical, thermal or enzymatic methods to extract genomic material. The most common commercial kits used to extract genomic DNA from activated sludge showed that some were unable to extract DNA from many Gram-positive bacteria (Guo and Zhang, 2013).

Of all the methods found, PCR-based approaches were by far the most frequently used. PCR amplification from wastewater can be subject to inhibition (Zafeiriadou et al., 2024), though others have found that competitive amplification of similar sequences to be a major mechanism of reduced sensitivity (Volkmann et al., 2007). Both effects result in under-estimation of pathogen load. The key factor affecting PCR specificity is primer design (e.g. (Hong et al., 2009)). By extension, sequencing approaches (e.g. meta-taxonomics) will suffer from the same drawbacks. Digital and droplet digital PCR have been shown to be more sensitive to lower concentrations of target microbes compared to traditional qPCR and is not as susceptible to inhibition (Duong et al., 2023). Other molecular methods such as RT-LAMP has high-specificity for viral RNA without requiring the thermal amplification cycles that PCR methods require, resulting in relatively quick and less-expensive method for quantification, which has been increasingly used for SARS-CoV-2 detection (Bivins et al., 2022a, Bivins et al., 2022b).

Advanced methods such as spectrometry-based methods bypass issues associated with culture-based and nucleic acid-based approaches, therefore giving high sensitivity. However, they lack reference standards to allow high specificity and cannot distinguish between pathogenic and non-pathogenic strains.

Microfluidic technologies, biosensors and emerging technologies offer varying levels of sensitivity and specificity. In general, colorimetric and fluorescent sensors have poorer LODs than electrochemical sensors. On the other hand, phages are highly specific, capable of binding to a single bacterial species or strain, achieving low detection limits (down to 10 CFU). Their lytic cycle amplifies signals *in situ* by producing progeny phages, enhancing detection sensitivity. With functional surface groups, phages can be immobilised on sensor surfaces for improved integration (Bayat et al., 2021). Future research should pay attention to enhancing sensor sensitivity and specificity, but their potential to provide portable and cost-effective devices for diverse pathogens can improve public health protection and environmental monitoring.

Box 3. Sensitivity and Specificity

- Inconsistent reporting on sensitivity and specificity makes it difficult to evaluate
- Culture-based methods cannot detect viable but non-culturable (VBNC) organisms
- Nucleic acid-based methods are affected by DNA extraction bias
- PCR-based methods are affected by inhibition, competitive binding and primer bias
- Spectrometry-based methods bypass issues with VBNC and inhibition, but are affected by lack of reference standards
- Microfluidic technologies, biosensors and emerging technologies offer varying levels of sensitivity and specificity

2.7 Efficacy in wastewater

Since "wastewater" was used as a search term, almost all papers focused on application in wastewater. Where the technology was not applied to wastewater, authors state that the technology is ready to be applied to wastewater, though this may still require validation.

As mentioned previously, there were some details of specific technologies where pathogen detection in wastewater is an issue due to the presence of multiple pathogens. For PCR-based approaches, competitive amplification of similar sequences was found to be a major mechanism of reduced sensitivity (Volkmann et al., 2007). By extension, this would also apply to isothermal amplification-based approaches where primers are used, and sequencing-based approaches such as meta-taxonomics. Raman allows phenotypic characterisation at single-cell levels (Cui et al., 2021), however, it remains challenging to distinguish between pathogenic and non-pathogenic strains of the same species (Sil et al., 2020). We could not find any other details of how the other methods and technologies perform in the presence of multiple pathogens, especially given that some were specifically designed to pick up multiple pathogens (e.g. biosensors). This may become more apparent for emerging technologies as they become increasingly used in wastewater.

However, there are other factors that need to be considered for any of these technologies to be effective in wastewater.

Spatio-temporal changes in pathogen load will influence where and when to sample wastewater. Fluctuations in ammonia loads (a waste product present in urine) in wastewater was observed indicating diurnal activities (there were usually high fluxes in the morning) and movements of populations weekly (i.e. commuters) and seasonally (i.e. holidays) (Been et al., 2014). Fluctuations in pathogen load are likely to follow similar patterns to ammonia, so knowing when to sample is critical to ensure that early stages of pathogen emergence or re-emergence is captured. Moreover, distinct trends for SARS-CoV-2 were observed in sewersheds in an urban centre, outlying suburban areas and outlying urbanised districts (Haak et al., 2022), highlighting the need to identify specific areas for testing.

Further considerations when sampling are transportation times and preservation since samples will need to be transported from wastewater treatment plants (WWTPs) to testing laboratories, plus preservation in the laboratory until the samples can be processed. Cold storage between 4°C and -80°C has been shown to be essential for the preservation of wastewater markers (both bacterial and viral). Wastewater storage at 4°C maintained sample integrity for up to 12 weeks for most markers, indicating that transport at 4°C would protect the sample from degradation. It is noteworthy that island and remote rural sampling can be more challenging in terms of resourcing and sample transport arrangements. In terms of storage, viral markers were stable when frozen for up to one year, however, freeze-thaw cycles significantly degraded RNA viruses, leading to under-estimation of pathogen load (Webster et al., 2025). Considerations must also be given to availability of Category II laboratories with sufficient storage capacity at -80°C.

One of the biggest challenges with most technologies is the primary concentration of the target organism. The large volume of wastewater dilutes the relatively low pathogen loadings, so the primary concentration step allows the organism to be in sufficient amounts to be above the limit of detection of the technology or method. Dilution effects of wastewater are constantly changing and varies between WWTPs. Primary concentration was considered the most critical step in the detection of SARS-CoV-2 using WBE (Lu et al., 2020).

Box 4. Efficacy in wastewater

- Established technologies already validated for use in wastewater
- Some emerging technologies still require validation in wastewater
- PCR-based and spectrometry-based approaches may be less effective in wastewater where multiple pathogens are present
- Spatio-temporal trends in pathogen load will influence sampling
- Potential degradation during sample transport and storage
- Primary concentration crucial to allow detection from wastewater

2.8 Correlation between WBE and community cases

Variation in flow of wastewater within a WWTP and between WWTPs results in variable levels of dilution of pathogen loads, which presents a challenge in effectively presenting quantitative data. This highlights the need to normalise data with other parameters such as total bacterial population, flow rate and total population served by the WWTP, expressing results as unit/person/unit time (Tiwari et al., 2024).

