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A review of microbial indicators of faecal contamination – Final report

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A review of microbial indicators of faecal contamination – Final report

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Glossary

ATP	Adenosine triphosphate
CFU	Colony forming units
CRI	Compliance risk index
CST	Chemical source tracking
DVGW	German Technical and Scientific Association for Gas and Water
DWI	Drinking Water Inspectorate
eDNA	Environmental DNA
EU	European Union
FCM	Flow cytometry monitoring
FIB	Faecal indicator bacteria
FIO	Faecal indicator organism
FWC	Fluorescence whitening compounds
H_2S	Hydrogen sulphide
HPC	Heterotrophic plate count
ICC	Intact cell count
ICC-PCR	Integrated cell culture PCR
LRV	Log removal value
MALDI-TOF MS	Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry
MAV	Maximum acceptable value
MST	Microbial source tracking
NAV	New Appointments and Variations
NGS	Next-generation sequencing
NHMRC	National Health and Medical Research Council (Australia)
NLF	Non-lactose fermenter
PCR	Polymerase chain reaction
PCV	Prescribed concentration or value
PFU	Plaque forming units
PMA	Propidium monoazide
PMMoV	Pepper mild mottle virus
QMRA	Quantitative microbial risk assessment
qPCR	Quantitative PCR
RA	Risk assessment
SCA	Standing Committee of Analysts
SOP	Standard operating procedure
SVGW	Swiss Association for Gas and Water
TCC	Total cell count

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TLF	Tryptophan-like fluorescence
TVC	Total viable count
US	United States
USEPA	US Environmental Protection Agency
VBNC	Viable but not culturable
WHO	World Health Organization
WSP	Water safety planning

Executive summary

Faecal contamination of drinking water is a significant public health concern due to the risk of exposure to enteric (intestinal) pathogens. Traditionally, water quality monitoring has relied on detecting faecal indicator organisms (FIOs) such as *Escherichia coli*, coliform bacteria, and enterococci, which are abundant in faeces and relatively easy to detect. However, these indicators have limitations, particularly in estimating risks arising from non-bacterial pathogens, e.g. norovirus and other enteric viruses, or *Cryptosporidium*. In recent years new faecal indicators and analytical techniques have been developed, but their adoption in regulations remains limited due to issues of standardisation, cost, and uncertainty regarding their relevance to health risks.

The aim of this report is to inform regulatory and operational practices on the usefulness and applicability of indicators and novel indicator detection technologies for safeguarding drinking water quality. To achieve this, first we performed a literature review (Section 1) to evaluate the effectiveness, limitations, and regulatory application of faecal indicators and detection technologies relevant to drinking water. We identified the following groups of faecal indicators and emerging technologies that are used or potentially could be incorporated in faecal pollution monitoring of water:

Faecal indicators	Technologies
• *Escherichia coli* (*E. coli*) • Enterococci • Coliform bacteria • *Clostridium perfringens* (and spores) • Heterotrophic plate count (HPC) bacteria • Somatic coliphages (viruses infecting *E. coli* and related bacteria) • *Pseudomonas* • Non-lactose fermenting (NLF) bacteria • Microbial source tracking markers • *Cryptosporidium*/*Giardia* • *Campylobacter*	• PCR-based methods • Next-generation sequencing • Flow cytometry • Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) • In-situ enzymatic sensors • In-situ fluorescence sensors

Following the review, we conducted an online survey followed by online interviews to gather real-world perspectives from water industry stakeholders in the UK and internationally on the adoption, practical challenges, and perceived value of the identified indicators and technologies (Section 2). Using the knowledge from the literature review and from stakeholders, we then ranked the indicators and technologies for an objective overview of their applicability in routine monitoring of faecal contamination in water (Section 3). Finally, we provided recommendations on the potential inclusion of these indicators and technologies in legislation.

Key findings:

- No single indicator or method provides a complete measure of sanitary water quality. International guidance recommends a multi-barrier, risk-based approach that integrates multiple lines of evidence.
- *E. coli* remains the most widely used and reliable indicator for faecal contamination and is required to be absent from all treated drinking water in most countries. Coupled with emerging rapid detection methods and typing approaches (e.g. MALDI-TOF MS), *E. coli* surveillance could be more efficient.
- Coliforms and enterococci are also commonly used, but each has limitations. Coliforms can originate from non-faecal sources, and enterococci are less sensitive than *E. coli*.
- *Clostridium perfringens* and coliphages are more resistant indicators used in some jurisdictions to assess protozoan and viral risks during water treatment, respectively.
- Newer methods such as flow cytometry, PCR-based microbial and chromatography-based chemical source tracking show promise but are not yet standard practice due to cost, complexity, and limited evidence linking them to health risks.
- Stakeholder insights reveal that while traditional indicators remain central, there is growing interest in adopting new technologies and more flexible, risk-based approaches. However, practical challenges, such as standardisation, cost, and regulatory clarity, must be addressed before these innovations can become routine.

Based on the findings of this review, the following recommendations and next steps are proposed to advance the monitoring and management of faecal contamination in drinking water:

- Maintain *E. coli* and enterococci as core compliance indicators.
- Gradually adopt new methods (e.g., somatic coliphages, MST markers, MALDI-TOF MS, PCR, flow cytometry) as evidence and standards develop.
- Use a multi-indicator, multi-technology approach for more robust water quality assessment.
- Develop clear guidance for interpreting results from both traditional and novel methods.
- Prioritise research on MST marker prevalence and specificity in UK waters; applicability of somatic coliphages, MST markers, and flow cytometry in water treatment monitoring, defining operational baselines for HPC bacteria and building a collaborative research and data-sharing framework.
- Ensure regulatory frameworks remain flexible and risk-based.

1. Literature review of indicators of sanitary significance

1.1 Summary

The purpose of this review is to describe and assess microbial indicators of sanitary significance currently included in national and international drinking water regulations, and to evaluate emerging, non-regulatory indicators and techniques for their potential application in monitoring drinking water quality.

Key findings:

- Across all jurisdictions reviewed, culture-based monitoring remains central to regulatory compliance. Novel techniques and indicators are recognised as valuable supplementary tools for operational and investigative purposes, but their routine regulatory adoption is limited by issues of standardisation, cost, and uncertainty over health risk relevance.
- Established faecal indicator organisms (FIOs) such as *Escherichia coli*, coliforms, and enterococci are widespread in compliance frameworks internationally, though their limitations, especially for indication of viral and protozoan risk, are increasingly acknowledged.
- *Clostridium perfringens* and coliphages (i.e. viruses infecting *E. coli* and related bacteria) are more resistant indicators used in some jurisdictions for assessing protozoan and viral risks and fate respectively.
- Flow cytometry is a promising culture-independent tool that is increasingly used to assess general microbial water quality in operational settings, though it detects all microbes not only faecal microorganisms.
- Microbial source tracking (MST) enables attribution of human vs animal faecal pollution sources but faces challenges regarding standardisation, interpretation, and application to treated waters.
- Chemical markers show potential for faecal source identification but have limited application in drinking water due to typically low contaminant concentrations.

While traditional indicators remain central to water safety monitoring, no single method is perfect. Combining different tests and approaches gives the best chance of detecting contamination and protecting public health. New technologies are being explored, but more research and standardisation are needed before they become routine.

1.2 Introduction

Ensuring the microbiological safety of drinking water is essential for the protection of public health. Traditional faecal indicator organisms, such as *E. coli* and enterococci, have long served as the cornerstone of compliance monitoring, but their limitations, particularly in indicating viral and other emerging risks, have become increasingly apparent. While new technologies and alternative indicators are being developed, their adoption into routine practice is often limited due to the lack of evidence on their implementation.

The objectives of this review are as follows:

- To describe and assess the benefits, limitations, and regulatory application of sanitary indicators used for the purposes of compliance monitoring and operational risk assessment of drinking water in the United Kingdom and internationally.

- To describe and assess the benefits, limitations, and regulatory potential of recognised sanitary indicators that are not currently present in drinking water regulation.

For the purposes of this review:

'Sanitary indicator' is defined as a microorganism, chemical compound, or analytical technique that can be used to indicate the microbiological quality of drinking water and/or microbiological health risks to consumers. This definition encompasses faecal indicator organisms, microbiological operational monitoring parameters, pathogens, chemical indicators, and analytical methods, thus providing a broad view of the potential methodologies available for the assessment of microbiological water quality in potable water supplies.

'Microbiological compliance parameter' is defined as a microbiological water quality parameter for which compliance is required under Regulation 4 (Wholesome water) of the Water Supply (Water Quality) Regulations 2016, or national/international equivalent.

'Operational monitoring parameter' is defined as a microbiological water quality parameter for which compliance is required or recommended outside of Regulation 4 (e.g. for supply zone monitoring), or national/international equivalent.

1.3 Methodology and sources

This review draws upon the following sources:

1) International regulations and guidelines

The regions and nations and their respective regulatory standards considered in Section 1.4 (International Guidelines and Regulations) are summarised in Table 1.1. To assess any variations in implementation of the EU Drinking Water Directive across Europe, the Netherlands

and Hungary have been selected as representative nations for Western and Central Europe respectively, with Norway selected as a representative non-EU nation.

Table 1.1 Jurisdictions included in Section 1.4 (International Guidelines and Regulations)

Region	Jurisdiction	Regulations
United Kingdom	England	Water Supply (Water Quality) Regulations 2016
	Wales	Water Supply (Water Quality) Regulations (Wales) 2018
	Scotland	Public Water Supplies (Scotland) Regulations 2014
	Northern Ireland	The Water Supply (Water Quality) Regulations (Northern Ireland) 2017
Europe	European Union	EU Drinking Water Directive (2020/2184)
	Ireland	European (Drinking Water Regulations) S.I. No. 99/2023
	The Netherlands	Dutch Drinking Water Decree
	Hungary	201/2001. (X. 25.) edict
	Norway	Regulations on water supply and water intended for human consumption (Drinking Water Regulations)
North America	USA	National Primary Drinking Water Regulations
	Canada	Guidelines for Canadian Drinking Water Quality
Australasia	Australia	Australian Drinking Water Guidelines 2011
	New Zealand	Water Services (Drinking Water Standards for New Zealand) Regulations 2022

2) Grey literature

To complement our understanding of the scientific basis of current international standards and best practice, and to determine key developing areas of interest for regulatory bodies, the following grey literature sources were drawn upon:

- Regulatory guidance documents – e.g. WHO Guidelines for Drinking Water Quality; DWI, USEPA, Health Canada guidance
- Government publications and research – e.g. USEPA, European Commission-led research studies
- Independent research reports – e.g. UKWIR, WHO, WRc reports
- Standard methodologies – e.g. Standing Committee of Analysts (SCA), USEPA methods

3) Peer-reviewed academic literature

A literature search was conducted based on a set of key terms relevant to the outlined topics, using scientific literature databases including Scopus and PubMed. Following initial screening based on relevance of title and abstract, full texts were assessed in detail and selected for inclusion in the review.

A tabulated summary of the sanitary indicators identified in this review is provided in Table 1.2.

Table 1.2 Summary of indicators and analytical methods identified for the detection and monitoring of faecal contamination

Indicator/method	Regulated?	Description	Benefits	Limitations
Escherichia coli	Yes	Bacterial indicator (faecal)	Well established compliance parameter Specific to faecal contamination High faecal shedding rate/highly sensitive Minimal environmental replication Correlations with bacterial enteric pathogens	Cannot reliably indicate presence of protozoan and viral pathogens Cannot reliably indicate persistent/longer-lived pathogens Lengthy incubation periods (>18h depending on confirmation tests)
Coliform bacteria	Yes	Bacterial indicator (general/faecal)	Well established compliance/operational monitoring parameter High faecal shedding rate/highly sensitive Associated with faecal contamination	Common in non-faecal contexts; can be environmental in origin or become naturalised in the environment Replication/biofilm formation in distribution Unreliable indicator of health risk Definition varies internationally Lengthy incubation periods (>18h depending on confirmation tests)
Enterococci	Yes	Bacterial indicator (faecal)	Specific to faecal contamination Minimal environmental replication Can indicate presence of more persistent pathogens	Less sensitive than *E. coli* Cannot reliably indicate presence of protozoan and viral pathogens Lengthy incubation periods (>48h depending on confirmation tests)
Heterotrophic plate count	Yes	Bacterial indicator (general)	Well established operational monitoring parameter Provides insight into general microbial conditions during treatment and supply	Does not indicate health risk Not specific to faecal contamination Lengthy incubation periods (40-72h) Does not account for unculturable species
Clostridium perfringens (and spores)	Yes	Bacterial indicator (faecal)	Specific to faecal contamination Highly persistent – can indicate historical/intermittent faecal contamination and presence of more persistent pathogens Can indicate fate of persistent cell types (spores, (o)cysts) through treatment stages	Less sensitive than *E. coli* Cannot be used as indicator of recent faecal contamination due to environmental persistence Varied correlations with protozoan pathogens e.g. *Cryptosporidium*, *Giardia* Lengthy incubation periods (24h)
Coliphages	Yes	Viral indicator (faecal)	Specific to faecal contamination	Lack of widespread laboratory capability (UK) Unclear association with health risk

Indicator/method	Regulated?	Description	Benefits	Limitations
			Can indicate fate of enteric viruses through treatment and supply; increasingly used as treatment process indicators	Varied correlation with enteric viruses Lengthy incubation periods (24h)
Ammonia	Yes	Chemical indicator (faecal)	Moderate to strong correlations with faecal indicator bacteria Rapid tests available	Unclear and indirect association with faecal contamination in drinking water Does not indicate health risk Not faeces-specific – can be derived from multiple sources including fertiliser and decaying organic matter
Molecular markers (microbial source tracking)	No	Microbial/nucleic acid indicator (faecal)	Specific to faecal contamination Indicates specific sources of faecal contamination Can be applied to source attribution for catchment/source water assessment and during drinking water-associated disease outbreaks Same day results can be produced	Does not indicate viability/infectivity Unclear association with health risk Limited application in drinking water Variation in marker abundance/persistence by location/population/environmental conditions Lack of standardisation Data interpretation challenges
Adenosine triphosphate (ATP)	No	Microbial/surrogate indicator (general)	Rapid and sensitive measure of microbial activity Real-time monitoring feasible	Association with health risk is unclear Not specific to faecal contamination Non microbe specific; can detect animal/plant sources Limited practical application in drinking water
Caffeine	No	Chemical indicator (faecal)	Specific to human faecal contamination	Varying correlations with FIOs Environmental persistence and transport are unclear Limited application in drinking water
Faecal sterols	No	Chemical indicator (faecal)	Specific to faecal contamination	Low sensitivity of existing methods for drinking water and variable correlation with microbial indicators
Artificial sweeteners	No	Chemical indicator (faecal)	Some show a positive correlation with faecal indicator bacteria	Low concentrations in drinking water and complex analysis
Fluorescence whitening compounds	No	Chemical indicator (faecal)	Specific to human wastewater and detectable using simple fluorometric techniques	Lack of quantitative accuracy and variable detection reliability

Indicator/method	Regulated?	Description	Benefits	Limitations
Hydrogen sulphide	No	Microbial indicator (faecal)	Low-cost and simple test	Not specific to human faeces and typically only gives non-quantitative results
Flow cytometry	No	Analytical method (general)	Not reliant on culture-based methods Detects and quantifies entire microbial community, including non-culturable cells Rapid and high throughput Extensive application in drinking water contexts, including treatment process evaluation and regrowth in distribution Low limit of detection	Not specific to faecal contamination Does not indicate health risk Lack of consistent correlation with FIOs Does not provide taxonomic resolution Does not detect viruses Challenges regarding data interpretation and gating strategies
MALDI-TOF MS	No	Analytical method (general/faecal)	Provides taxonomic resolution of heterotrophic bacteria that may not be possible via culture-based/biochemical methods Alternative to biochemical confirmation tests for HPC, coliforms etc.	Limited by coverage of reference library Cannot distinguish between closely related species Relies on culture-based methods and is therefore subject to their limitations Does not account for non-culturable cells High upfront cost
Molecular approaches	No	Analytical method (general/faecal)	Not reliant on culture-based methods Provides a high level of taxonomic resolution depending on primer selection PCR-based techniques are rapid Multiple applications in drinking water including FIO and pathogen detection, MST, community analysis	Does not indicate infectivity/viability Unclear association with health risk PCR primer selection can impact specificity Inhibitory substances can impact efficiency NGS limited by high cost, long turnarounds, and data complexity Lack of standardised protocols
Tryptophan-like fluorescence	No	Analytical method (faecal)	Can provide real time *E. coli* surrogate detection data	Does not perform well in low contamination situations Poor detection of large, short-term fluctuations in water quality Organic matter can impact reliability of detection
Sensor technologies	No	Analytical method (general/faecal)	Real time data collection Automation	Often limited to surrogate measurements Challenges interpreting data in comparison with traditional measures e.g. CFU Variable sensitivity and specificity depending on water type and target organism High costs

1.4 Indicators identified in international guidelines and regulations

The objective of this section is to discuss and evaluate the sanitary indicators currently included in national and international drinking water regulations and guidelines. Table A.1 provides a detailed summary of the microbiological compliance and operational monitoring parameters present in national and international regulations, including their associated prescribed concentration or value (PCVs) and point(s) of compliance. Organisms that are regulated for reasons other than indicating faecal contamination and/or sanitary water quality, such as *Legionella*, which is primarily associated with premise plumbing and aerosol transmission, have been excluded, as they fall outside the scope of this assessment. For more information on the regions/nations selected for inclusion in this section, see Section 1.3.