Within the Scottish COVID-19 Wastewater monitoring program, an early study from 2021 (Fitzgerald et al., 2021) observed a nation-wide rank correlation between daily viral RNA concentration and the number of COVID-19 cases detected in the corresponding catchment area in the previous week of 0.79, but also reported considerable site-specific correlation. An evaluation study conducted by Public Health Scotland (Scotland, 2024) reports overall national-level correlations with wastewater COVID-19 levels smoothed by 7-day moving averages of 0.86 for ONS prevalence estimates and 0.70 for cases detected through diagnostic testing. This study also shows that the correlation was higher when the Delta variant dominated and dropped considerably once Omicron took over. Yang et. Al. (2025) estimated that rates of viral shedding were lower when the Omicron variant dominated vs. the Delta variant (both of which were lower than those estimated for the earlier variants). This indicates that the relationship between virus levels measured in wastewater and cases can also depend on the specific variant. However, other variables can also impact correlations between wastewater and population level indicators. For example, temperature (and therefore seasonality) and presence and concentrations of chemicals and/or biological components in the wastewater may affect RNA degradation and persistence in sewer systems is uncertain (Li et al, 2023). Further, different phases of the pandemic and impacts of control measures may have influenced correlations.

A WBE study in Hawaii used culture-based methods to monitor *Salmonella* spp. in municipal wastewater serving the Honolulu area for a 54-week period. Health data on salmonellosis cases was also obtained from the Hawaii Department of Health State Laboratories covering the same period as the WBE study. Results showed positive and significant linear and rank correlation with clinical salmonellosis case numbers. Notably, one out-break associated Paratyphi B strain was captured by both WBE and clinical cases, demonstrating the feasibility of using bacterial pathogens for timely indication of enteric disease in the community (Yan et al., 2018).

In another WBE study in USA, specifically Houston and El Paso, SARS-CoV-2, influenza virus, and MPOX virus were measured in municipal wastewater using sequencing methods. Results showed a positive correlation between case data and positivity rate for SARS-CoV-2 summer and winter waves, and the wastewater signal in Houston (R=0.5–0.78) and case data from El Paso (R = 0.59 - 0.73). This finding was confirmed by qPCR of SARS-CoV-2 for both Houston (R=0.64) and El Paso (R = 0.84) (Tisza et al., 2023).

In the same study, Influenza A sequencing data was consistent with case reporting in Houston (R = 0.9) and was confirmed by qPCR (R = 0.57 – 0.73). Influenza variants H3N2 and H1N1 were detected, which was consistent with clinical subtyping of this flu season in Texas (Tisza et al., 2023). MPOX was detected at low abundance in Houston wastewater samples though 1050 cases were reported (R = 0.46). MPOX was not detected in El Paso wastewater, consistent with very few reported clinical cases (only 10 reported) (Tisza et al., 2023).

One of the stakeholders noted that a complication for developing correlations between wastewater analyses and population levels of infection is the availability and access to clinical data. In their experience, this has not always been readily available in a suitable time-frame due to the existing pressures on staff administering the data (Appendix 4).

Box 5. Correlations to the population

Demonstrated correlations with community cases:

- SARS-CoV-2
- *Salmonella* spp.
- Influenza virus
- MPOX virus

3 Results - O2. The cost-effectiveness of wastewater surveillance compared to traditional surveillance methods (e.g., clinical testing, active case finding).

3.1 Costs associated with WBE

To calculate costs of WBE per person, considerations need to be made for:

- Personnel salaries (e.g. sampling team, laboratory technicians, bioinformaticians and specialists)
- Sampling equipment
- Sample transport
- Laboratory equipment
- Laboratory consumables and reagents

The cost of WBE surveillance was estimated for SAR-CoV-2 from pilot sites in Malawi and Nepal. The Malawi sites were within Blantyre (~200km^2) which is dominated by informal wastewater disposal via urban rivers. Eighty sites, representing the entire city were sampled fortnightly for a year. Their WBE costs included consumables, equipment, human resource time costs (i.e. salaries) and overhead costs. The Nepalise sites were in Kathmandu, also sampled fortnightly at 23 sites for six months. The size of geographic areas covered was not reported but the population size was ~ 2.7 million people. Sampling sites included municipal sewers, squatter areas, sewers from housing, wastewater treatment plant influent, river sites, and a SARS-COV-2 hospital sewer. The cost per person per year in the catchment areas ranged from $USD0.07 – $USD0.10 in Malawi and $USD0.07 – $USD0.13 in Nepal (Ngwira et al., 2022). This study did not give costs for diagnostic testing.

The cost of WBE surveillance for SAR-CoV-2 was also estimated for rural and urban sites in the USA. Their estimated costs per person per year were $0.10 in rural areas and $0.005 in urban areas (Gawlik et al., 2021). This difference suggests that factors such as population size, consumables, labour costs and proximity to testing laboratories may influence costs for WBE.

WBE cost estimates could not be found for the UK. The cost estimates derived from the literature indicate very different costs per head of population for WBE vs. clinical diagnostics, however they fulfil different purposes – as clinical testing is unlikely to be applied at whole population level. It is anticipated that WBE complements existing clinical testing rather than being an alternative. Further research to understand the impact of WBE on reducing morbidity and mortality would provide a clearer understanding of cost-effectiveness. Referring to management of endemic SARS-CoV-2, Wu et al (2022) highlight that WBE and clinical testing can be integrated for cost-effective mass surveillance.

It should be noted that, even in cities, open defaecation is practiced by 17% of the population, so WBE would not capture this.

3.2 Sampling costs

General information on sampling costs was provided by Scottish Water (Appendix 4). Costs are mainly associated with organisational costs (administration and management) based on staff hourly rate and time spent. Additional costs may come from courier costs to the laboratory analysing the samples, also charged at an hourly rate and may be included in sampling hourly rate if appropriate. There was confirmation that there would be capacity to conduct additional sampling for WBE as long as these costs could be met. If there was to be a significant workload increase, the administration and management time may be higher.