1.4.1 Microbial indicators

Exposure to pathogenic microorganisms derived from faecal contamination is considered to be the most significant health risk associated with the consumption of drinking water (WHO, 2022). Although pathogenic protozoa, viruses, and bacteria pose a significant risk to the sanitary quality and safety of drinking water, the direct detection of these organisms is not routinely required in most regulatory frameworks. This is primarily due to the practical and technical challenges associated with pathogen monitoring. Target organisms often occur at very low concentrations in treated water, necessitating large-volume sampling, labour-intensive concentration and recovery steps, and detection methods that are expensive and lack standardisation across laboratories. As a result, regulators commonly adopt indirect approaches to manage pathogen risks, using faecal indicator organisms (FIOs), general microbial indicators, source water assessments, and treatment performance validation rather than routine pathogen monitoring.

FIOs are enteric microorganisms shed in faeces. Their presence in water indicates faecal contamination, and consequently the potential presence of faecal pathogens. FIOs are widely used in regulatory frameworks worldwide due to their practicality and established correlations with contamination risk.

The WHO Guidelines for Drinking Water Quality (WHO, 2022) outline the key criteria for FIOs. Specifically, they should:

- Be universally present in faeces in humans and animals in large numbers
- Not multiply in natural waters
- Persist in water in a similar manner to faecal pathogens
- Respond to treatment processes in a similar fashion to faecal pathogens
- Be readily detected by simple, inexpensive culturing methods

This section reviews the FIOs and other general measures of microbial water quality that are currently present in national and international regulations.

Escherichia coli

Escherichia coli is a thermotolerant coliform bacterium that can be distinguished from other coliforms by its ability to produce indole from tryptophan and by the expression of the enzyme β-glucuronidase. It is an ubiquitous inhabitant of the intestinal tract of warm-blooded animals and is the most abundant coliform bacteria in faeces, shed in concentrations of up to 10^9 cells/g (SCA, 2002). Due to its exclusive faecal origin and high prevalence in human and animal excreta, *E. coli* is considered a highly sensitive faecal indicator organism (USEPA, 2006a; WHO, 2022). It is consequently widely present in international drinking water regulations as a measure of microbiological water quality (**Error! Reference source not found.**).

Presence in regulation

The WHO Guidelines for Drinking Water Quality classify *E. coli* as an essential parameter of water quality, stating that its detection provides “conclusive evidence for human faecal pollution” and that it should be absent from all drinking water. This principle has been adopted by regulatory bodies globally, with *E. coli* listed as a mandatory compliance parameter in every national regulatory framework reviewed. A consistent parametric value of 0 (or <1) colony forming units (CFU) per 100 mL treated drinking water is applied across all jurisdictions (**Error! R eference source not found.**). In Australia, *E. coli* is the sole microbiological compliance parameter, though the use of additional indicators to complement *E. coli* monitoring is recommended (NHMRC, 2022). In England and Wales, routine *E. coli* compliance monitoring is required at three compliance points: the outlet of the water treatment works, the service reservoir, and the customer tap. Specific compliance points vary throughout international regulation, but all require the absence of *E. coli* in treated supplies.

Beyond compliance, *E. coli* is widely used as an operational monitoring parameter for source water quality, treatment efficacy, and disinfection performance (WHO, 2022). The detection of *E. coli* in a treated water supply is generally attributed to either recent faecal ingress due to distribution system failure or inadequate treatment performance and warrants immediate investigation by the responsible authorities.

Value as a sanitary indicator

The universal presence of *E. coli* in mammalian faeces and its high shedding rates make it a sensitive and reliable indicator of faecal contamination. Importantly, growth of *E. coli* in the environment, particularly in temperate regions, is rare, and it is therefore generally not observed in the absence of faeces (WHO, 2022). However, naturalised strains capable of limited growth in environmental settings have been identified in tropical and sub-tropical climates, including soils and sediments (Fujioka *et al*., 1998; José Figueras *et al*., 2010; Jang et a., 2017). For example, rare *'E. coli* blooms' have been documented in Australian drinking water reservoirs,

prompting the use of multiple indicators, such as enterococci, to verify faecal sources (Power *et al*., 2005; NHMRC, 2022). However, the low water temperatures, poor nutrient availability, and presence of residual disinfectant in a well-managed temperate distribution system would be highly unlikely to support the growth of *E. coli* and enteric pathogens (SCA, 2002; WHO, 2022). *E. coli* can therefore be considered solely of faecal origin for the purposes of water quality assessment in the UK.

E. coli shares similar environmental survival and removal profiles to bacterial pathogens such as *Salmonella* and *Shigella*, and is relatively short-lived in environmental and treated waters, supporting its use as an indicator of recent faecal input (SCA, 2002; Health Canada, 2020). However, *E. coli* is highly sensitive to disinfection, and its absence from disinfected water does not necessarily imply the absence of more resistant pathogens. For example, protozoan (e.g. *Cryptosporidium*) and viral pathogens (e.g. norovirus) are more resistant to treatment and may persist when *E. coli* is undetectable (WHO, 2004; USEPA, 2006a). Additionally, the physical removal of *E. coli* during treatment is not predictive of the removal of smaller or denser microorganisms such as viruses or protozoan cysts, with the latter often capable of surviving for longer periods in distribution than *E. coli* (Health Canada, 2020). To address these limitations, WHO recommends the inclusion of additional FIOs, such as spores (see *Clostridium perfringens*) and bacteriophages (see Somatic Coliphage), within monitoring programmes, particularly when catchment assessments indicate elevated protozoan or viral risks (WHO, 2022). Similarly, the European Commission recommends the use of more resistant microorganisms in the validation of disinfection efficiency when implementing the EU Drinking Water Directive (Niegowska *et al*., 2022).

Coliform bacteria

Coliform bacteria, or coliforms, are a functional group of bacteria within the family *Enterobacteriaceae* that are defined based on shared biochemical and physiological characteristics. Since their initial characterisation, the definition of coliforms has evolved in response to advances in analytical methods and shifting regulatory priorities, resulting in a broader group that includes a wide range of species (Stevens *et al*., 2003). In the UK, the current definition, outlined in the Microbiology of Drinking Water: Part 4 (SCA, 2016), classifies coliforms as "*organisms which are oxidase-negative, produce acid from lactose or express β-galactosidase, and form all shades and sizes of yellow colonies on membrane filters after incubation at 30 °C for 4 hours followed by incubation at 37 °C for 14 hours*." Common genera isolated from drinking water include *Escherichia*, *Klebsiella*, *Hafnia, Citrobacter, Enterobacter,* and *Yersinia* (WHO, 2004).

The terms 'thermotolerant coliform' and 'faecal coliform' refer to coliforms with the ability to grow and ferment lactose at 44.5 ± 0.2°C (SCA, 2016). *E. coli* constitutes the vast majority of thermotolerant coliforms detected in water supplies, with *Klebsiella* and *Enterobacter* also falling under this definition (USEPA, 2006a). While WHO recognises thermotolerant coliform monitoring as an acceptable alternative to *E. coli* detection (WHO, 2022), this approach has seen limited international uptake due to the availability of more specific *E. coli* methods (Health

Canada, 2020). Monitoring of thermotolerant coliforms is not a requirement in any UK regulations, though methods for distinguishing them from the total coliform group are included in standard methods (SCA, 2016).

Coliforms have historically been used as indicators of faecal pollution due to the high concentrations in which they are shed in faeces. However, it is now understood that many coliform species including *Klebsiella* spp., *Enterobacter* spp., and *Serratia* spp. are commonly found in natural environments, including soils and water, independently of faecal contamination (Leclerc *et al*., 2001; Stevens *et al*., 2003; USEPA, 2006a; Figueras et a., 2010). Their ability to grow within distribution system biofilms further undermines their utility as reliable indicators of faecal pollution.

Presence in regulation

The regulatory status of coliforms varies internationally. In the UK, total coliforms are a mandatory compliance parameter in England, Wales, and Northern Ireland, and must be absent from water leaving treatment works and service reservoirs (**Error! Reference source not f ound.**). In contrast, Scottish regulations do not require mandatory coliform monitoring in alignment with the EU Drinking Water Directive, though they are still present in regulation as a non-mandatory operational monitoring parameter.

The value of coliforms as regulatory indicators has been subject to increasing scrutiny. WHO has expressed concerns regarding their ambiguous interpretation, limited faecal specificity, and poor correlation with health-based outcomes (WHO, 2004; WHO, 2022). In a 2017 review, WHO recommended the use of taxonomically defined faecal indicators, such as *E. coli*, to reduce uncertainty in data interpretation (WHO, 2017). Similarly, Australia, New Zealand, and the EU have removed coliforms from their mandatory compliance frameworks. In support of this decision, NHMRC stated that total coliforms are of limited use for assessing faecal pollution due to their ability to proliferate in distribution systems, frequent environmental origin, and inconsistent prevalence during waterborne disease outbreaks (Stevens *et al*., 2003). The report concluded that the sanitary significance of coliform detections is difficult to interpret in the absence of *E. coli*.

Nonetheless, many nations have maintained the use of coliforms in regulation. In the United States, the Recast Total Coliform Rule (USEPA, 2013) continues to govern coliform monitoring in water supplies, with USEPA maintaining that coliforms have value as indicators of faecal pathogens in drinking water. They also remain present as operational parameters in Irish and Canadian regulations. In the UK, a 2005 UKWIR study assessed the implications of removing coliforms from regulations. While acknowledging operator uncertainty about their public health relevance, the report concluded that removing coliforms from compliance monitoring would reduce the likelihood of detecting treatment or distribution failures and recommended retaining them as a conservative safeguard (UKWIR, 2005).

Value as a sanitary indicator

Despite regulatory variability, coliforms remain widely used in operational monitoring to assess treatment efficacy, detect distribution system issues, and provide early warning of biofilm formation or ingress. In England and Wales, routine monitoring is undertaken at customer taps to assess distribution system integrity, and coliforms are used throughout the water production process to evaluate treatment effectiveness and detect post-treatment ingress. Coliform detections in the absence of *E. coli* are typically interpreted as evidence of regrowth, biofilm detachment, or environmental contamination rather than recent faecal pollution (USEPA, 2006a; Ministry of Health NZ, 2019).

Coliforms are valued for their simple detection using culture-based and enzymatic methods. However, their usefulness as sanitary indicators is limited by their existence in non-faecal contexts, potential for regrowth, and unreliable association with public health risks (Gruber *et al*., 2014). These limitations have led to the exploration of alternative indicators, such as heterotrophic plate count (HPC) (see below) and flow cytometry (Section 1.5.3).

Enterococci

Enterococci are Gram-positive, facultatively anaerobic cocci belonging to the *Enterococcus* genus. In regulatory and academic literature, they are also referred to as 'intestinal enterococci' and 'faecal streptococci', the latter reflecting their former classification within the genus *Streptococcus*. Enterococci are catalase-negative, possess the Lancefield Group D antigen, and are capable of growth in the presence of bile salts, sodium azide, and at temperatures of 44°C (SCA, 2012). The genus comprises over 30 species, with *E. faecalis* and *E. faecium* most commonly associated with the intestinal tracts of warm-blooded animals (Health Canada, 2020). Enterococci are shed in faeces in concentrations of 10^3-10^7 CFU/g, typically several order of magnitude lower than *E. coli* (Health Canada, 2020; NHMRC, 2022). Enterococci are considered specific indicators of faecal contamination and are included as secondary faecal indicators in multiple international drinking water frameworks **Error! Reference source not f ound.**).

Presence in regulation

Although not as universally adopted as *E. coli*, enterococci are widely recognised in drinking water regulation. WHO supports the use of enterococci as supplementary faecal indicators, stating that they should be absent from 100 mL of treated drinking water (WHO, 2022). In the 1998 revision of the EU Drinking Water Directive, enterococci were included for the first time as a mandatory microbiological compliance parameter, effectively replacing coliforms as the preferred secondary faecal indicator in Europe (**Error! Reference source not found.**). This is r eflected in the regulations for all UK nations, where enterococci is a mandatory compliance parameter to be monitored at the customer tap. The most recent revision of the EU Drinking Water Directive (2020/2184) further recommends enterococci monitoring at the point of abstraction or in raw water (Niegowska *et al*., 2022).

Enterococci are less commonly applied as a mandatory compliance parameter outside of Europe. They are not mandated in US federal drinking water standards, though enterococci monitoring of untreated groundwaters following a positive coliform detection is often a requirement at state level, in adherence with the USEPA Ground Water Rule (USEPA, 2006b). Enterococci are similarly absent from Australian, New Zealand, and Canadian microbiological compliance criteria, but are recognised by all respective regulatory bodies as useful operational indicators for assessing water quality in both raw and treated water (Ministry of Health NZ, 2019; Health Canada, 2020; NHMRC, 2022).

Value as a sanitary indicator

Enterococci are generally regarded as effective indicators of faecal pollution in drinking water systems, though they are typically less sensitive and less consistently present than *E. coli* (USEPA, 2006; Health Canada, 2020). Proliferation of enterococci in the environment is uncommon, particularly in treated water supplies where only limited associations with biofilms have been documented (Health Canada, 2020). Some species, such as *E. casseliflavus* and *E. mundtii*, are associated with non-faecal sources, including plants (UKWIR, 2005), and other species have been detected in non-faecal contexts in terrestrial and aquatic environments (Byappanahalli *et al*., 2012). The specificity of enterococci detections to faecal pollution can vary depending on the analytical method used; some target faecal-associated species specifically, while others detect a broader range of environmental species (Niegowska *et al*., 2022). Overall, for the purposes of water quality monitoring in the UK, enterococci can be considered as specific indicators of faecal pollution (SCA, 2016; WHO, 2022).

Enterococci offer several key advantages over other established FIOs. They exhibit greater resistance to environmental stressors than *E. coli* or other coliform bacteria, enabling them to persist for longer periods in aquatic environments (Byappanahalli *et al*., 2012; WHO, 2022). Monitoring of enterococci in water supplies can therefore provide a better measure of the risk of more environmentally persistent faecal pathogens. Their detection in distribution systems, particularly in the absence of *E. coli,* may indicate post-treatment ingress and has been useful in post-installation assessments of new or repaired water mains (WHO, 2022), as well as in groundwater monitoring where faecal contamination is of particular concern (USEPA, 2006b; Health Canada, 2020).

Enterococci also demonstrate increased resistance to disinfection in comparison to other FIOs, though not to the extent that they can reliably indicate the inactivation of highly resistant viral and protozoan species (USEPA, 2006a). It is unclear whether the use of enterococci to evaluate treatment efficiency would provide any tangible benefits over established indicators such as *E. coli*, coliforms, and colony counts (Health Canada, 2020), though monitoring may still be carried out in the interest of maintaining a multi-barrier risk-based approach.

This group faces similar challenges to *E. coli* with regard to correlations with specific pathogens through treatment and distribution (Health Canada, 2020).

Heterotrophic plate counts (HPC)

Heterotrophic plate counts (HPCs), also known as colony counts, are used to estimate the number of heterotrophic bacteria in drinking water. Heterotrophic microorganisms require organic carbon for growth and include a broad range of aerobic and facultatively anaerobic bacteria and may also sometimes encompass fungi and yeast species (WHO, 2022). HPCs are not indicators of faecal contamination and have no direct link to public health outcomes (WHO, 2003; USEPA, 2006a) but are routinely monitored as part of operational surveillance to assess general microbial water activity in treatment and supply (SCA, 2020; WHO, 2022).

HPCs are measured using culture-based methods in which water samples are incubated on non-selective nutrient-rich media under defined conditions of time and temperature (SCA, 2020). Colony counts are most commonly carried out at 22°C and/or 35-37°C, with the choice of incubation temperature influencing the types of bacteria recovered. Incubation at 22°C favours the growth of environmental microorganisms, particularly those associated with biofilms and system regrowth (SCA, 2020; WHO, 2022). Incubation at 37°C was formerly used to infer faecal contamination, however this interpretation is now seen as unreliable, and the requirement for monitoring at 37°C has been removed from many regulations due to redundancy (SCA, 2002; WHO, 2003; UKWIR, 2005). Incubation time can span from a few days to a week (SCA, 2020; WHO, 2022), which makes the assays hard to implement in routine monitoring.

Presence in regulation

HPCs are not used as microbiological compliance parameters in international drinking water regulations, instead serving as operational monitoring parameters for the assessment of general microbiological water quality, typically as a supplementary measure to the mandatory monitoring of FIOs (**Error! Reference source not found.**). WHO support their use as part of a b roader strategy for assessing treatment efficiency and distribution system integrity but emphasise that absolute HPC values have limited interpretive value (WHO, 2022). Rather, changes in HPC counts over time, particularly sharp increases, are considered more meaningful (WHO, 2003; WHO, 2004; Niegowska *et al*., 2022). A comprehensive review of the use of HPCs in water quality assessment was conducted by WHO in 2003, the findings of which have informed international guidance and best practice (WHO, 2003).

In the UK, colony counts at 22°C are listed in regulation for operational monitoring at the water treatment works, service reservoir, and customer tap. In line with revisions to the EU Drinking Water Directive, the requirement for enumeration at 37°C was removed from regulations for UK nations with the exception of Northern Ireland. Parametric values are not defined; rather, ‘no abnormal change’ must be observed. The manner in which this requirement is defined can vary in practice, with DWI guidance, historical data, and statistical analysis employed to establish operational thresholds (UKWIR, 2005). Several nations, including the Netherlands, Norway, Hungary, and the United States, provide baseline standards against which significant deviations are used to trigger an investigation. HPCs are absent from regulations in New Zealand, though

recommended baselines for untreated and treated waters for colony counts at 22°C and 35°C are provided in associated guidelines (Ministry of Health NZ, 2019). The Australian Drinking Water Guidelines supports the use of HPCs and advises that counts are best interpreted through system-specific baselines rather than universal thresholds (NHMRC, 2022).

Value as a sanitary indicator

HPCs provide insight into the general microbial conditions of water at various stages of the treatment and supply process. They are frequently used to assess the performance of filtration and disinfection, and to track regrowth potential in distribution systems (WHO, 2003; USEPA, 2006a; Health Canada, 2013). Increases in HPC values may indicate stagnation, biofilm detachment, ingress, loss of disinfectant residual, or inadequate maintenance of infrastructure such as storage tanks or filters (WHO, 2004; NHMRC, 2022). As HPCs originate from diverse sources, they are particularly valuable for identifying changes in microbial ecology that may not be captured by routine compliance testing.

HPCs do not provide a measure of health risk and show no consistent correlations with FIOs or waterborne disease (WHO, 2003; Health Canada, 2013). The organisms recovered by HPC methods represent only a small proportion of the microbial population and cannot distinguish between environmental bacteria and pathogens (SCA, 2002). Additionally, culture-based methods used for HPC enumeration cannot detect viable but not culturable (VBNC) organisms (WHO, 2003). These restraints have led to increased interest in more advanced cell counting approaches such as flow cytometry (see Section 1.5.3).

Clostridium perfringens

Clostridium perfringens is an anaerobic, Gram-positive, spore-forming bacterium capable of reducing sulphite (SCA, 2021). It inhabits the gut of approximately 13-35% of humans and is also common amongst warm-blooded animals (WHO, 2022). *C. perfringens* is considered specific to faecal contamination, though it is shed in substantially lower concentrations than other faecal indicator bacteria (NHMRC, 2022). *C. perfringens* spores are highly resilient and may persist in water environments for extended periods of time due to their resistance to heat, disinfection, and desiccation (Ministry of Health NZ, 2019). This organism is used as an indicator of intermittent or historical contamination, treatment performance, and the potential presence and fate of environmentally persistent pathogens.

Presence in regulation

C. perfringens has been adopted primarily in Europe as an optional or risk-based operational monitoring parameter (**Error! Reference source not found.**). The EU Drinking Water Directive (2020/2184) lists *C. perfringens* (and its spores) as a parameter subject to monitoring where indicated by risk assessment. Specifically, it is recommended to assess in supplies originating from surface waters or groundwaters influenced by surface waters (Niegowska *et al*., 2022). This approach is reflected in regulations governing UK nations, where *C. perfringens* is listed

as an operational monitoring parameter. *C. perfringens* should be absent from 100 mL treated water at the supply point. In Wales, the Water Supply (Water Quality) Regulations (Wales) 2018 dictate that *C. perfringens* operational monitoring is applicable to all supplies regardless of surface water influence; the requirement for surface water influence is also absent from regulation in Northern Ireland and The Netherlands.

WHO guidelines do not assign a parametric value to *C. perfringens* but endorse its use as a conservative process indicator where enteric protozoa (e.g. *Cryptosporidium*, *Giardia*) are a concern, especially for validating filtration performance or assessing historical faecal contamination. It is recommended as a possible 'resistant' indicator organism as part of a multi-barrier risk-based framework (WHO, 2022).

C. perfringens is less commonly included in drinking water regulation outside of Europe. In New Zealand, *C. perfringens* is not listed in drinking water standards but is recommended for use in groundwater assessments following the die-off of more vulnerable faecal indicator bacteria (Ministry of Health NZ, 2019). The Australian Drinking Water Guidelines also recommend its use in filtration performance validation but emphasise its limited sensitivity as a faecal indicator due to its low prevalence (NHMRC, 2022). *C. perfringens* is absent from US and Canadian standards and guidelines.

Value as a sanitary indicator

The primary value of *C. perfringens* in drinking water monitoring lies in its environmental persistence and resistance to conventional treatment barriers. Its spores are substantially more resistant to chlorine and UV than vegetative bacteria, and are more difficult to remove by coagulation and filtration due to their small size and physicochemical characteristics (Ministry of Health NZ, 2019). As such, *C. perfringens* is considered a conservative indicator of treatment performance (Stevens *et al*., 2003). Its presence post-treatment may indicate inadequate physical removal (e.g. filtration failure), backwashing inefficiencies, or ingress (NHMRC, 2022). Its environmental persistence also allows detection of historical faecal contamination where shorter-lived indicators may no longer be present.

Numerous studies have examined the relationship between *C. perfringens* and protozoan pathogens in environmental waters, and, to a lesser extent, through treatment processes, in order to assess the suitability of this organism to indicate protozoan risk. A review conducted by the European Commission highlighted the poor or inconsistent correlations reported in academic research between *C. perfringens* and *Cryptosporidium* oocysts and/or *Giardia* cysts in ambient waters, concluding that *C. perfringens* may not be a reliable indicator of their presence in aquatic ecosystems (Lamy *et al*., 2020). Research pertaining to the co-occurrence of *C. perfringens* spores, oocysts, and/or cysts through drinking water treatment processes is somewhat limited. Varied susceptibilities of *C. perfringens* and protozoa have been observed during different treatment and disinfection processes (Lamy *et al*., 2020). The ability of *C. perfringens* to indicate the presence of protozoan pathogens is therefore not universally valid,

though it has been recommended as a surrogate the assess protozoan removal by WHO Europe (WHO Europe, 2017; Lamy *et al*., 2020).

C. perfringens demonstrates limited applicability as a real-time faecal indicator due to its low sensitivity to faecal contamination and long-term environmental persistence. For this reason, *C. perfringens* is not typically recommended as a primary microbial indicator and is best interpreted in conjunction with other microbiological parameters (NHMRC, 2022).

Coliphages

Coliphages are bacteriophages that infect *E. coli* and related coliform bacteria. In the context of water quality assessment they are typically subdivided into two major groups based on their manner of infection, namely somatic coliphage and F-specific (F-RNA) coliphage (also known as F+ or male-specific coliphages). Both groups are excreted in faeces and can thus serve as indicators of faecal contamination (Singh *et al*., 2022; WHO, 2022). Due to their shared morphological characteristics and manner of replication, coliphages are increasingly used as viral treatment process indicators, and have been proposed as more reliable indicators than faecal indicator bacteria of the fate and behaviour of human enteric viruses in water environments, though correlations between coliphages and viruses in the environment are variable (UKWIR, 2021; NHMRC, 2022).

Presence in regulation

Coliphages have been increasingly recognised as operational monitoring and treatment process indicators in international regulation (**Error! Reference source not found.**). In the EU, t he 2020 recast Drinking Water Directive (2020/2184) implemented somatic coliphages as an operational parameter for raw water monitoring, marking the first inclusion of a viral indicator in EU drinking water legislation. A reference value of 50 plaque-forming units (PFU)/100 mL is applied at the point of abstraction. Upon exceedance, the directive recommends monitoring after treatment to assess viral removal efficiency. Although monitoring is not mandatory in treated water, member states are expected to consider the parameter in their risk assessments for source water and treatment system validation (Niegowska *et al*., 2022). This approach has been adopted into Scottish regulations.

WHO does not specify a guideline value for coliphages in drinking water but supports their inclusion in operational monitoring where viral removal is required. WHO guidance notes that coliphages are appropriate surrogates for enteric viruses in bench-scale and full-scale process verification and can support the demonstration of log removal values (LRVs) for disinfection and filtration processes (WHO, 2022).

In the US, the USEPA Ground Water Rule lists coliphages as one of three possible faecal indicators that should be used to assess microbial contamination in groundwaters, with indicator selection governed at state level (USEPA, 2006). The USEPA have undertaken considerable investigations into the application of coliphages as water quality indicators, both in the context

of drinking water treatment and recreational water quality monitoring, and are generally in support of their use as alternative faecal indicators (USEPA, 2006c, 2015, 2016).

National regulators in Australia and New Zealand do not currently require coliphage monitoring in treated water, but the guidelines of both countries acknowledge their potential value for source water characterisation and treatment validation, particularly for bench- and pilot-scale assessment of different treatment processes (Ministry of Health NZ, 2019; NHMRC, 2022). The Australian Drinking Water Guidelines recommends that if applied as an indicator, coliphages should be absent from 100 mL of treated water (NHMRC, 2022). The Drinking Water Quality Guidelines for New Zealand do not specify any maximum acceptable values (MAVs) for any viral indicators, but note that a viral parameter will likely be established in the future (Ministry of Health NZ, 2019).

In the UK, with the exception of Scotland, coliphages are not currently included in public water supply regulations. A 2024 technical advisory group, overseen by the DWI, concluded that while somatic coliphages show potential as operational indicators of virus removal, significant limitations prevent their application as operational monitoring parameters. These include the limited availability of accredited laboratory capacity in England and Wales, unclear links with health risk, uncertainties over interpretation in the absence of corresponding virus data, and concerns over cost-effectiveness and added value in the context of existing risk-based approaches. Coliphages were therefore not recommended for immediate inclusion in regulation, but were listed for further investigation (DWI, 2024). In 2021, an UKWIR report assessing the suitability of coliphages as indicators of the sanitary quality of drinking water in UK water supplies gave the following recommendation:

"Routine monitoring of coliphages is not necessary for improving effectiveness of water treatment. Coliphages may have value, however, as sanitary indicators of the virus risks posed by surface derived sources of drinking water but would require more specialty techniques to permit enumeration of the types of coliphage that are more representative of the presence of human enteric viruses."

Value as a sanitary indicator

Coliphages offer several advantages as viral indicators in drinking water systems. As viruses, they are generally more resistant to disinfection and environmental inactivation than bacterial indicator organisms (USEPA, 2016; Jofre *et al*., 2016). Coliphages typically exhibit removal profiles through treatment processes more similar to those of pathogenic human enteric viruses such as norovirus, adenovirus, and enterovirus, though considerable variations have been observed (USEPA, 2016; Lamy *et al*., 2020; Singh *et al*., 2022).

Among the two main groups of coliphage indicators, somatic coliphages are generally more abundant in raw water, allowing for greater recovery rates which is of benefit for routine monitoring (Jofre *et al*., 2016). F-specific coliphages, in contrast, are considered more specific to faecal sources due to their narrow host range and low likelihood of environmental replication.

Although, they typically occur at lower concentrations than somatic coliphages and hence it may be more difficult to detect them consistently (Jofre *et al*., 2016; UKWIR, 2021; NHMRC, 2022; WHO, 2022). Somatic and F-specific coliphages have been considered acceptable viral indicators in international guidance, and several protocols, particularly those used by the USEPA, recommend the use of both groups in parallel to increase coverage (USEPA, 2016; Jofre *et al*., 2016; WHO, 2022).

The principal limitation of coliphages is their lack of direct correlation with the presence of human enteric viruses. Coliphages are continuously shed by both humans and warm-blooded animals, whilst human pathogenic viruses are typically shed intermittently and only by infected individuals (Stevens *et al*., 2003; WHO, 2022). Numerous studies have shown that the presence or absence of coliphages is not a reliable predictor of virus occurrence in source or treated waters (USEPA, 2006a), with a review conducted by the European Commission stating that the majority of studies reviewed reported no correlation between coliphages and enteric viruses in surface waters (Lamy *et al*., 2020). There is a general consensus across international guidance that coliphages should not be interpreted as health-based indicators of virological risk, but rather as process indicators for verifying treatment performance (Stevens *et al*., 2003; NHMRC, 2022; Ministry of Health NZ, 2019; WHO, 2022). In particular, coliphages have been applied in validation studies to demonstrate the log removal efficiency of treatment processes including membrane and media filtration, UV disinfection, and chlorination, with specific types such a MS2 (F-specific) phage now widely used for this purpose (Ministry of Health NZ, 2019; Lamy *et al*., 2020; UKWIR, 2021; Singh *et al*., 2022; WHO, 2022).

1.4.2 Chemical indicators

While the drinking water quality regulations in each jurisdiction (**Error! Reference source not found.**) do require monitoring for a number of chemical analytes (ammonia, chloride, etc.), none of them explicitly state that they can be used to assess microbiological risk. However, some regulators do acknowledge their potential use as indicators of human faecal pollution in drinking water. For example, the Irish EPA state that ammonium concentration is a possible indicator of sewage pollution in drinking water (EPA, 2014).