3.3 Technology costs

Reporting of costs were inconsistent, making direct comparisons difficult (Appendix 3). Costs were not always reported, and where they were, often qualitative indications were given (e.g. "low cost", "cost-effective", "expensive", etc.) or they make qualitative comparisons to other methods (e.g. "more cost-effective than PCR") rather than specific values.

Culture-based methods or detection of whole organisms (e.g. microscopy, immune assays) are low cost to conduct, but due to the labour-intensive and time-consuming nature of these methods, the high cost comes from personnel salaries. Nucleic acid-based methods have high initial set up costs, e.g. the majority of thermal-cyclers for qPCR start at $20,000. Advanced techniques, such as spectrometry-based methods also involve high initial setup costs. However, these methods offer low per-sample costs once established. With sequencing costs decreasing, this is also becoming a more viable option. Some emerging technologies are low cost (e.g. paper-based biosensors). The COVID-19 pandemic raised manufacturing demand of sensors, which called for low-cost, high availability sensors that covered the total population in all geographical areas. The advancement of 3D printing and machine moulding could help increase production levels (Matheri et al., 2023).

3.4 The cost-effectiveness of wastewater surveillance compared to traditional surveillance methods

Costs for clinical testing or active case finding also could not be found in the literature and were not provided via conversations or emails with stakeholders, therefore cost comparisons to WBE could not be provided. Given international experience (see previous sections), it is anticipated that WBE would incur a lower cost per person, however other surveillance is unlikely to be applied at population level, therefore comparisons are difficult to evaluate. Stakeholders commented that a direct comparison would be difficult due to the very different nature of clinical vs. WBE type programmes (Appendix 4).

Box 6. Cost of WBE

- Costs for WBE should consider:
  - Personnel salaries
  - Sampling equipment
  - Sample transport
  - Laboratory equipment
  - Laboratory consumables and reagents
- WBE for SARS-CoV-2 estimated $0.07 – $0.10/person in Malawi and $0.07 – $0.13/person in Nepal.
- WBE for SARS-CoV-2 estimated $0.10/person in rural areas and $0.005/person in urban areas of the USA.
- No WBE cost estimates for the UK.
- Sampling costs mostly incurred from personnel salaries.
- Costs for specific technologies difficult to assess as reporting was poor.

4 Results - O3. Ethical/ legal considerations

The review by Doorn (2022) gives a comprehensive assessment of ethical considerations associated with WBE (Doorn, 2022), summarised as follows:

Privacy and the protection of personal data – although it has not yet been demonstrated that WBE data can be traced back to an individual, the technology in principle does allow this or at least to groups of individuals. Moreover, the smaller the catchment or for defined wastewater sources such as prisons, or nursing homes the higher the possibility of tracing back to an individual.

Data analytics, big data & decision making – wastewater has been proposed to complement census data for assessing population socio-economics including inference of education level, proficiency of English language, and presence of a physical or mental disorder. This may allow third parties to target specific groups of individuals, for example, insurance companies may apply higher insurance premiums in areas where wastewater data suggest an unhealthy lifestyle.

Public health ethics – within this context, WBE may offer a more equitable solution to disease surveillance because it is independent of socio-economic status. For example, not all areas are well represented in clinical test facilities.

Potential non-public health uses of wastewater data – consideration also needs to be given around the potential for wastewater data to be utilised for non-public health areas. For example, the detection of non-pathogenic substances such as drugs, both legal and illegal, and the implications of sharing wastewater data for purposes beyond public health (e.g. in prison settings).

Research ethics –The use of data derived from WBE could, depending on government or public health policies, be utilised to identify high-risk premises, which will then have negative implications for those residents if disproportionally strict measures are imposed. For example, if a single virus shedder is detected in a residential area, all of those residents will receive restrictive measures to protect the wider community, however, such measures may not be proportional to the situation (e.g. considering vaccination rates within that community).

Environmental and water ethics – Decisions on wastewater treatment may be made based on the presence of residues that affect human health, but different decisions may be made when the impact on aquatic animals or ecological health is considered.

The legal considerations of WBE were outlined in Gable et al (2020) using the COVID-19 pandemic as a case study (Gable et al., 2020). In the US, the key issue with WBE was how the data would be used. To protect the community, any positive results from WBE may lead officials to restrict the activities or movements of individuals (e.g. stay at home orders). However, enforcement of such orders could potentially violate the Fourth Amendment protections against unreasonable searches and seizures by the government.

If WBE is used in a way where policy directs it to lead to conditional screening programs, which requires individuals to be tested prior to being able to leave their home, enforcement of such programs potentially violates an individual's autonomy and right to refuse testing. While those individuals may not be physically forced to be tested, they may be subjected to other measures such as more restrictive conditions or a fine. In the US, for example, this raises Fourth amendment concerns.

WBE could lead to imposition of broader restrictions on whole communities (e.g. stay at home orders, sanitary cordons or mass quarantine order) without further testing. However, this form of civil confinement would need to meet strict scrutiny standards.

In addition, WBE could lead to public health authorities to impose social distancing such as business closures (leading to economic implications), prohibiting social gatherings and limiting activities. However, if measures are imposed on religious institutions, which leads to contact tracing and further restrictions, concerns about privacy and discrimination arise.

Perrault and Goodridge (2025) highlight the issue of human genomic bycatch, that presence of human DNA in wastewater, inadvertently sampled, could facilitate genomic analyses that can reveal sensitive information about genetic ancestry, health predispositions, and disease prevalence. This generates specific concerns around small, closed populations such as indigenous communities, in that these data heighten the risk of re-identification and could be misused to reinforce stereotypes, justify discriminatory policies, or exert social control. Without robust ethical governance, such applications may erode trust and exacerbate historical injustices. WBE is also used across Europe for monitoring of psychoactive substances (Avery et al., 2025), and the potential to couple these data with genomic data through which specific groups could be identified risks further marginalisation.