Ammonia

As a by-product of microbial degradation of proteins, ammonia has received attention as a potential chemical indicator of faecal contamination (Tillett *et al*., 2018). Research has shown a moderate to strong positive correlation between ammonia concentration and faecal indicator bacteria, and while not entirely specific to wastewater, ammonia monitoring could offer a cost effective and fast indicator of faecal contamination (Baral *et al*., 2018; Tillett *et al*., 2018; Reynolds *et al*., 2021). There is very little research to support the use of ammonia as an indicator of microbial risk in the context of drinking water. Furthermore, ammonia is not an ideal indicator because it has multiple potential sources in water, including inputs from agriculture wastewater and the decay of organic matter (Reynolds *et al*., 2021). Further studies would be

necessary to assess the usefulness of ammonia as a microbial quality parameter in drinking water.

1.4.3 Analytical techniques

In England, approved analytical methods for microbiological monitoring are prescribed under Regulation 5 of the Water Supply (Water Quality) Regulations (2016) and standard methods are published by the Standing Committee of Analysts. Equivalent provisions exist in other jurisdictions reviewed, where regulators stipulate permitted methods through legislation or associated technical standards. Analytical approaches across all jurisdictions remain broadly consistent with international standards, reflecting a continued reliance on culture-based methods for microbiological water quality assessment.

Pathogens and quantitative microbial risk assessment (QMRA)

Quantitative microbial risk assessment (QMRA), which estimates health risks associated with microbial hazards in drinking water, is central to many regulatory frameworks globally and is recommended by WHO as a central element of microbial risk management in water supply systems (WHO, 2022). The Australian framework is widely regarded as a leading implementation of QMRA in drinking water regulation. The Australian Drinking Water Guidelines use QMRA to calculate the log reduction requirements needed to meet a target of ≤1 infection per 10,000 people per year. Pathogen risk assessments are conducted based on local catchment characteristics, historical monitoring data (where available), and pathogen removal efficiency across treatment barriers. Direct pathogen testing is reserved for investigative purposes, such as treatment upsets or incident response (NHMRC, 2022).

Similar approaches have been adopted elsewhere. In New Zealand, total pathogenic protozoa are included as a mandatory compliance parameter in regulatory standards, having been identified as an important health risk in surface waters nationally (Ministry of Health NZ, 2019; **Error! Reference source not found.**). Treatment systems must demonstrate compliance t hrough log credit calculations and process verification, though direct testing may be required in exceptional circumstances. Similar approaches, based on QMRA log removal values, exist in Canada, the US, and Europe. Though not specified in regulation, many UK water companies employ QMRA models to support compliance with Regulation 4 (wholesome water), (WRc, 2024).

1.5 Indicators identified in peer-reviewed literature or published reports

1.5.1 Microbial indicators

Molecular markers and microbial source tracking

Molecular markers of faecal contamination are nucleic acid sequences that originate from host-associated microorganisms or biological material present in faeces. These markers can be derived from a range of sources, including enteric bacteria such as *Bacteroides*, enteric viruses,

and mitochondrial DNA. Importantly, many markers exhibit host specificity, enabling differentiation of faecal pollution originating from different host groups (e.g. humans, ruminants, birds).

Microbial source tracking (MST) encompasses a range of molecular techniques that use molecular markers to identify the origin of faecal contamination in water. These methods are broadly classified into library-independent and library-dependent approaches. Library-independent techniques, such as quantitative polymerase chain reaction (qPCR), target individual host-specific markers directly (García-Aljaro *et al*., 2019; Paruch and Paruch, 2022). Library-dependent methods use sequencing techniques to compare microbial community profiles in water samples against reference datasets, which can be beneficial in complex pollution scenarios with multiple sources (Mathai *et al*., 2020).

Value as a sanitary indicator

The molecular markers used for MST address a key limitation of faecal indicator bacteria, namely their inability to differentiate between human and non-human contamination, thus providing a method of faecal pollution source attribution (Holcomb and Stewart, 2020). MST has been used to investigate pollution events, FIO non-compliances, and disease outbreaks across a range of aquatic environments both in the UK and internationally, including fresh and marine waters, recreational waters, groundwaters, and to a lesser extent, drinking water (Nguyen *et al*., 2018; Paruch and Paruch, 2022; Zan *et al*., 2022; Karunakaran *et al*., 2024).

Current MST methods face several methodological and interpretive challenges. Marker abundance and persistence vary with geographic region, host population, and environmental conditions such as water temperature and disinfectant residual. This can complicate comparisons between sites and studies (Ballesté *et al*., 2020), and it is generally acknowledged that the performance of an MST assay is to some extent dependent on the specific circumstances of the study location (Reischer *et al*., 2013). Additionally, differences in sample preparation, DNA extraction, and amplification protocols can impact marker recovery and lead to inconsistent results (Ballesté *et al*., 2020). As a result of these challenges, standardisation of MST protocols is very limited, with only one standard marker assay protocol (USEPA's HF183/BacR287 assay) endorsed by a regulatory body at present (USEPA, 2019).

Correlations between MST markers and pathogen presence have been explored but remain inconsistent. Some studies suggest tentative associations (Harwood *et al*., 2014; González-Fernández *et al*., 2021), while others report weak or no correlation due to differing persistence and decay rates (Vadde *et al*., 2019). Integrated cell culture methods and quantitative microbial risk assessment (QMRA) models have been applied to address this, with some success in estimating site-specific health risks (Ahmed *et al*., 2024), but these approaches remain complex and rarely implemented in routine monitoring.

Importantly, at present the application of MST in treated drinking water systems remains limited. In high-performing supply systems, such as those in the UK, microbial compliance failures are

rare, intermittent, and typically involve very low concentrations of faecal material. Detecting faecal molecular markers in such conditions requires high sensitivity and is often constrained by operational and logistical challenges. Additionally, disinfectant residuals and long residence times in distribution can degrade marker DNA, complicating source attribution. The use of MST in drinking water contexts has focused on understanding changes in microbial composition and dynamics throughout water supply, using sequencing-based tools such as SourceTracker (Knights *et al*., 2011; Liu *et al*., 2018; Wang *et al*., 2023). Quantitative PCR-based methods have also been successfully used retrospectively in disease outbreak investigations. For example, a norovirus outbreak in Pennsylvania, USA, was traced back to a contaminated drinking water well using the human-associated HF183 marker (Mattioli *et al*., 2021).

A 'toolbox' approach, wherein MST analysis is used to complement additional water quality metrics such as FIO compliance data and sanitary surveys, is increasingly advocated for MST investigations in water environments (USEPA, 2011).

Regulatory potential

MST methods are not currently approved for routine microbiological compliance monitoring under UK or international drinking water regulations. However, MST is increasingly recognised in regulatory frameworks for its value in source water and catchment assessments. In the US, USEPA provides technical guidance on the use of MST to support development of Total Maximum Daily Loads (TMDLs) for waterbodies impaired by faecal pollution under the Clean Water Act. MST data are used alongside FIB, sanitary surveys, and watershed data to guide remediation and regulatory actions (USEPA, 2011). Despite this, MST remains a supplementary tool and is not used to determine compliance with microbiological standards. Globally, similar positions are held by regulators in Australia and New Zealand, where MST is viewed as a valuable tool for catchment risk characterisation but not for treated water monitoring (NHMRC, 2022; Ministry of Health NZ, 2019).

Adenosine triphosphate (ATP)

As the main source of energy for all living cells, ATP can act as a key marker for bacterial activity in drinking water (Chen *et al*., 2024). The majority of ATP monitors rely on bioluminescence to quantify the amount of ATP in a sample, and this measurement can then be used to infer the level of bacterial contamination.

The reaction between luciferin and ATP, catalysed by luciferase, produces an intermediate which releases a photon on reaction with oxygen (Capuano *et al*., 2024). To isolate the contribution of ATP from viable cells, two assays are performed, an initial assay to determine extracellular ATP, and a further assay to determine total ATP using a lysis reagent to release the ATP within viable cells. The difference between the results of these two assays represents the proportion of ATP present in viable cells such as bacteria (Hansen *et al*., 2019).

Value as a sanitary indicator

ATP monitoring has been shown to be much faster (even real-time, see Section 1.5.3) and potentially more sensitive than traditional culture-based methods, but further work is required to identify risk-based ATP thresholds (Burnet *et al*., 2025). Furthermore, ATP is not entirely specific, and its measurement will include contributions of ATP from bacterial, animal and plant sources (Sattar *et al*., 2022). While established in other industries, only a few studies have explored the use of ATP monitoring as a measure of faecal contamination in drinking water (Burnet *et al*., 2025).

1.5.2 Chemical indicators

Similarly to conventional microbial indicators, chemical markers (also referred to as tracers) which are specific to wastewater can be used to identify the presence and source of faecal contamination in water, as part of a methodology known as chemical source tracking (CST). While not currently required or recommended in regulation, they have several potential benefits over conventional FIO-based methods, including specific faecal source attribution and rapid methods of detection (Hagedorn and Weisberg, 2009; Lim *et al*., 2017). The speed and reliability depend on the specific chemical marker and the analytical method used to determine its concentration (Hagedorn and Weisberg, 2009). Chemicals markers are chosen for their specificity to human wastewater and are therefore able to provide greater source differentiation due to the lack of regrowth in the environment when compared to some biological indicators (Hagedorn and Weisberg, 2009).

Chemical indicators can provide useful information regarding the presence of faecal contamination, but further research is required to understand the environmental fate of each marker in order for them to be representative of microbial risk (NRC, 2004). If correlation is established between microbial contamination and chemical indicators then health risks could be quantified using chemical parameters (Devane *et al*., 2019).

Studies have investigated the correlation between FIOs and certain chemical parameters, e.g. fluorescence brightening agents, ammonium and caffeine. However, it's important to note that very little research has been done to directly linked these chemical indicators to the risk of illness (Glassmeyer *et al*., 2005). As most research correlates them with FIOs, the chemical indicators act as double surrogates for health risk. While these chemicals can be useful for tracking human faecal pollution, they currently have limited value for guiding public health decisions and further research is required.

The following chemical indicators have been identified in the literature as receiving attention for use in microbial quality assessment. Although most of the literature focuses on surface, ground and wastewater, the use in drinking water is conceptually very similar. The key limitations on their use in drinking water are that concentrations in drinking water are magnitudes lower and that there is little evidence to prove their link to illness. Therefore, more sensitive techniques and epidemiological studies are required.

Caffeine

Caffeine is a widely consumed substance which degrades slowly in the environment after release via wastewater after excretion. The presence of caffeine indicates exclusively human faecal pollution and thus indicates specifically human pathogenic risk (Spence, 2015). A study by Daneshvar *et al*. found a strong positive correlation between caffeine and traditional faecal indicators during analysis of wastewater (Daneshvar *et al*., 2012). However, there is little agreement on the presence or strength of this relationship, with different research outputs finding this correlation at varying degrees (Ebrahimzadeh *et al*., 2021). Additionally, the environmental persistence and transport characteristics of caffeine require further research before it can be considered an ideal indicator (Hagedorn and Weisberg, 2009).

While several studies have shown the potential benefits of caffeine as an indicator of faecal contamination, none of these studies are specific to drinking water. Therefore, it is difficult to compare caffeine with conventional indicators of microbial risk.

Faecal sterols

Faecal sterols are a metabolic product of cholesterol which is present in the gut and faeces of both humans and animals. Faecal sterol profiling across a number of animals has shown that there are differences significant enough to differentiate between human and animal faecal contamination (Leeming *et al*., 1996). For example, coprostanol is a sterol with the highest abundance in human faeces and can therefore indicate the presence of human faecal contamination in water (USEPA, 2006).

However, the applicability of coprostanol to microbial quality assessment of drinking water is limited in two main ways. Firstly, the methods developed may not be sensitive enough to detect the low concentrations of corpostanol present in drinking water as they were mostly developed for environmental monitoring. Secondly, the correlation between *E. coli* and faecal sterols is subject to seasonal and geographical variation meaning that sterols may only be useful as indicators in certain circumstances (Lu *et al*., 2016).

Artificial sweeteners

Certain artificial sweeteners including acesulfame and cyclamate have been investigated as potential indicators of faecal contamination as both are widely used in the food and beverage industries (Tran *et al*., 2014). Neither of these compounds are metabolised by the body and so they are excreted and eventually introduce to the environment via wastewater, linking their presence to potential faecal contamination (Van Stempvoort *et al*., 2013; Li *et al*., 2020).

One particular study found a positive correlation between cyclamate and faecal indicator bacteria, suggesting that cyclamate could be used as an early screening tool to identify waters contaminated with human sanitary waste (Spoelstra *et al*., 2017).

For analysis of artificial sweeteners in water samples, high performance liquid chromatography and mass spectrometry are required, making analysis costly and slow (Ramos *et al*., 2022). Concentrations in drinking water are likely to be below the limit of detection unless advanced mass spectroscopy methodologies are used (Dietrich *et al*., 2021).

Fluorescence whitening compounds (FWCs)

Fluorescence whitening compounds (FWCs) are used to enhance the whiteness of clothes and can be found in most modern laundry detergent. Hence, these compounds are highly specific to domestic wastewater, therefore can indicate of anthropogenic pollution (Cao *et al*., 2009; Dubber and Gill, 2017). FWCs are not rapidly biodegradable and hence persist in the environment long enough for detection to take place. However, some degree of photodegradation does occur on exposure to sunlight (Assaad *et al*., 2014).

Since the detection of FWCs follows a simple fluorometric method, there has been a considerable amount of research into their potential use as a marker of human faecal contamination (Cao *et al*., 2009). However, there is very limited research in their use in the context of drinking water microbial quality. The accuracy of detection is variable and gives no quantitative measurement for FWCs in water, therefore this fluorescence approach can only give a simple presence/absence measurement (Dubber and Gill, 2017).

Hydrogen sulphide (H_2S)

The hydrogen sulphide test detects the presence of H_2S gas, which can be produced by certain bacteria of faecal origin, indicating faecal contamination (Wright *et al*., 2012). The H_2S test has been promoted for water quality testing in settings where conventional microbiological methods may be impractical due to cost, infrastructure, or technical limitations (WHO, 2002). This test is most accurate when faecal contamination is high and therefore unlikely to provide useful assessment of faecal contamination in the highly developed UK drinking water industry (Weppelmann *et al*., 2014).

Most literature focuses on simple presence or absence H_2S tests which fail to give a quantitative assessment of drinking water quality (WHO, 2002; Khush *et al*., 2013). Furthermore, the bacteria that produce H_2S are not specific to human waste meaning that concentrations of H_2S are not necessarily indicative of human faecal contamination (WHO, 2002).

1.5.3 Analytical techniques

Reliable analytical methods are central to the detection and characterisation of microorganisms in drinking water. Regulatory methods are currently based on culture-based techniques, which remain the foundation of microbiological compliance monitoring in the UK and internationally. However, recent years have seen substantial progress in the development of alternative methods offering greater sensitivity, faster turnaround times, and enhanced taxonomic resolution. This section outlines the principles and applications in drinking water, and regulatory potential of several key analytical techniques relevant to microbiological water quality

assessment. These techniques include flow cytometry, MALDI-TOF MS, molecular and sensor-based approaches. The chromatography methods used for chemical source tracking not implemented for drinking water monitoring in the UK or internationally are not discussed here.

Flow cytometry

Flow cytometry monitoring (FCM) is a culture-independent analytical technique that quantifies individual microbial cells in a liquid sample using light scatter and fluorescence. In drinking water applications, FCM is typically applied with fluorescent dyes such as SYBR Green I (which stains all cells) and Propidium Iodide (which stains cells with compromised membranes), allowing the measurement of total cell count (TCC) and intact cell count (ICC), respectively (Van Nevel *et al*., 2017; Cheswick, 2019).

Unlike culture-based cell counting methods like HPC, which detect only a small fraction (<1%) of the microbial community capable of growing on solid media, FCM quantifies the entire bacterial population (Van Nevel *et al*., 2017). This is particularly valuable in the context of disinfection, where viable but not culturable (VBNC) organisms, induced by disinfectant stress, may persist undetected using traditional techniques (Cheswick *et al*., 2019). FCM typically provides results within 15-30 minutes and is capable of analysing over 10,000 particles per second (Cheswick *et al*., 2019). The detection limit of FCM generally falls between 200 and 1000 cells/mL depending on instrument sensitivity.

Value as a sanitary indicator

Flow cytometry provides quantitative data on microbial cell concentrations and has been used to assess water quality across source, treatment, and distribution stages. It is currently employed by many UK water companies, either routinely or as part of investigative monitoring, and an industry user group exists to share operational and academic insights (Cheswick, 2019).

FCM has been used to investigate a range of treatment processes including clarification, membrane and media filtration, and disinfection (Cheswick *et al*., 2019). A series of bench- and pilot-scale studies published by Cheswick *et al*. explored the use of FCM in treatment process evaluation, with a focus on chlorination efficiency (Cheswick 2019; Cheswick *et al*., 2019, 2022). These studies demonstrated that FCM can be used to characterise microbial reductions across different treatment stages and to quantify log removal of bacterial cells with a level of resolution not achievable by traditional culture-based methods (Cheswick *et al*., 2019, 2022). Notably, chlorine disinfection was shown to selectively reduce ICC, without affecting TCC, making FCM particularly useful for assessing disinfection efficacy (Cheswick *et al*., 2022). This research ultimately informed an assessment of the disinfection practices at Scottish Water (Cheswick, 2019).

In chlorinated and unchlorinated distribution systems, FCM has been used to support operational assessments of biofilm activity, ingress, and microbial regrowth (Liu *et al*., 2013; Schonher *et al*., 2021; Claveau *et al*., 2024b; Pluym *et al*., 2024). Recent research has focused

on the optimisation of biofilm sampling for FCM analysis of distribution systems (Pick and Fish, 2024). FCM has also proven valuable in the assessment of microbial loadings in source waters, and can provide insight into seasonal variations, particularly in groundwaters (Cheswick, 2019).

It is worth noting that FCM results do not correlate consistently with traditional FIOs (Van Nevel *et al*., 2017). For example, although elevated ICC or TCC may coincide with coliform detections in some cases, coliform positive samples have also been observed at low cell counts, and high cell count samples are often coliform negative (Cheswick *et al*., 2019). Additionally, FCM does not provide taxonomic resolution or detect specific pathogens and cannot be relied upon to detect small viral particles (Singh *et al*., 2022). As such, FCM data is not recommended to be used to infer health risk or the presence of faecal contamination, and should be used as a complementary measure to existing FIO monitoring (Cheswick *et al*., 2019). Additionally, data interpretation remains a key challenge for this method. Most UK water companies apply fixed gating strategies to classify intact and total cells; however, dynamic gating methods and fingerprint-based analyses have been recommended to improve reproducibility in variable systems (Claveau *et al.*, 2024a).

Regulatory potential

Flow cytometry is not currently approved for microbiological compliance monitoring in the UK. Nevertheless, it is increasingly used by UK water companies as an operational tool to support Regulation 27 risk assessments, treatment optimisation, and investigative monitoring.

Internationally, the Swiss Association for Gas and Water (SVGW) has recently incorporated flow cytometry into national guidance and published a standard method (MW102; SVGW, 2023). WHO Europe has recognised the value of FCM in the evaluation of regrowth in the distribution network, particularly in comparison to less sensitive methods such as HPC. However, WHO do not currently recommend its inclusion in the EU Drinking Water Directive at present, primarily due to the lack of widespread implementation of FCM methods across the EU and reported variations in sensitivity, though it remains listed for reconsideration at the next revision (WHO Europe, 2017).

MALDI-TOF MS

Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) is a proteomics-based identification method that classifies culture microorganisms by comparing their protein mass spectra (primarily ribosomal) to reference databases (SCA, 2020). It is typically applied to pure colonies obtained from membrane filtration or plate count methods and provides genus or species-level identification.

Value as a sanitary indicator

The primary role of MALDI-TOF MS in drinking water microbiology is the identification and characterisation of heterotrophic bacteria isolated using traditional culture-based techniques

from drinking water, source water, and treatment systems. This method has been applied in water treatment to identify the dominant culturable strains across different treatment processes. For example, Sala-Comorera *et al*. (2017) used MALDI-TOF MS in a full-scale drinking water treatment plant to track changes in heterotrophic populations from raw water through sand filtration, ultrafiltration, reverse osmosis, and chlorination, with distinct shifts in community composition observed at each treatment stage. MALDI-TOF MS has also been used to investigate microbial community dynamics and the presence of pathogens in treated water, bottled water, and source waters, including groundwater (Sala-Comorera *et al*., 2020).

While MALDI-TOF MS provides species- or genus-level identification for many isolates, its resolving power is limited by the coverage of the reference library. Identification of drinking water isolates is often incomplete or incorrect when using general-purpose libraries, and the taxonomic resolution may be insufficient to distinguish between closely related species, such as *E. coli* and *Shigella* spp. (SCA, 2020; Ashfaq *et al*., 2022). Recent efforts have focused on the expansion of reference libraries to encompass drinking water-specific strains, which has been shown to improve correct identification rates (Pinar-Méndez *et al*., 2021). Additionally, the reliance of MALDI-TOF MS on colonies isolated via culture-based techniques means that it suffers from the same limitations faced by culture-based identification, namely that it cannot be used to analyse VNBC cells, and is thus limited to a small proportion of the overall microbial population (Sala-Comorera *et al*., 2020).

Regulatory potential

MALDI-TOF MS is not currently present in UK water regulations but is included in the SCA Microbiology of Water and Associated Materials (SCA, 2020) as an optional tool to identify colonies isolated via approved culture-based methods. It is recommended that laboratories employing MALDI-TOF MS for water quality assessment should assess the suitability of reference libraries via desktop review and empirical testing, with the option of including additional strains/species given proper validation (SCA, 2020). MALDI-TOF MS analysis is offered by several external companies in the UK, in addition to in-house facilities developed by some water companies. MALDI-TOF MS has also been applied internationally; for example, in Germany, the Technology Centre for Water (TZW) and German Technical and Scientific Association for Gas and Water (DVGW) have conducted several studies on the use of MALDI-TOF MS to identify a range of water-relevant microorganisms, including coliforms, *Campylobacter*, *Pseudomonas aeruginosa*, and enterococci, in drinking water and groundwater (TZW, 2025).

Molecular approaches

Molecular techniques are based on the detection of biomolecules, mainly proteins and nucleic acids, though the assays targeting proteins are usually not sensitive for low concentration target identification. On the other hand, nucleic acid identification has increasingly been used in water microbiology to detect and characterise microorganisms. Key approaches in the context of drinking water include polymerase chain reaction (PCR) and next-generation sequencing

(NGS). PCR amplifies specific nucleic acid sequences to detect and quantify microorganisms of interest. As it is a rapid, sensitive and relatively cheap method, it has been applied to the detection of FIOs, MST markers, and a wide range of waterborne pathogens in drinking water (DWI, 2013; Maheux *et al*., 2024; NHMRC, 2022). NGS methods allow for the high-throughput sequencing of DNA, often via 16S rRNA gene amplicon sequencing, which can be used to characterise the microbial community structure of a water sample (Werner *et al*., 2022). These approaches do not rely on culture and can identify a broad range of taxa, including those not readily detected using conventional methods.

Value as a sanitary indicator

Molecular detection methods can offer significant advantages in terms of speed, specificity, and breadth of detection relative to culture-based methods, though they are often limited by cost, the requirement for large sample volumes and concentrations steps, the presence of inhibitory substances, and issues regarding cell viability and infectivity, as detailed below.

Quantitative PCR (qPCR) enables the rapid detection of specific organisms, including those that are difficult or impossible to culture. It is particularly valuable in outbreak investigations and source attribution and has been used to achieve greater taxonomic resolution for indicators such as coliforms (Maheux *et al*., 2014; Holcomb and Stewart, 2020; Pluym *et al*., 2024). However, qPCR does not confirm organism viability or infectivity, which limits its utility in directly assessing health risk (WHO, 2022). Complementary approaches such as propidium monoazide (PMA) treatment and integrated cell culture PCR (ICC-PCR) have been used to address this limitation (DWI, 2013; Cheswick, 2019), however they require further validation prior to implementation in water quality assessment.

NGS approaches have value in the assessment of microbial community composition across source waters, treatment, and distribution (Sala-Comorera *et al*., 2020) and can give insights into community variations and microbial activity. Recent advances in this technology have allowed for rapid and field-deployable platforms (Werner *et al*., 2022). However, the application of NGS to operational settings remains generally limited by high costs, long turnaround times, and complex data interpretation. Similarly to PCR-based approaches, NGS does not indicate viability or infectivity. These methods do not at present therefore give a direct measure of sanitary water quality. As such, in the water industry they are typically used as complementary tools for investigative purposes.

Regulatory potential

Molecular methods are not currently approved for routine compliance monitoring in UK or international drinking water regulations. Regulatory frameworks remain focused on culture-based enumeration of standard indicator organisms, and while PCR assays for viruses and protozoa are recognised in national and international guidance documents (DWI, 2013; Ministry of Health NZ, 2019; NHMRC 2022; WHO, 2022), their use is generally restricted to outbreak investigation or research contexts (WHO, 2022). Challenges regarding protocol

standardisation, cost, data complexity, and unclear links to health risk have been noted by regulators as limits on routine operational use (Ministry of Health NZ, 2019; WHO, 2022).

Tryptophan-like fluorescence

Tryptophan-like fluorescence (TLF) refers to the fluorescence emission peak observed between 280 and 350 nm, which is typically associated with the presence of aromatic amino acids such as tryptophan. This signal can be measured using fluorescence microscopy to characterise natural organic matter (Sorensen *et al*., 2021). This analytical approach has been applied to assess microbial contamination in water due to the relationship between TLF emission intensity and faecal indicator organisms, particularly *E. coli* (Ward *et al*., 2021). This relationship exists in part because *E. coli* excretes compounds which fluoresce in the TLF region. In addition to excreting tryptophan, *E. coli* can hydrolyse tryptophan to form indole, a compound that fluoresces even more strongly within the TLF spectral range (Sorensen *et al*., 2018). As a result, elevated TLF signals may serve as an indirect indicator of *E. coli* contamination.

Value as a sanitary indicator

There are many purported benefits of TLF spectroscopy when compared to traditional culture based microbial assessment (Sorensen *et al*., 2018). The instantaneous nature of this approach means that it can provide real-time screening for microbial contamination while the absence of additional reagents makes it well suited to online implementation (Sorensen *et al*., 2018).

This approach has been shown to struggle in low contamination situations, with Sorensen *et al*. showing that it is not effective at classifying samples with *E. coli* levels lower than 10 CFU/100mL (Sorensen *et al*., 2018). Additionally, TLF spectroscopy is poor at detecting large, short-term fluctuations in microbial water quality (Ward *et al*., 2021). A further issue highlighted in literature is the impact of other organic matter on the strength of the TLF signal. For instance, high concentrations of organic matter can both strengthen and weaken the signal (Nowicki *et al*., 2019). With all of this in mind, TLF spectroscopy is suggested as a useful early screening tool to be used in conjunction with traditional culture methods (Ward *et al*., 2021).

Sensor technologies

A range of in-situ sensor technologies are currently applied in the water industry to support rapid microbiological monitoring. The specific details of sensors currently used in the water industry are summarised in Table 1.3. These sensors offer near real-time data and improved operational awareness but are typically limited to surrogate or proxy measurements, require calibration against laboratory methods, and vary in sensitivity and specificity depending on water type and target organism.

Table 1.3 Selected commercially available sensors for microbial parameters

Technology name	Type	Parameter	Sample point	Operational principle
ColiMinder [1]	Enzymatic detection	*E. coli* Enterococci Total microbiological activity	Source water Treated drinking water	Addition of enzyme specific fluorescent indicators which fluoresce to varying degrees based on enzyme activity. Measurement of this fluorescence is used to quantify microbial contamination. Because each parameter/microorganism has unique enzymes, different reagents must be used for each [2, 3]
MicroLAN: BACTcontrol [4]	Enzymatic detection	*E. coli* Enterococci Total microbiological activity	Source water Treated drinking water	
bNovate: Bactosense [5]	Flow cytometry	Total microbial cell count	Source water Treated drinking water	Samples are dyed and incubated before being excited with a monochromatic light source. The resulting cellular fluorescence is then measured in order to determine the total microbial cell count [6]
Proteus Water Quality Probe [7]	In-situ tryptophan-like fluorescence	*E. coli*	Source water Treated drinking water	Spectroscopic method which measures the fluorescence of compounds with similar fluorescence characteristics to tryptophan. The known positive correlation between tryptophan fluorescence and microbial concentration is then applied to assess the degree of microbiological contamination [8, 9]
Virridy: Lume [10]	In-situ tryptophan-like fluorescence	*E. coli*	Source water Treated drinking water	
EZ-ATP [11]	Adenosine triphosphate (ATP) detection	Total microbiological activity	Source water Treated drinking water	ATP is quantified through a reaction which induces the release of visible light proportional to the concentration of ATP in the sample. ATP is present in all bacterial cells and can therefore be used as a proxy measurement of microbial contamination [12]

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Technology name	Type	Parameter	Sample point	Operational principle
	11. AppliTek (2014) “EZ ATP brochure.” Available at: https://www.applitek.com/wp-content/uploads/2016/06/EZ-ATP-leaflet-EN-1.pdf (Accessed: June 10, 2025). 12. Vang, Ó.K. *et al.* (2014) “Evaluation of ATP measurements to detect microbial ingress by wastewater and surface water in drinking water,” *Water Research*, 64, pp. 309–320. Available at: https://doi.org/10.1016/j.watres.2014.07.015.			

2. Insights from stakeholders on the use of novel faecal indicators and technologies

2.1 Summary

In this section, we aimed to develop a practical understanding of how indicators are currently used for operational risk assessment and regulatory compliance across the water industry. We constructed an online survey and a series of in-depth interviews with stakeholders from UK water utilities, companies that manage water distribution networks (i.e. New Appointments and Variations – NAVs), and international organisations. We explored the application, confidence, and perceived value of both traditional and emerging faecal indicators and analytical techniques, highlighting differences in practice and perspective across stakeholder groups.

Key findings:

- Core faecal indicators (*Escherichia coli*, coliforms, enterococci) remain central for compliance; *E. coli* is considered the most reliable and actionable.
- Confidence in total coliforms, heterotrophic plate count (HPC), and non-lactose fermenters is mixed because they are not specific to faecal contamination, making interpretation challenging.
- UK water companies are exploring advanced techniques, e.g. matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) for identifying bacteria, flow cytometry monitoring (FCM) for counting microbes and polymerase chain reaction (PCR) for targeted genetic detection, but these are not yet standard practice.
- International respondents show greater adoption of advanced and pathogen-focused monitoring, often driven by research or regulatory differences.
- Stakeholders expressed interest in flexible, proportionate regulatory guidance, especially for interpreting HPC, and integrating novel methods in routine monitoring.
- Interviewees call for clarity on indicator organism purpose (contamination detection, process control, health risk) and evidence-based frameworks tailored to each use case.

While traditional indicators continue to underpin regulatory practice, there is growing momentum internationally toward multi-indicator, risk-based approaches that incorporate emerging technologies.

2.2 Introduction

The literature review in the previous section provided an in-depth assessment of traditional and emerging microbial indicators and analytical methods. However, it did not reflect on the operational and compliance utilisation of the indicators and approaches. In order to gain a practical understanding of how indicators of microbiological water quality are currently used or could be used, we conducted an industry-wide survey, followed by 1-2-1 interviews with water industry representatives. The responses enabled the assessment of the usefulness and potential of indicators and novel methods for operational risk assessment and regulatory purposes in drinking water quality assessment.