Human DNA is rarely a target in applications where DNA is extracted directly from the environment (eDNA), including in wastewater. Therefore, issues arising relate directly to the techniques applied to the extracted DNA. Where specific (non-human) targets are detected or amplified, as in the use of q-PCR-based techniques to detect and enumerate SARS-CoV-2 gene copies in wastewater, this is less likely to present an issue. However, the application of shotgun sequencing of eDNA would also obtain and sequence human DNA which could potentially be used to identify

individuals (Whitmore et al., 2023). Further, eDNA can be stored frozen for many years without degradation which could be considered to be an archive containing human as well as other environmental DNA.

Whitmore et al (2023) note that for the purpose of scientific publication and open data requirements, sequence data need to be deposited in public repositories. They note that open questions include whether sequencing data should be pre-filtered to remove human sequence data (likened to anonymisation of patient data), how filtering criteria would be defined and made transparent and who would be responsible for enforcing this. They also consider the possibility of applying blockers to prevent sequencing of human DNA, but report that studies have found human sequences present despite the application of blockers.

This underscores the necessity for stringent data governance frameworks and meaningful community engagement to prevent misuse and protect autonomy.

Stakeholder comments considered a number of aspects relating to ethical considerations. These included:

- Ensuring equity for rural and island populations in terms of access to WBE approaches.
- Awareness that certain age groups (e.g. infants or elderly people using incontinence pads or nappies) or communities (e.g. some Gypsy/Traveller communities) may be excluded from WBE monitoring.
- Information use should be planned.
- There may be issues around consent relating to smaller populations/potential identification of source.
- Ethical issues had not been encountered during COVID work but WWTPS were large and sources not identifiable.
- High profile infection data associated with specific locations could lead to stigmatisation.
- There are ethics around those working with samples containing pathogens, especially those that are of human origin (in the literature, Whitmore et al., 2023) also highlight that human sequences can arise from contamination from those working with samples, adding a further layer of complexity to ethics associated with laboratory and sampling team members.

4.1 Limitations of the Study

The short duration of this study was such that literature searches were largely restricted to higher level documents i.e. review papers. Rapid reviews and use of review papers as source material can be vulnerable to error and bias and use of review papers. Interviews can be subject to bias in selection of those interviewed and different priorities and interests of interviewees. However, the combined use of both approaches mitigates some of these vulnerabilities and provides complementarity.

5 Results - O4. Gaps in the research, recommendations including additional studies needed to improve global health monitoring.

5.1 Research gaps

- No WBE cost estimates could be found for the UK. Moreover, costs comparisons between WBE and traditional methods (e.g. clinical testing) will need to be conducted by relevant stakeholders to assess the cost-effectiveness of WBE.
- Spatial (i.e. between catchments, WWTPs) and temporal (e.g. diurnal, seasonal) fluctuations in pathogen loads is still not known for Scotland.
- The effects of sewer sample transport and sample storage on pathogen degradation are unclear.
- The effect of primary concentration methods on pathogen detection is still unclear.
- Reference standards are still required for some advanced technologies (e.g. MALDI-TOF MS).
- Validation is still required for emerging technologies.
- Even if the technology has been found to work in wastewater, validation is still required for performance on specific pathogens.

6 Recommendations to improve global health monitoring

6.1 Recommendations pertinent to Scotland

Towards furthering the application of WBE for wastewater monitoring in Scotland we make the following recommendations:

- Determine priority pathogens from Tables 1-5 based on likelihood, prevalence and severity of health risk.
- Define populations of interest for priority pathogens.
- Develop and implement protocol to assess ethical aspects of inclusion/exclusion, anonymity and human genomic bycatch.
- Identify best available methodology for prioritised pathogens and determine technical capability.
- Determine monitoring requirements (e.g. sampling intensity and numbers of sites required to be representative of populations of interest).
- Determine cost of applying WBE for prioritised pathogens based on all costs identified in Section 3.4.
- Establish External Quality Assessment Schemes (i.e. interlaboratory trials) to ensure comparability across institutions.

6.2 Methodological recommendations

- Follow up of this study to determine for which priority pathogens, WBE has been applied successfully in a research or applied context worldwide.
- Research is required to fully understand the effect of the methodological biases and limitations on the accuracy of WBE to capture infectious disease circulation in the population.
- Further research into the relative shedding of different pathogens by the human population into the wastewater systems, and the methods that link pathogen signals back to human numbers in a catchment.
- Rigorous testing and validation of emerging technologies, especially those not yet used in wastewater is required.
- Standardised reporting of technological specifications should be established to allow comparison.
- Establish External Quality Assessment Schemes (i.e. interlaboratory trials) to ensure comparability across institutions and countries.

6.3 Recommendations for pathogen reporting

- Constant re-assessment or 'horizon scanning' of emerging pathogens with pandemic potential should be conducted at regular intervals (e.g. annually).
- Further research is required to elucidate and minimise methodological biases and limitations on accuracy of WBE to represent infectious disease circulation in populations, including better understanding of rates of pathogen shedding to wastewater, impact of sewer transit/sample storage and optimal statistical and data analysis pipelines.
- Implementation of WBE trials for monitoring of prioritised pathogens should be undertaken to provide evidence of utility, efficacy and integration with clinical testing and contact tracing.
- We identified a wide range of detection methods, each with their own advantages and limitations. However, agreement on a standard detection method for WBE that could be implemented globally (especially in resource-limited areas) is recommended to allow direct comparisons.
- Standardised reporting of WBE results is recommended to allow direct global comparison.
- Establish External Quality Assessment Schemes (i.e. interlaboratory trials) to ensure comparability across institutions and countries.

6.4 Recommendations for coordinated monitoring

- Following generation of evidence of cost-effectiveness, significant investment in global infrastructure for sampling, transport and testing laboratories would be required to facilitate coordinated WBE at an international level.
- Development of a global open access database where WBE data and clinical data can be deposited is recommended for constant pathogen surveillance and vigilance. Database must be accessible and fit for purpose.
- Analysis of deposited data should take place in a timely manner and lead to action. Therefore, development of modelling or a decision support tool to translate data into resource planning is recommended.
- Formulation of pandemic action plans should include potential use of WBE to aid disease monitoring.