2.3 Methodology

2.3.1 Survey

A short (~15 min) Google Forms questionnaire was designed to gather information on the use of microbial indicators and analytical techniques in the water industry, and the confidence of the responders in the value of these indicators/techniques for the assessment of microbial water quality. The first five questions focused on personal data whereas questions 6-21 were technical. The questionnaire was shared with UK water industry contacts via the DWI and international water industry contacts via WRc. Responses were collected over a period of approximately 3 weeks (June-July 2025).

The survey received a total of 23 responses: 13 from UK companies, and 10 from international contacts (Table 2.1). Results were visualised using R v4.5.1 (inclusive colour palette), Microsoft 365 Copilot and Google Forms.

2.3.2 1-2-1 interviews

A total of 15 1-2-1 interviews were conducted during August-September 2025. Organisations interviewed (Table 2.2) included 8 UK representatives (4 water utilities, 4 NAVs) and 7 international representatives spanning Western/Central Europe and Australasia (2 water utilities, 4 government research organisations, and 1 academic research organisation).

Interviews were 30-60 minutes in duration and conducted virtually using Microsoft Teams. Questions were designed beforehand and included general questions that were common across all interviews, and questions tailored to the specific survey responses of the interviewee. Interview notes were sifted and summarised using Microsoft 365 Copilot.

Table 2.1 Summary of survey respondents

Organisation	Location	Type
Southern Water	UK	Water utility
SES Water	UK	Water utility
Dŵr Cymru Welsh Water	UK	Water utility
Wessex Water	UK	Water utility
Anglian Water	UK	Water utility
United Utilities	UK	Water utility
Affinity Water	UK	Water utility
Severn Trent Water	UK	Water utility
Matrix Water	UK	NAV
Advanced Infrastructure Networks	UK	NAV
Independent Water Networks (IWNL)	UK	NAV
ESP Water	UK	NAV
Albion Water	UK	NAV
Jersey Water	Jersey	Water utility
Waternet Amsterdam	The Netherlands	Water utility
Eau de Paris	France	Water utility
National Centre for Public Health and Pharmacy	Hungary	Research (government)
Public Health and Forensic Science	New Zealand	Research (government)
Swiss Federal Institute of Aquatic Science and Technology (Eawag)	Switzerland	Research (government)
Luxembourg Institute of Science and Technology	Luxembourg	Research (government)
Commonwealth Scientific Industrial Research Organisation (CSIRO)	Australia	Research (government)
TU Wien	Austria	Research (academic)
Universidade Federal de Viçosa	Brazil	Research (academic)

Table 2.2 Summary of organisations interviewed

Organisation	Location	Type
Southern Water	UK	Water utility
Sutton and East Surrey Water	UK	Water utility
Dŵr Cymru Welsh Water	UK	Water utility
Wessex Water	UK	Water utility
Matrix Water	UK	NAV
Advanced Infrastructure Networks	UK	NAV
Independent Water Networks (IWNL)	UK	NAV
ESP Water	UK	NAV
Waternet Amsterdam	The Netherlands	Water utility
Eau de Paris	France	Water utility
National Centre for Public Health and Pharmacy	Hungary	Research (government)
Public Health and Forensic Science	New Zealand	Research (government)
Swiss Federal Institute of Aquatic Science and Technology (Eawag)	Switzerland	Research (government)
Luxembourg Institute of Science and Technology	Luxembourg	Research (government)
TU Wien	Austria	Research (academic)

2.4 Findings

For the purposes of reporting and analysis, the survey and 1-2-1 findings have been separated into three groups: UK water utilities/incumbents, UK NAVs, and international organisations. Individual organisations have been anonymised and randomly assigned numbered labels.

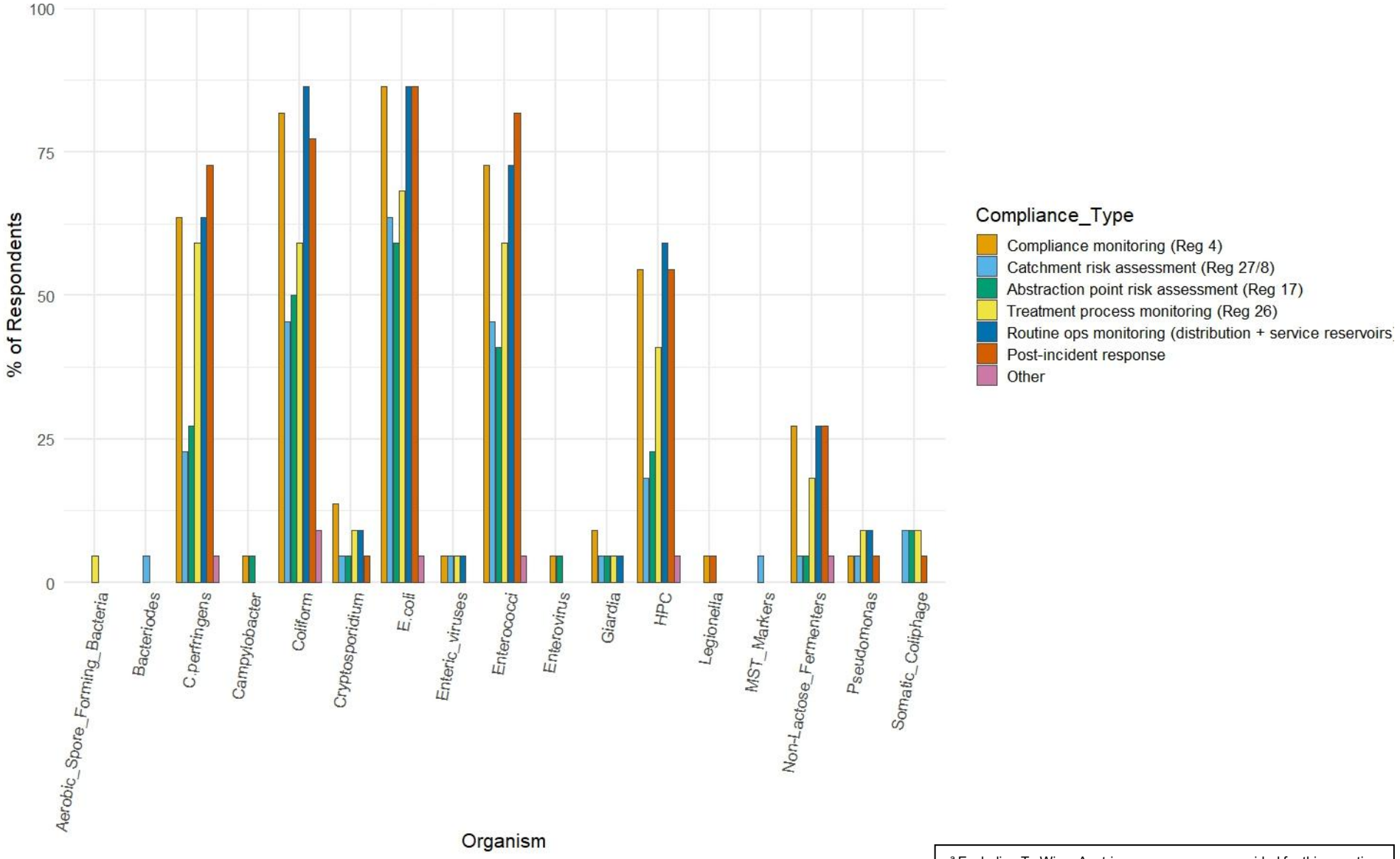
The results of the survey provide a broad-ranging and high-level view of the application of microbial indicators and related analytical techniques across the water industry, both nationally and internationally. UK water utilities demonstrate a broadly integrated use of both traditional and alternative methods, reflecting their regulatory obligations and operational scope, though adoption of more advanced/emerging methods and indicators is more limited. NAVs, operating primarily at the distribution level, are constrained to compliance indicators and techniques only, and rely on monitoring data from incumbent water utilities. International respondents, many of whom are research-focused or operate in different regulatory contexts to the UK, show strong engagement with emerging technologies and MST, and more commonly conduct direct pathogen monitoring to support microbial risk assessment approaches. A detailed summary of survey results, including all graphs and figures, is provided in Table 1.2.

2.4.1 General feedback on microbial indicators

Core faecal indicator bacteria – *E. coli*, coliforms, and enterococci – are consistently applied across organisations for both compliance and operational monitoring. Confidence in *E. coli* and enterococci is generally high, whereas views on coliforms are more varied, particularly among international respondents. General indicators of microbial water quality, such as non-lactose fermenters (NLFs) and heterotrophic plate counts (HPC), tend to inspire moderate to low confidence. NLFs are commonly used by UK water utilities but are applied minimally or not at all in international contexts. UK water utilities also demonstrate moderate use of non-regulatory indicators, including *Pseudomonas*, *Cryptosporidium*, and NLFs, though these are not employed by NAVs. Internationally, a broader range of indicators is used – often reflecting differing regulatory frameworks – including somatic coliphage for assessing treatment process efficiency, *Aeromonas* for network monitoring, and various pathogens such as *Legionella*, mycobacteria, enteric viruses, *Cryptosporidium*/*Giardia*, and *Campylobacter*. In some countries, direct pathogen monitoring at source waters or abstraction points is mandatory and contributes to quantitative microbial risk assessments (QMRAs).

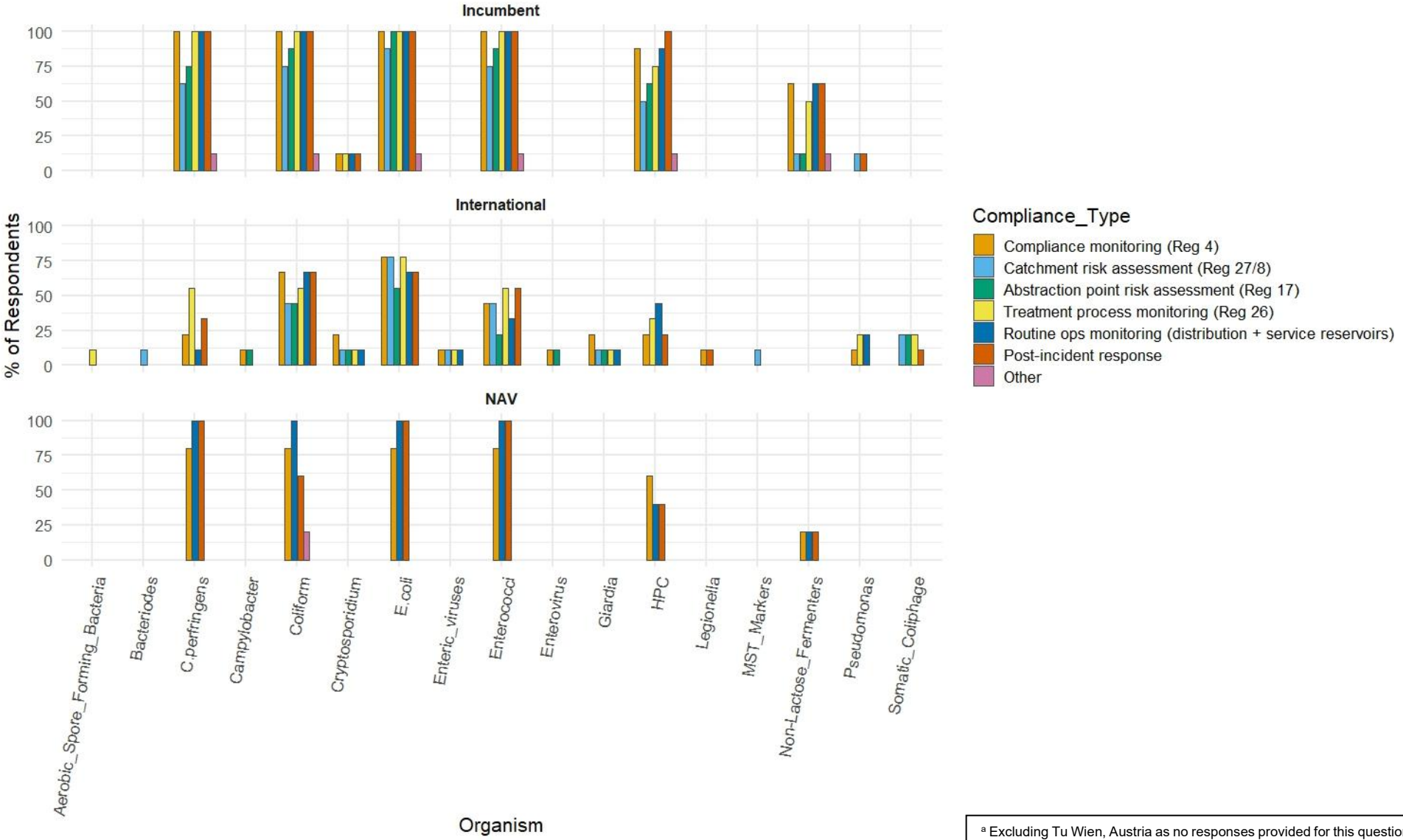
A graphical overview of the use of microbial indicators by compliance type is provided in Figure 2.1 (total respondents) and Figure 2.2 (separated by respondent type).

Figure 2.1 Percentage of respondents using indicator organisms for each application (total respondents n=22[a]).



[a] Excluding Tu Wien, Austria as no responses provided for this question

Figure 2.2 Percentage of respondents (n=22[a]) using indicator organisms for each application, separated by respondent type.



[a] Excluding Tu Wien, Austria as no responses provided for this question

2.4.2 General feedback on analytical techniques

Most UK water utilities employ alternative methodologies beyond those listed in Schedule 5 of the regulations to assess microbial water quality, whereas NAVs do not use such methods. PCR-based techniques are commonly applied by UK utilities for post-incident response, with high confidence in their effectiveness. Internationally, PCR usage is widespread but more diverse in purpose, with significant emphasis on MST. Flow cytometry is widely adopted in the UK for routine operational and treatment process monitoring, although confidence in its reliability is mixed. Its use by international respondents is more limited but is currently being explored. MALDI-TOF MS is another method in which many UK utilities express confidence, particularly for compliance monitoring and post-incident analysis.

More advanced or novel approaches – such as next-generation sequencing, chemical source tracking, and fluorescence-based detection – are more frequently used by international respondents, many of whom are research-focused. UK exploration of these techniques remains limited. In-situ sensors are used to some extent in the UK, though confidence in their performance ranges from low to moderate, in contrast to the higher confidence reported internationally. Around a quarter of UK water utility participants are trialling or planning to trial novel indicators and methods, including MALDI-TOF MS, metagenomics, endotoxin testing, fluorescence, and environmental DNA (eDNA) monitoring. Internationally, a broader array of techniques is under trial, including MST, flow cytometry, viral monitoring (targeting human viruses and indicators such as crAssphage), and machine learning integrated with metagenomics.

A graphical overview of the use of analytical techniques by compliance type is provided in Figure 2.3 (total respondents) and Figure 2.4 (separated by respondent type).

Figure 2.3 Percentage of respondents using technique for each application (total respondents n=23).