Appendices

Appendix 1. Search methods

To confirm emerging and re-emerging pathogens in Scotland, literature was searched for in Web of Science and Google Scholar. Search terms were: "infectious diseases" OR "endemic diseases" OR "pathogen" OR "emerging pathogens" OR "re-emerging pathogens" OR "enteric viruses" OR "blood-borne viruses" AND "Scotland" OR "UK" OR "temperate regions". We placed a time limit of 5 years, geographical limit to the UK only and article type to review articles only. Furthermore, grey literature was searched for in Google using the same search terms; no limits could be placed on this search.

To search for methods of detection in wastewater, literature was searched for in Web of Science only. This is because a large number of papers were returned, so we did not need to use Google Scholar also. Search terms were: "wastewater-based epidemiology" OR "WBE surveillance" OR "wastewater surveillance" OR "sewage surveillance" OR "wastewater monitoring" OR "sewage monitoring" AND "Detection methods" OR "Analytical methods" OR "Sensors" OR "Markers" OR "Indicators for tracking" AND "infectious diseases" OR "endemic diseases" OR "pathogen" OR "emerging pathogens" OR "re-emerging pathogens" OR "enteric viruses" OR "blood-borne viruses" OR "SARS-CoV-2" OR "influenza". We placed a time limit of 10 years and article type to review articles only. We did not place any geographic limits on this search. Other areas of interest with respect to the methods were sensitivity and specificity, (especially where multiple pathogens are present) and cost, however, we did not perform separate searches for these as they were already covered in the literature that was returned.

Appendix 2. Grey literature used to identify priority pathogens

Table A1: Pathogens with the potential to emerge or re-emerge in the UK and their potential to be tracked by WBE.

Disease/pathogen	Impacts the UK (Y/N)	Reference*	Able to track by WBE? (Y/N + method)	Reference
Measles	Y- cases ae seen across UK	Confirmed cases of measles in England by month, age, region and upper-tier local authority: 2025 - GOV.UK	Y - culture	(Benschop et al., 2017)
Mumps	Y- cases are seen across UK	Mumps: notifications and confirmed cases by oral fluid testing in England, 2013 to 2022 by quarter - GOV.UK	Y - RT-ddPCR	(Wu et al., 2024)
Rubella	Y- cases are seen across UK	Rubella (German measles): guidance, data and analysis - GOV.UK	Y - dPCR	(McCarthy et al., 2025)
Meningococcal	Y- cases are seen across UK	Meningococcal disease: guidance, data and analysis - GOV.UK	Presence has not yet been confirmed in wastewater. Detectable in urine (Neisseria meningitidis)	(Van Poelvoorde et al., 2025)
Varicella	Y	JCVI statement on a childhood varicella (chickenpox) vaccination programme - GOV.UK	Y	(Gentry et al., 2023)
Pertussis	Y	Information for individuals who have whooping cough - GOV.UK	Y (rarely observed) - qPCR	(Kim et al., 2024)
Tuberculosis	Y	With tuberculosis (TB) on the rise again, how can we prevent further spread? – UK Health Security Agency	Y - ddPCR	(Reddy et al., 2022)
Streptococcus spp.	Y	Group A streptococcal infections: first update on seasonal activity in England, 2023 to 2024 - GOV.UK	Y - qPCR	(Shrestha et al., 2024)
Influenza	Y	EoESIT_FluSlides_Sept2024.pdf	Y – RT-ddPCR	(Boehm et al., 2023)
Avian Influenza	Y	Bird flu (avian influenza): latest situation in England - GOV.UK	Y - biosensor	(Bonyadi et al., 2023)
MPOX	Y- control measures in place	Biological principles for control of MPOX in the UK: 4 nations consensus statement - GOV.UK	Y - PCR	(Chen and Bibby, 2022, Julian et al., 2024)
Poliomyelitis	Y- though infection/ illness rates are very low polio has been traced in UK	Polio - NHS	Y – cell culture and PCR	(Bowes, 2024)
Rotavirus	Y	National norovirus and rotavirus report, week 13 report: data to week 11 (data up to 16 March 2025) - GOV.UK	Y - PCR	(Chacón et al., 2021)
Norovirus	Y	National norovirus and rotavirus report, week 13 report: data to week 11 (data up to 16 March 2025) - GOV.UK	Y – review article	(Guo et al., 2022)
Adenovirus	Y	Data and surveillance - Adenovirus - Infectious diseases - Health protection - Population health - Public Health Scotland	Y – PCR & qPCR	(Shaheen et al., 2024)
Campylobacter spp.	Y	Campylobacteriosis Case Rates in the UK: An Expert Elicitation Exercise | Published in FSA Research and Evidence	Y – review article	(Zhang et al., 2023)
Escherichia coli	Y	Escherichia coli (E. coli): guidance, data and analysis - GOV.UK	Y - culture	(Mbogo, 2023)
Salmonella spp.	Y	Non-typhoidal Salmonella data 2013 to 2022 - GOV.UK	Y - culture	(Yan et al., 2018)
Shigella spp.	Y	Warning after rise in extremely drug-resistant Shigella - GOV.UK	Y – review article	(Khan et al., 2025)
Yersinia enterocolitica	Y	Forgotten but not gone: Yersinia infections in England, 1975 to 2020 - UK Health Security Agency	Y - PCR	(Kim et al., 2024)