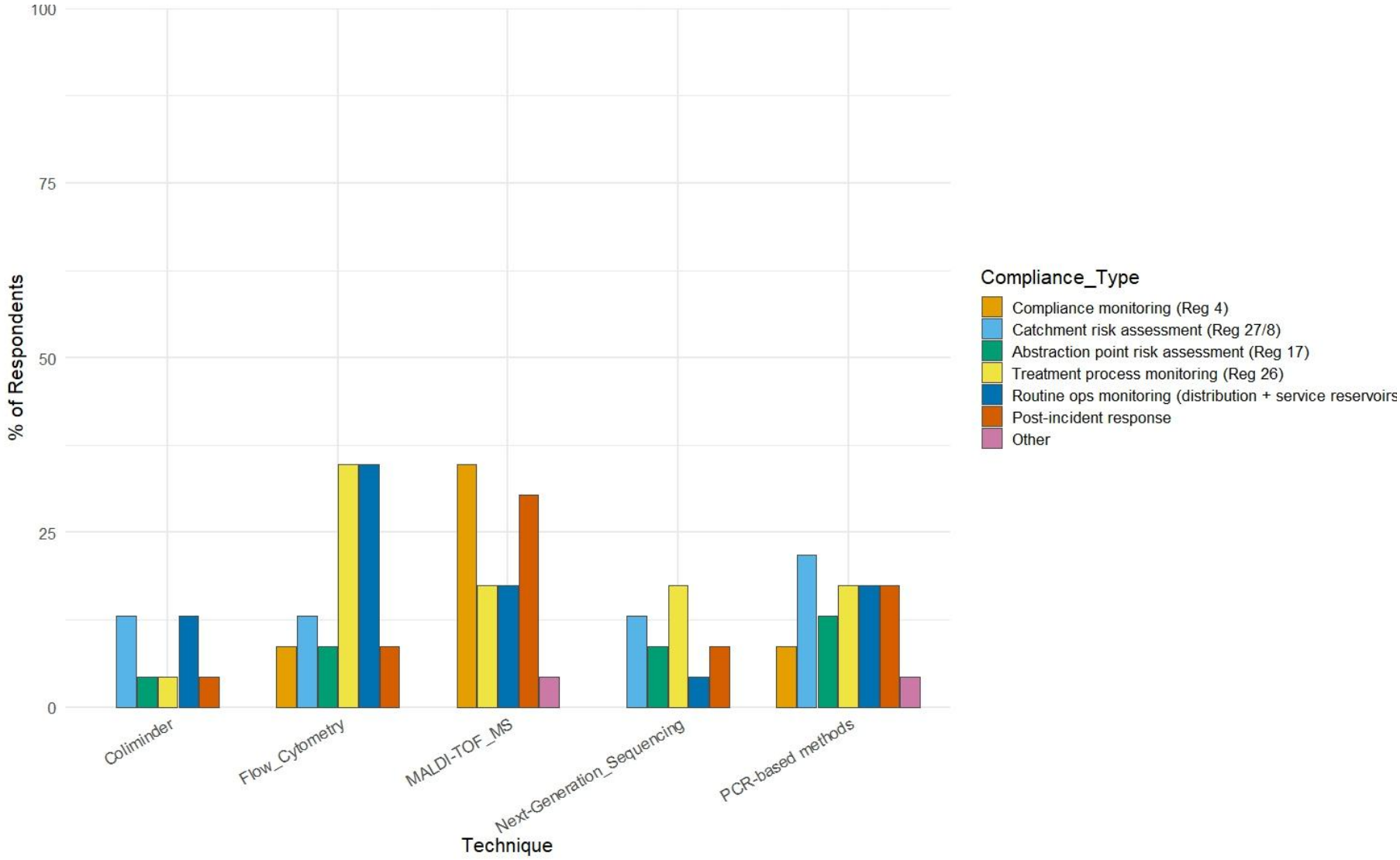
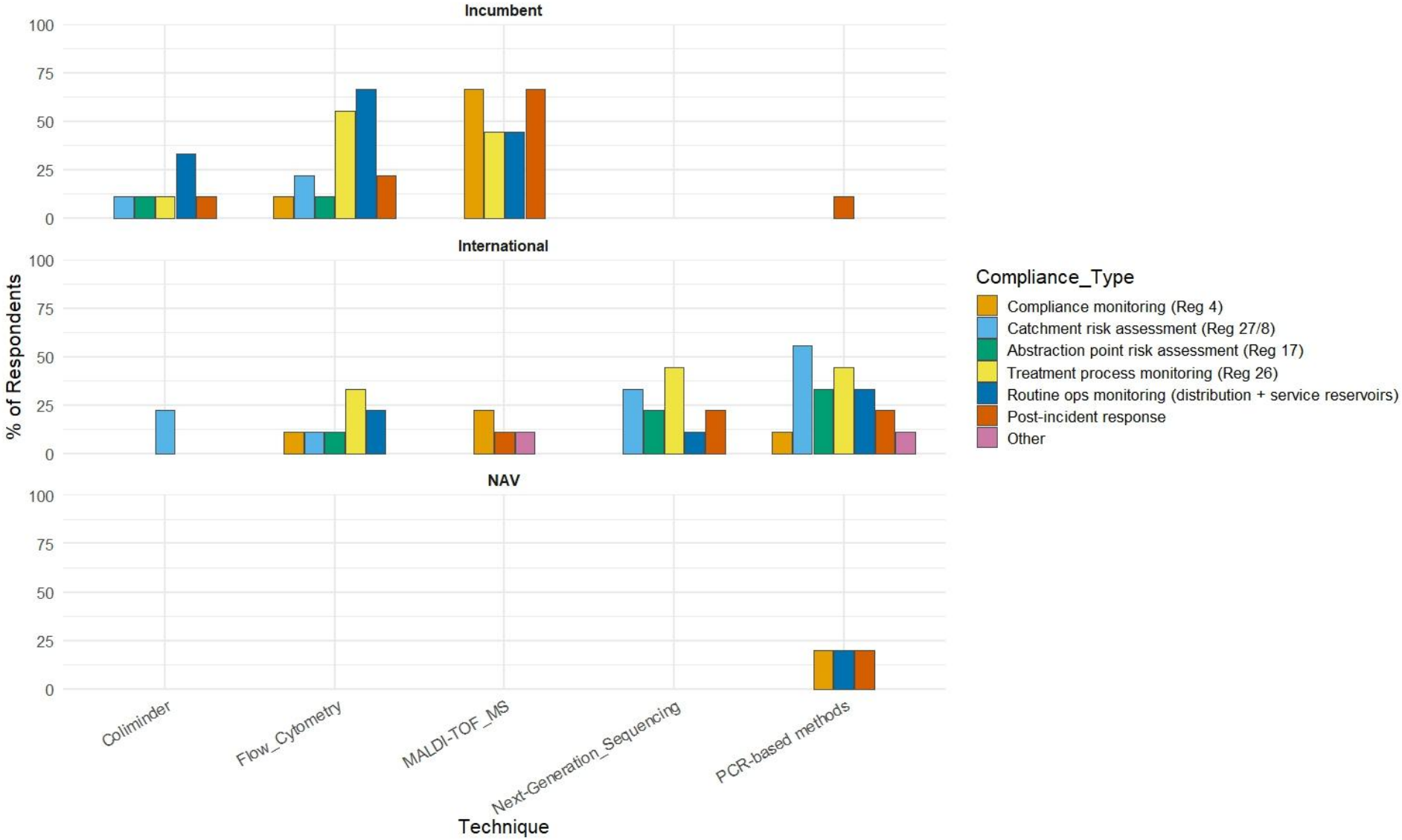


Figure 2.4 Percentage of respondents (n=23) using analytical techniques for each application, separated by respondent type.



2.4.3 Detailed insights from UK water utilities/incumbents

Across all four companies, *E. coli* remains the operational anchor for confirming faecal contamination, while supporting indicators are used to triage investigations and understand context. Coliforms are widely deployed for early warning (with mixed enthusiasm), NLFs are valued by some as precursor signals, and HPC/TVC often carry low operational value – interpreted via trends/baselines where retained, but rarely decisive for rapid actions. MALDI-TOF stands out as a high value confirmatory tool that can prevent unnecessary public actions; views on FCM range from promising for operational insight to not yet reliable as an early warning predictor.

Regulatory and operational context

In the regulatory and operational context, there is a call for greater flexibility and proportionality in guidance to help avoid unnecessary and prolonged incidents. For example, a single detection of *E. coli* at a customer tap may indicate point-of-use contamination that could be resolved within a day, whereas defaulting to extended boil water advice may introduce its own risks and costs. Several utilities have requested clearer definitions and expectations regarding HPC results, particularly around what constitutes 'no abnormal change'. Interview responses revealed a variety of approaches to interpreting this guideline, ranging from minimal operational use of HPC data, to the routine application of context-specific thresholds and action triggers.

There is also interest in formally recognising MALDI-TOF mass spectrometry due to its practical value, alongside a more limited proposal to consider flow cytometry in regulation as an optional operational metric rather than a mandated replacement. No specific additional microbial indicator organisms were identified by this group for inclusion in future regulatory updates; there is interest in viral indicators, but cost and feasibility challenges associated with viral testing were also raised.

2.4.4 Detailed insights from UK New Appointments and Variations (NAVs)

E. coli remains the most valued indicator across NAVs, providing the clearest confirmation of abnormal conditions. Coliforms are in use but often viewed as ambiguous due to frequent, sometimes non-repeatable positives, while HPC is widely regarded as low-specificity, mainly useful for trend or baseline tracking rather than decisive action. NAV priorities are shaped by their distribution-only role: reliance on incumbent compliance datasets, variability in data format and access, and the need for proportionate monitoring requirements. Appetite for novel methods is minimal, with additional measurements (e.g. *Cryptosporidium* count) applied only in response to notified risks. Rapid operational checks (chlorine residual, turbidity, taste and odour) remain central to on-site triage. Risk assessment approaches typically include water safety planning/risk assessment (WSP/RA) matrices aligned to compliance risk index (CRI) impact logic, with small percentage shifts or baseline deviations acting as triggers. NAVs

request clearer guidance on HPC trending, proportional scaling of testing for small zones, consideration of *Legionella* and *Pseudomonas* in distribution/biofilm contexts, improved data standardisation and sharing from incumbents. Viral indicators are acknowledged in principle but considered of limited practical value under current constraints.

Regulatory and operational context

Several companies highlighted concerns around proportionality for NAVs, noting that they face similar testing burdens as incumbent water utilities – even for small zones or new builds – which can lead to duplication and potentially excessive testing relative to actual risk. As a result, there is support for scaled requirements. In line with views expressed by incumbents, multiple NAVs also called for clearer guidance on HPC 'no abnormal change', favouring interpretive clarity over strict numerical limits. There was a suggestion that *Pseudomonas* and *Legionella* should be considered for inclusion in drinking water regulations due to their relevance to biofilms and domestic plumbing systems. Additionally, several NAVs emphasised the value of chemical parameters, such as total organic carbon (TOC) and chlorine residual, as contextual indicators of microbial risk.

Practical challenges unique to NAVs were also discussed, including delays in response due to limited laboratory access and costs, difficulties in event sampling due to access constraints, and limitations in portal tooling, which currently lacks trending and analytics capabilities. A recurring theme was the need for more standardised data sharing from incumbent utilities.

2.4.5 Detailed insights from international organisations

E. coli remains the central faecal indicator organism internationally, valued for actionability and historic comparability, while coliforms and HPC are widely questioned for risk relevance, retained mainly for process or trend value. Human viruses and MST markers are increasingly recognised as important but not yet regulated, with several stakeholders advocating for inclusion. Molecular methods (quantitative and digital PCR) and rapid/online tools (e.g. flow cytometry, ColiMinder) show promise for speed and sensitivity but face barriers of cost, training, standardisation, and unclear action thresholds. Risk assessment practices are evolving from compliance-based monitoring towards multi-indicator, risk-based approaches (including WSP, QMRA, and emerging MST integration), supported by high-frequency/event-driven sampling. Stakeholders emphasise the need for clearer regulatory frameworks on method equivalence, indicator purpose, and data use, alongside improved catchment-based approaches and alignment with global safe water goals.

Regulatory and operational context

Several interviewees expressed support for a shift toward a risk-based approach to microbial water quality monitoring, advocating for a move away from reliance on single indicator organisms in favour of multi-indicator and pathogen-focused strategies tailored to local context. Many organisations are actively trialling new techniques, including PCR-based MST, flow

cytometry, metagenomics, and online sensors. While there is broad support for the future inclusion of these methods in regulations, interviewees emphasised the importance of validating and standardising laboratory procedures and data interpretation, alongside the development of clear, science-backed guidance and parametric values. Some participants also highlighted the need for greater flexibility in regulations – not only in terms of which parameters are monitored, but also in how they are measured. Calls were made for clearer guidance on the intended purpose of regulatory indicator organisms, whether for contamination detection, process control, or health risk inference, and for supporting evidence tailored to each use-case.

A global perspective was also raised, with reference to alignment with WHO Sustainable Development Goals and the principle of equitable access to safe water, highlighting the importance of ensuring that methodological advances lead to practical, scalable improvements.

3. Multi-criteria analysis of faecal indicators and emerging technologies

3.1 Summary

This section presents a structured summary of the comparative evaluation of both established and emerging indicators and technologies for detecting faecal contamination in drinking water. The multi-criteria analysis was designed to provide an objective, transparent framework for ranking the suitability of each option, taking into account scientific validity, operational practicality and industry needs.

Key findings:

- Traditional indicators such as *Escherichia coli* and enterococci remain the most suitable for routine monitoring, scoring highest for sensitivity, specificity, and regulatory acceptance. Their widespread use is supported by robust standard methods and clear interpretive frameworks.
- Emerging indicators like somatic coliphages and microbial source tracking (MST) markers offer valuable supplementary information, particularly for viral risk assessment and source attribution. However, their adoption is currently limited by factors such as laboratory capacity, cost, and lack of standardised protocols.
- Technological innovations such as matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), polymerase chain reaction (PCR)-based methods, and flow cytometry, rank highly for their potential to deliver rapid, actionable results and enhanced taxonomic resolution. These methods are increasingly being trialled or adopted for operational and investigative purposes, though challenges remain around standardisation, training, and initial investment.
- Sensor-based and next-generation sequencing approaches show promise for real-time or high-resolution monitoring but currently face barriers related to cost, data complexity, and lack of regulatory frameworks.

The analysis confirms that a multi-indicator, multi-technology approach is optimal for safeguarding drinking water quality. While traditional indicators should remain the foundation of compliance monitoring, gradual integration of advanced methods can enhance responsiveness, specificity, and overall risk management. The findings also highlight the importance of the development of clear, evidence-based guidance to support the adoption of new technologies.

3.2 Introduction

The selection of appropriate indicators and analytical methods is critical for effective monitoring and management of faecal contamination in drinking water. Building on the comprehensive review of current and novel practices and stakeholder perspectives presented in the previous sections, this section applies a structured, multi-criteria analysis to both established and emerging faecal indicators and emerging technologies. By systematically evaluating each option against key criteria this analysis aims to identify the most suitable tools for risk assessment and regulatory compliance. The findings provide an evidence-based framework to support decision-making in the adoption and prioritisation of indicators and methods for safeguarding water quality now and in the future.

3.3 Methodology

3.3.1 Scoring exercise for indicators

All indicators (existing or potential) that were identified during the literature review and the stakeholder engagement exercise were listed (Table 3.1). This includes existing faecal indicators that are currently used by the water industry, as well as those that have potential to be included in risk assessment and monitoring for faecal pollution. Pathogens (e.g. *Cryptosporidium*, enteric viruses) were also included as those are frequently included in risk assessments overseas. For enteric viruses, Human *Mastadenoviruses* (formerly known as Human Adenoviruses) was selected as a representative enteric virus for scoring. Human *Mastadenoviruses* have been used most frequently for risk assessment due their relative ease of detection in water using both culture-based and molecular methods.

Table 3.1 The list of faecal indicators and technologies included in the scoring exercise

Faecal indicators	
Bacteria	*E. coli*
	Enterococci
	Coliform bacteria
	Clostridium perfringens (and spores)
	Heterotrophic plate count (HPC) bacteria
	Pseudomonas
	Non-lactose fermenting (NLF) bacteria
	Campylobacter
Viruses	Somatic coliphage
	Enteric viruses (*Human Mastadenovirus* as a representative)
Protozoa	*Cryptosporidium*/*Giardia*
Other	Microbial source tracking markers (human and animal)
	Chemical sanitary indicators
Technologies	

Nucleic acid-based detection	PCR-based methods (e.g. quantitative PCR (qPCR))
	Next-generation sequencing
Sensors	In-situ enzymatic sensors (e.g. Colimínder)
	In-situ fluorescence sensors
Other	Flow cytometry
	matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS)

The scoring method was then established, as detailed in Table 3.2 . Seven criteria that ideal faecal indicators should meet were identified based on the WHO recommendations (WHO, 2017). For each criterion, a scoring scale from 1 to 5 was established, with a score of 5 reflecting the most suitable indicator for that criterion, and a score of 1 reflecting the least suitable indicator. Descriptions of each score were developed to maintain a consistent scoring process for each indicator Table C.1.