Cryptosporidiosis	Y	Preliminary investigation of a significant national Cryptosporidium exceedance in the United Kingdom, August 2023 and ongoing - UK Health Security Agency	Y – review article	(Zahedi et al., 2021)
Entamoeba histolytica	Y	Interim Public Health Operational Guidelines for Amoebiasis	Y – meta-taxonomics	(Rozo-Montoya et al., 2023)
Giardia spp.	Y	Giardia duodenalis in the UK: current knowledge of risk factors and public health implications | Parasitology | Cambridge Core	Y – review article	(Zahedi et al., 2021)
human immunodeficiency virus (HIV)	Y	UK HIV statistics - National AIDS Trust	Y - biosensor	(Jiménez-Rodríguez et al., 2022)
hepatitis B (HBV)	Y	Hepatitis B in England 2024 - GOV.UK	Y – LS/MS MS	(Hou et al., 2020)
Hepatitis C	Y	Hepatitis C in England 2024 - GOV.UK	Y – PCR then sequencing	(Cheshomi et al., 2025)
HPV	Y	United Kingdom: Human Papillomavirus and Related Cancers, Fact Sheet 2023	Y - PCR & qPCR	(Shaheen et al., 2024)
Rhinovirus	Y	Other Respiratory Viruses | UKHSA data dashboard	Y - RT-ddPCR	(Boehm et al., 2023)
Coxsackievirus	Y	Coxsackievirus A6 U.K. Genetic and Clinical Epidemiology Pre- and Post-SARS-CoV-2 Emergence - PubMed	Y - qPCR	(Shrestha et al., 2025)
Astrovirus	Y- studied	Enteric virus unit (EVU): diagnostic and reference services - GOV.UK	Y - RT-ddPCR	(Wolken et al., 2024)
Hepatitis A	Y- Low risk	Hepatitis A | NHS inform	Y - RT-PCR	(Fantilli et al., 2023)
Hepatitis E	Y	Hepatitis E: symptoms, transmission, treatment and prevention - GOV.UK	Y - RT-qPCR	(Rau et al., 2024)
West Nile fever mammals/birds zoonotic	Y- probability of infection in UK population is very low	Qualitative assessment of risk West Nile virus presents to UK health	Conflicting Reports	(O'Brien and Xagoraraki, 2019)
Eastern equine encephalitis	Y- Very rare- little to no risk currently	Case report: Eastern equine encephalitis virus imported to the UK - Harvala - 2009 - Journal of Medical Virology - Wiley Online Library	Y - qPCR	(McCall et al., 2020)
Parainfluenza	Y	National flu and COVID-19 surveillance report: 20 February (week 8) - GOV.UK	Y - RT-ddPCR	(Boehm et al., 2023)
Human metapneumovirus (hMPV)	Y	HMPV cases rise in England - should we be worried about it? | UK News | Sky News	Y - RT-ddPCR	(Boehm et al., 2023)
Sapoviruses	Y- has been detected in past- limited literature	Characterization of sapoviruses collected in the United Kingdom from 1989 to 2004 - PubMed	Y - qPCR	(McCall et al., 2020)
Henipaviruses	Low Risk	Qualitative Release Assessment to Estimate the Likelihood of Henipavirus Entering the United Kingdom - PMC	Potential	(Grassly et al., 2024)
SAR-CoV-2	High Risk	Priority Pathogens: The disease families which require urgent scientific research – UK Health Security Agency	Y – RT-qPCR	(Amman et al., 2022)

*NB. Further list of grey literature used to find these diseases/pathogens:

Causes | Background information | Gastroenteritis | CKS | NICE

Communicable (Infectious) Diseases | Health and Safety Department

What are blood-borne viruses? - Blood borne viruses (BBV)

Emerging infections: how and why they arise - GOV.UK

Appendix 3. Detection methods and technologies

Information extracted from the review articles can be found as an Excel spreadsheet, titled 'Appendix 3'. It includes the methods, a brief description of how they work, sensitivity, specificity, costs and technology readiness.

Appendix 4. Stakeholder Interviews

A limited process of seeking stakeholder views was undertaken to feed into the objectives. Stakeholder contacts were derived from discussions with the PSG and existing contacts of the research team of relevance to the project. We contacted the organisations represented on the PSG (though often different individuals we were directed to) and also academics known to the research team. Responses were received from SEPA, NHS Highland, Scottish Water, academic sector.

Question	Stakeholder Sector providing answer	Answers
Which pathogens are emerging/ re-emerging in Scotland?	Health (NHS)	• Measles – re emerging • MPOX emerging (clade 2 outbreak in 2022; recent increase in incidence of MPOX clade 1. However, there have not been any cases of clade 1 MPOX in Scotland to date) • Hepatitis E • Hepatitis A-E increased in 2022 but unlikely to class as emerging
Specifically are there any re-emerging blood borne viruses?	Health (NHS)	• Commitment to eliminate HIV transmission (by 2030) • Commitment to eliminate Hepatitis C virus as major public health threat (by March 2025) • Hepatitis B virus – significant public health concern
Are any of these emerging/ re-emerging pathogens different in Scotland from the rest of the UK that you are aware of?	Health (NHS)	No
What are you aware of that is a potential future threat e.g. coming in from other areas of the world?	Health (NHS)	UK Health Security Agency (UKHSA) produces a monthly report which provides up-to-date surveillance data and monitoring of infectious diseases affecting human and animal health across the UK1. European Centre for Disease Prevention and Control (ECDC) weekly communicable disease threats report offers a comprehensive analysis of ongoing and potential threats to public health within the European region2. UKHSA also produces a guideline that explores the epidemiology and global distribution of emerging infections over a nearly two-decade span on how and why they arise, including a timeline of emerging infections since 20233. With respect to a subjective view from ourselves, we feel that vector-borne diseases could be a potential future threat. Similarly, the world is very highly accessible now with significant travel and thus significant scope for potential future threats.
What pathogens do you currently monitor/test for?	Health (NHS); Academic	Through wastewater: SARS-CoV-2 RNA and polio Notifiable diseases (Public Health etc. (Scotland) Act 2008 reported to relevant NHS HPT Part of a project focussed on developing the methods to detect COVID in wastewater leading to NERC project on early detection of virus in sewage.

Where and how?	Health (NHS)	The WBE programme initiated by CREW collects approximately 200 wastewater samples weekly from treatment works across Scotland, representing about 75% of the population and all local authorities4. The components of the SARS-CoV RNA monitoring programme in Scotland include5: 1. Scottish Government: Operational leadership and also acting as a data stakeholder. 2. 2Scottish Water: Wastewater sampling at treatment works. 3. Scottish Environment Protection Agency (SEPA): Conducting qPCR analysis for SARS-CoV RNA. 4. Biomathematics and Statistics Scotland (BioSS): Performing data processing, including normalisation, smoothing, and visualisation. 5. NHS Lothian: Carrying out genome sequencing. 6. PHS Bioinformatics: Investigating SARS-CoV-2 variants. 7. NHS Boards, PHS, and the public: Acting as key data stakeholders. From a more general perspective, there are many different approaches to monitoring and testing
What are the costs associated with that?	Health (NHS)	Currently, the SARS-CoV RNA wastewater surveillance programme is funded annually as part of the broader respiratory surveillance funding package4. In March 2021, the Scottish Government announced an additional £2.3 million in funding for COVID-19 wastewater testing, ensuring the programme's operation through March 20226. More general costs - laboratory colleagues best placed to answer
What infections do you think are most likely to be missed by traditional monitoring approaches?	Health (NHS)	• Case based surveillance – there are challenges in relation to identifying infections that primarily rely on case based surveillance7. Identifying outbreaks of endemic infections in a timely manner in this way can be a challenge. There can be many biases present in case-based surveillance indicators due to, for example test sensitivity, changing testing behaviours and the co-circulation of pathogens with similar symptom profiles. • Infections with long latent periods or long incubation periods. • Novel pathogens for which we do not have adequate tests • With respect to specific infections, we would consider BBVs as quite likely to be missed as we tend to focus on risk factors although there are some pilots of population based screening such as opt-out BBV testing within ED departments.
What infections do you think need to be monitored that are not currently monitored (by traditional approaches) (reasons would be useful if you can give them)	Health (NHS)	• In light of climate change and the resultant increase in risk, there could be additional monitoring for vector-borne diseases although it is acknowledged that there are some surveillance programmes already in place. • Consideration of more wide-spread polio testing, although testing sensitivity is deemed to be relatively low9.
Are there infections that ARE currently monitored by traditional approaches that you think would benefit from further monitoring (either by traditional approaches or wastewater monitoring?)	Health (NHS)	• In light of the long period prior to the development of complications, hepatitis C could benefit from further monitoring. This could then direct further population-wide testing. • Surge capacity may be beneficial for illnesses like measles or polio if there is an outbreak or periods of higher incidence.

What are the ethical and legal issues that you are aware of, associated with wastewater monitoring of pathogens? If none, do you know who is best placed to have an understanding of these aspects?	Health (NHS); Academic, Water Industry (Scottish Water), academic	• Ensuring equity of access and effectiveness at rural level and island populations in NHS Highland. Sewerage coverage vs septic tank coverage needs to be considered10. • Similarly, it should be recognised that wastewater surveillance will potentially exclude some age-groups such as infants secondary to use of nappies. • There would need to be a clear plan of what the information would be used for. • In small areas, there could be issues around consent as detections from wastewater would then prompt further action and could lead to identification at an individual or community level. • The ethical issues appear to have been explored in the literature (links to examples provided) • None encountered in our SARS-CoV-2 detection work. Large areas were served by the WWTPs so data couldn't be identified back to the human source. Ethical permissions were required for use of prison wastewater samples. There could be stigma associated with specific numbers of infections – need to understand what that equates to. • See previous information from PHS • Containment protocols for working with highly pathogenic samples in wastewater are crucial for ensuring safety. This is especially important as the pathogens in sewage are already human-adapted – they aren't going to need to cross a species barrier so protecting those working on the samples is important. Work on risk for COVID – was there infectious virus still present at the point of entry to sewage treatment plant. It was never detected. They were also able to obtain samples close to source so detected higher virus concentrations but still never able to culture it. However, could culture other enteric viruses. • No ethical issues encountered – reporting was in relation to sewage works that served large areas so there was nothing that could be identified back to a human source or specific postcode. Ethical permissions were required for samples from prisons. Ethical issues could come into play e.g. if you find a surge in Ebola and you're going to want to track that back to the population and that means you're flagging that populations as being highly infectious for something. So that could create stigma. So when you start to report back, there may be ethical issues depending on your questions and what you are finding.
Do you have any comments on how well infectious disease markers in wastewater correlate with population estimates from traditional monitoring approaches?	Health (NHS), academic	Review of COVID-monitoring as per PHS report shows that wastewater was a closely correlated with ONS testing results during moderate outbreak activity levels (Delta COVID variant), but showed lower correlation when compared to ONS at time of high-levels of infection (Omicron COVID variant) 11. Of note, NHS Highland was one of the least accurate health boards in Scotland versus traditional methods of monitoring for infections, and this was hypothesised to be due to rural commuting populations and potentially low levels of prevalence in isolated populations. In addition, there is discussion regarding the challenges of septic tank usage in rural communities in relation to wastewater monitoring. This is a concern as sensitivity and specificity ranges were very wide and low at time during covid wastewater surveillance in NHS Highland. Wastewater data provides insights quickly, but relating it to clinical outcomes requires challenging access to human data. Obtaining clinical data from public health agencies is difficult due to red tape and lack of manpower.

If there are any key papers you are aware of that relate closely to questions above, please let us know the citation	Health (NHS); Water Industry (Scottish Water)	UK Health Security Agency. Emerging infections: monthly summaries. Available at: https://www.gov.uk/government/publications/emerging-infections-monthly-summaries European Centre for Disease Prevention and Control. Weekly communicable disease threats report. Available at: https://www.ecdc.europa.eu/en/publications-and-data/monitoring/weekly-threats-reports UK Health Security Agency. Emerging infections: how and why they arise. Timeline of newly identified emerging infections and notable outbreaks of diseases in humans in new regions between 2003 and 2022. Available at: https://www.gov.uk/government/publications/emerging-infections-characteristics-epidemiology-and-global-distribution/emerging-infections-how-and-why-they-arise Public Health Scotland. Scotland's wastewater monitoring programme: 3-year strategic plan. Available at: https://publichealthscotland.scot/media/28584/scotlands-wastewater-monitoring-programme-3-year-strategic-plan.pdf Public Health Scotland. Evaluating the public health utility of wastewater-based surveillance of SARS-CoV-2 in Scotland: Final Report. Available at: https://publichealthscotland.scot/media/26992/evaluating-the-public-health-utility-of-wastewater-based-surveillance-of-sars-cov-2-in-scotland-final.pdf Envirotec Magazine. Scotland's COVID-19 wastewater monitoring programme extended until March 2022. Available at: https://envirotecmagazine.com/2021/03/25/scotlands-covid-19-wastewater-monitoring-programme-extended-until-march-2022 Royal Society Open Science. Available from: Challenges in the case-based surveillance of infectious diseases | Royal Society Open Science The Lancet Microbe. Global wastewater surveillance for pathogens with pandemic potential: opportunities and challenges. Available from: Global wastewater surveillance for pathogens with pandemic potential: opportunities and challenges - ScienceDirect Lasen et al. PLOS: Global Public Health. Available from: Non-detection of emerging and re-emerging pathogens in wastewater surveillance to confirm absence of transmission risk: A case study of polio in New York | PLOS Global Public Health Cohen et al. Making waves: The benefits and challenges of responsibly implementing wastewater-based surveillance for rural communities. Available from: https://www.sciencedirect.com/science/article/abs/pii/S004313542301535X Public Health Scotland. Evaluating the public health utility of wastewater-based surveillance of SARS-CoV-2 in Scotland: Technical Report. Available from: Evaluating the public health utility of wastewater-based surveillance of SARS-CoV-2 in Scotland: Technical Report A. Corbishley., D. Gally., S. Fitzgerald., A. Tidswell., S. Mcateer (2020). Tracking SARS-CoV-2 via municipal wastewater. CD2019_06. Scotland's Centre of Expertise for Waters (CREW). Nick Gilbert, Isabel Fletcher, Catherine Lyall, Livia C. T. Scorza, Tomasz Zieliński, Sumy V. Baby and Andrew J. Millar (2022). SARS-CoV-2 monitoring in Scottish wastewater: Variant Detection, FAIR data Outputs, and Lessons Learned. CD2021_03 Natalie Sims, Lisa Avery, Barbara Kasprzyk-Hordern (2021), Review of wastewater monitoring applications for public health and novel aspects of environmental quality (CD2020_07). Scotland's Centre of Expertise for Waters (CREW). Lisa Avery, Kristin E. Ceniccola-Campos, Claus-Deiter Mayer, Wakene Negassa, Zulin Zhang, Sebastian Sprick and Eulyn Pagaling. (2025). Review of psychoactive substances wastewater monitoring approaches and recommendations for the feasibility of applying different approaches in Scotland – Main report. CRW2023_10. Scotland's Centre of Expertise for Waters (CREW).
What are the costs associated with sampling specifically for emerging pathogens in wasteewater (is SARS a good example, and if so what is the sampling frequency and what are the laboratory costs)	Water Industry (Scottish Water)	Organisational costs (administration and management time) - based on staff hourly rate and time spent Sampling - hourly rate Analysis - test cost, per sample Potentially courier costs to the laboratory analysing the samples - hourly rate (may be included in sampling hourly rate if appropriate) Sharing actual costs of example monitoring campaigns (e.g. COVID) is sensitive information for both SW commercial activity and Scottish Government (COVID monitoring programme).

Is there any capacity to take on additional sampling and if so would it be at similar costs to above or required additional resource e.g. staffing	Water Industry (Scottish Water)	Yes if fully funded, based on above costs. If a significant workload increase the administration and management time may be higher
Do you undertake any of the laboratory analyses associated with the monitoring of these pathogens (including non-microbiological analyses that are used to normalise data back to the population e.g. ammonia)	Water Industry (Scottish Water)	ammonia/nitrate. Coliform, *E. coli*, Enterococci
What are the costs of laboratory analyses (and any other costs) for emerging pathogens you are currently monitoring or have looked into?	SEPA	NHS Lothian laboratory contacts provided (SEPA samples associated with COVID were processed here).
Any other comments made	academic	We couldn't prove it statistically, but we did think that there was potential that sewage could provide an early warning system of a surge in human infection. I think the accuracy was far greater for this with the larger populations served waste treatment plants come compared to the slightly smaller ones. Detection isn't causation, its not necessarily telling you that a given percentage of your population has a given infection. You need clinical data to see what that finding in sewage equates to in terms of infection levels. COVID was a brilliant scenario in which to do that because it was new, and we had case numbers and access to data. It can take too long to get clinical data.

*NB NHS laboratories were contacted on the basis of two stakeholder suggestions but we did not receive a reply. This was not pursued once we became aware that sampling cost information would not be able to be shared with us as this rendered it impossible to undertake any comparison of costs of traditional clinical monitoring vs. WBE based monitoring.

Follow up on stakeholder engagement

The list of notifiable diseases, as mentioned in a resource by a stakeholder ((Public Health etc. (Scotland) Act 2008), identified is as follows:

- Anthrax
- Botulism
- Brucellosis
- Cholera
- Clinical syndrome due to *E.coli* O157 infection
- Coronavirus disease 2019 (COVID-19)
- Diphtheria
- Haemolytic Uraemic Syndrome (HUS)
- Haemophilus influenzae type b (Hib)
- Measles
- Meningococcal disease

- MPOX
- Mumps
- Necrotising fasciitis
- Paratyphoid
- Pertussis
- Plague
- Poliomyelitis
- Rabies
- Rubella
- Severe Acute Respiratory Syndrome (SARS)
- Smallpox
- Tetanus
- Tuberculosis (respiratory or non-respiratory)
- Tularemia
- Typhoid
- Viral haemorrhagic fevers
- West Nile fever
- Yellow Fever

The CREW SARS-CoV-2 monitoring project on variant detection reported that the best method to detect SARS-CoV-2 variants in wastewater was DNA-based next generation sequencing (Gilbert et al., 2022). This does not mean it is the best method for detection and enumeration of viruses or other pathogens more broadly, but is pertinent to the question being asked, for example, where the emergence of different strains, clades or sub-types are important. Furthermore, sequencing approaches more broadly can detect novel pathogens whereas detection methods based on markers selected a priori will not capture this information. The authors reported that the methodology for SARS-CoV-2 detection could be modified to monitor different pathogens. They note the rapid development of wastewater-based monitoring for SARS-CoV-2 (within 6 months), but identified challenges with transitioning from the research phase to a routine testing programme.

A key difference noted by interviewees in the study by Gilbert et al. (2022) was that Scotland benefitted from having one national water body that has a clear mandate to act in the public interest. Commitment to wastewater testing was observed both from Scottish Water and SEPA.

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