Weighted scores were utilised as shown in Table 3.2 . Most criteria were weighted equally, however, correlation with enteric pathogens was subdivided into three categories – viruses, bacteria, and protozoa – to enable a more detailed evaluation. Therefore, these three criteria were proportionally weighted less than the others. Once the weighted scores were established, the indicators were ranked from most useful to least useful.

Table 3.2 Details for the criteria established for faecal indicator scoring

Criterium title	Description	Weight	Justification
Sensitivity	How common is the indicator in the faecal matter of warm-blooded animals (birds and mammals)	0.20	A faecal indicator should be abundant in the intestines of humans and other warm-blooded animals and consistently present in their faeces.
Specificity	How specific is the indicator to faecal contamination in water	0.20	A faecal indicator should not be found in environments that are absent of faecal contamination from warm-blooded animals to avoid false positive detections.
Indicator for enteric bacteria	How well the indicator correlates with enteric bacteria in water	0.07	A faecal indicator's presence should indicate a high likelihood of faecal bacteria being present in water.
Indicator for enteric viruses	How well the indicator correlates with enteric viruses in water	0.07	A faecal indicator's presence should indicate a high likelihood of faecal viruses being present in water.
Indicator for enteric protozoa	How well the indicator correlates with enteric protozoa in water	0.07	A faecal indicator's presence should indicate a high likelihood of faecal protozoa being present in water.

Indicator for operation	How well the indicator correlates with the removal of enteric pathogens during water treatment	0.20	A faecal indicator should have a survival rate and response to water treatment (such as chlorine resistance) similar to most resistant enteric pathogens.
Detection	How easy and reliable the detection of the indicator is, i.e. are standard methods available	0.20	The indicator should be easy to detect and quantify using simple, reliable and cost-effective laboratory methods.

3.3.2 Scoring exercise for emerging technologies

Similarly to indicators, a list of technologies to consider in the scoring exercise was established (Table 3.1), based on the outcomes of the literature review and the stakeholders' feedback described in the previous sections. This exercise focused on emerging methods that are currently not regulated in the UK but have been used or trialled to assess faecal pollution in water.

The scoring criteria was developed based on stakeholders' responses to questions on the usefulness of different methods in water quality testing (

Table 3.3). Similarly to indicators, a scoring scale from 1 to 5 was established for each criterion, with a score of 5 reflecting the most suitable technology for that criterion, and a score of 1 reflecting the least suitable technology. Descriptions of each score were developed to maintain a consistent scoring process for each indicator Table C.2.

The scoring weighting was established prioritising data interpretation and reliability which are the most relevant factors in water quality assessment. The cost and time associated with each method were deemed less important and training needs and the level of standardisation were considered the least important criteria (

Table 3.3). Once the weighted scores established, the technologies were ranked from most useful to least useful.

Table 3.3 Details for the criteria established for technology scoring

Criterium title	Description	Weight	Justification
Cost-efficiency	How expensive is to set up and run tests (i.e. equipment and consumables costs)	0.15	The technology should be affordable to set up and maintain.
Training needs	How complicated are the tests to learn	0.10	The technology should be easy to learn with limited troubleshooting between runs.
Time-efficiency	How long does the analysis take	0.15	The test should be rapid enabling timely response to potential contamination events.
Data interpretation	How easy is to achieve actionable results	0.25	The technology should provide data that is easy to interpret and representative of water quality, and hence actionable.
Reliability	How accurate and sensitive the test is	0.25	The technology should provide highly accurate data with no false negative or false positive results even at low target concentrations.
Standardisation/ industry recognition	How well recognised is the technique in the water industry, and how standardised are the methods	0.10	The technology should be validated and suited for water industry applications.

3.4 Findings

3.4.1 Ranking of faecal indicators

High-ranking indicators

As shown in Table 3.4 and Table 3.5, *E. coli* and enterococci scored the highest in this exercise, suggesting that they are the most suitable indicators for the assessment of faecal contamination in water. They both are abundant in the faeces of warm-blooded animals and only few strains are associated with other hosts or grow in the environment. *E. coli* and enterococci correlate well with pathogenic bacteria in water; however, they are less indicative for pathogenic viruses and protozoa. Additionally, they are generally more sensitive to water treatment and disinfection than some pathogens, especially viruses and protozoa. *E. coli* and enterococci are included in UK and international regulations and standard methods for detection are widely available and affordable for water testing.

Table 3.4 Ranking of faecal indicators

Final Ranking		Weighted score
1	*E. coli*	4.27
2	Enterococci	4.27
2	Somatic coliphage	4.00
4	Microbial source tracking markers (human and animal)	4.00
5	*Clostridium perfringens* (and spores)	3.80
6	Coliform bacteria	3.60
7	*Cryptosporidium*/*Giardia*	3.20
8	*Campylobacter*	2.87
9	Enteric viruses (*Human Mastadenovirus* as a representative)	2.80
10	Non-lactose fermenter bacteria	2.27
11	Heterotrophic plate count (HPC) bacteria	2.20
12	*Pseudomonas*	2.00
13	Chemical sanitary indicators	1.40

Table 3.5 Detailed scoring (non-weighted) for each faecal indicator against the criteria

Indicator	Sensitivity	Specificity	Indicator for enteric bacteria	Indicator for enteric viruses	Indicator for enteric protozoa	Indicator for operation	Detection
E. coli	5	5	5	3	2	3	5
Enterococci	4	5	5	3	2	4	5
Somatic coliphage	4	4	3	4	2	5	4
MST markers	4	5	5	5	5	3	3
***Clostridium perfringens* (and spores)**	3	4	2	1	3	5	5
Coliform bacteria	5	3	3	2	1	3	5
Cryptosporidium/Giardia	2	2	3	1	5	4	5
Campylobacter	2	2	4	1	2	3	5
Enteric viruses (Human *Mastadenovirus*)	2	3	3	5	1	4	2
Non-lactose fermenter bacteria	2	1	2	1	1	2	5
HPC bacteria	1	1	1	1	1	3	5
Pseudomonas	1	1	1	1	1	2	5
Chemical sanitary indicators	2	2	1	1	1	1	

Somatic coliphages and microbial source tracking (MST) markers both ranked highly. Somatic coliphages resemble the sensitivity and specificity of their host bacteria, however, they are slightly less abundant in faeces than *E. coli*. They correlate with enteric viruses more effectively than *E. coli* and other bacterial indicators; however, the exact correlations are hard to establish due to differences in detection methods (phage culturing vs. molecular detection). The correlation of somatic coliphages with pathogenic bacteria and protozoa is less known as they are primarily considered indicators to establish viral risks. While there is an ISO standard for the detection of somatic coliphages in water, only one laboratory in the UK is currently accredited for that test and hence it is less accessible than *E. coli* or enterococcus assays.

In contrast, MST markers (representing a wide group of indicators associated with the genomes of microorganisms found in the gut of certain animal groups and species and humans) can be

highly specific to faecal matter of warm-blooded animals, however, depending on the marker they may be less abundant than *E. coli*. As tailored markers can be used to indicate the presence of viral, bacteria and protozoan pathogens, they correlate well with each pathogen group. However, MST markers are detected using molecular assays targeting gene segments, which can be found in water after treatment when the pathogens have been disintegrated, thus posing no public health risks. Therefore, MST markers often overestimate the resistance of pathogens to water treatment, especially for disinfection, and consequently overestimate health risks. Various standard methods have been established for PCR-based detection of targets in water, either to provide general guidance (e.g. the ISO/TS 16099:2025 Water quality — Polymerase chain reaction [PCR] for the detection and quantification of microorganisms and viruses — General requirements, quality assurance and validation method) or specific protocols for the detection of certain genes (e.g. USEPA Method 1696: Characterization of Human Fecal Pollution in Water by HF183/BacR287 TaqMan® Quantitative Polymerase Chain Reaction [qPCR] Assay). Currently no single standard is available for the detection of common animal and human-associated faecal MST markers in water.

Clostridium perfringens and coliform bacteria also ranked relatively high. *C. perfringens* scored highly due to its persistence in the environment (often indicating former contamination events) and its resistance to water treatment procedures. It ranked lower on its abundance in the faeces of warm-blooded animals due to its intermittent presence and low concentrations and for its poor correlation with pathogens in water. Coliform bacteria are highly abundant in faeces, however, often associate with the environment as well, resulting in poor correlation with pathogens. Similar to *E. coli*, coliforms are also readily removed during water treatment. As regulatory indicators, standard methods for the detection of *C. perfringens* and coliform bacteria are widely available in the UK and internationally. Standard methods allow the detection of faecal/thermotolerant coliform bacteria that are more representative to faecal contamination, however, those techniques are not required for regulatory monitoring in the UK.

Mid-ranking indicators

Cryptosporidium/*Giardia* and *Campylobacter* ranked close to the average of scores. These are pathogens of animal origin. *Cryptosporidium*/*Giardia* infect both humans and animals including cattle, sheep, dogs, cats, rodents and birds, resulting in gastrointestinal illness. While some animals are common reservoirs for *Campylobacter*, it often causes gastrointestinal illness in both humans and animal species. These microorganisms only sporadically detected in the faeces of animals and humans during infection. Being pathogens themselves, they are self-indicative, and the presence of *Campylobacter* and *Cryptosporidium*/*Giardia* can also indicate the presence of other enteric bacteria and protozoa, respectively. However, they do not correlate well with other pathogen groups, especially with enteric viruses. These pathogens are also self-indicative during water treatment, with *Cryptosporidium*/*Giardia* being more resistant to disinfection than *Campylobacter*. Standard methods for the detection of both indicator groups are available in the UK and internationally.

Human enteric viruses, such as *Mastadenoviruses* (formally Human Adenovirus), are abundant in the faeces of humans during infection, which is common and often asymptomatic. These viruses are not present in the faeces of other warm-blooded animals and are therefore only representative of human faecal pollution in the aquatic environment. They are self-indicative and the presence of one enteric virus often correlates with the presence of other pathogenic viruses in water, however, they are more persistent than most bacteria and hence can overestimate bacterial health risks. Correlations between enteric viruses and protozoan pathogens in not well studied. *Mastadenoviruses* are relatively resistant to water treatment so can represent similarly resistant species. A USEPA Method (1615) has been developed for the enumeration of enteroviruses and noroviruses in water which may be adapted for other viruses, however, more validation would be necessary for adaptation.

Low-ranking indicators

Non-lactose fermenters (NLFs), heterotrophic plate count (HPC) bacteria and *Pseudomonas* all ranked low in this exercise compared to the indicators detailed above. These are generally environmental bacteria which do not associate specifically with faeces. While some non-lactose fermenter bacteria, for example *Salmonella,* are human pathogens, the current standard techniques do not allow such identification. These indicator groups do not correlate with enteric pathogens well in water and their removal does not represent the removal of pathogens during water treatment. However, HPC bacteria can indicate regrowth and the presence of general biofilm bacteria, and hence they are good indicator for treatment performance in general. For all three indicator groups, standard methods are available for detection and enumeration in water.

Out of all indicators included, chemical indicators scored the lowest. As reviewed in Section 2, chemical indicators represent a large number of chemical compounds that commonly associate with domestic wastewater, including diet-related compounds and drugs (e.g. caffeine, codeine, ibuprofen, naproxen, lisinopril, cimetidine) and household chemicals (e.g. laundry detergents). Some of these are found in mainly human faeces, however their abundance is sporadic. Some chemical markers have been shown to be useful for tracking wastewater-derived contamination in the aquatic environment; however, they do not directly associate with faeces and with enteric pathogens in the environment or during water treatment. Currently, no standard method is available for their detection in water and the methods used in research require specialised equipment and are not readily available for the water industry.

3.4.2 Ranking of emerging technologies

High-ranking technologies

In agreement with the outcomes of the stakeholders' engagements, MALDI-TOF MS scored the highest amongst the emerging technologies included in scoring (Table 3.6 and Table 3.7). Data generated are easy to interpret and they enable species, sometimes subspecies-level identification (e.g. for coliform bacteria), hence data is actionable. The data are highly reliable; however, outcomes depend on the quality and comprehensiveness of the refence library used,

and hence differentiation between closely related species is sometimes challenging. As the method is reliant on cultured samples, sample preparation often takes days. While the equipment itself requires a significant investment, the day to day running of the equipment is affordable. Furthermore, there is a standardised, Standing Committee of Analysts (SCA) method available in the UK for testing water-derived samples using MALDI-TOF MS. Some training is required for using the equipment, however, additional training for data generation/interpretation may be necessary. Custom library creation would require further expertise, however, that is not highly relevant in water quality assessment.

Table 3.6 Ranking of emerging technologies

Final Ranking		Weighted score
1	MALDI-TOF MS	3.95
2	PCR-based methods (e.g. qPCR)	3.75
2	Flow cytometry	3.70
4	In-situ enzymatic sensors (e.g. Coliminder)	2.75
5	In-situ fluorescence sensors	2.75
6	Next-generation sequencing	1.75

Table 3.7 Detailed scoring (non-weighted) for each technology against the criteria

Technology	Cost-efficiency	Training needs	Time-efficiency	Data interpretation	Reliability	Standardisation/ industry recognition
MALDI-TOF MS	3	3	3	5	4	5
PCR-based methods	3	3	4	3	5	4
Flow cytometry	4	2	5	3	4	4
In-situ enzymatic sensors	2	5	5	2	2	2
In-situ fluorescence sensors	2	5	5	2	2	2
Next-generation sequencing	1	1	1	2	3	1

PCR-based methods and flow cytometry also scored highly amongst the technologies included in the exercise. PCR-based approaches scored highly on reliability as with the inclusion of appropriate controls false detections are unlikely. The assays are often more sensitive than culturing-based approaches. The data derived from the PCR runs are easy to interpret, however, DNA/RNA detection does not indicate the presence of infectious cells or particles and hence the outcomes can overestimate the health risks. With recent advancements in viability qPCR testing, which includes a short preparation step for the expulsion of free DNA/RNA prior to PCR, health risks can be better estimated (Farkas et al., 2020). PCR-based methods are usually rapid, with only a few hours required for sample preparation. Results are usually available within 24-48 hours. Similar to MALDI-TOF MS, the initial investment to equipment can be substantial, especially if for digital PCR platforms. Quantitative PCR equipment is more affordable, and simplified, field-ready qPCR machines are now also available for a reduced price, though these devices require more maintenance and do not have all functions (e.g. multiplexing). The running costs of PCR-based approaches are slightly higher than for MALDI-TOF MS, but still affordable. Some training on the use of the equipment is required and understanding of molecular diagnostics and DNA extraction/purification is also needed. There are several international standards on PCR-based detection in water. As noted in the previous sections, the ISO/TS 16099:2025 standard sets the general requirements for the detection of targets using qPCR in water. Other standards describe the molecular detection of certain pathogens, e.g. norovirus (USEPA 1615), Legionella (ISO/TS 12869:2019) and human MST markers HF183 and BacR287 (USEPA 1696).

Flow cytometry is a valuable tool for assessing microbial water quality, particularly for treatment efficiency monitoring, as long as the equipment is used properly and the data is interpreted correctly. That can be challenging without having clear threshold values to distinguish noise from signal. A big advantage of FCM is that same-day results are provided, and some equipment can also be used for real-time monitoring. In general, the price of equipment is similar to qPCR, however, real-time measuring devices can be more expensive. The running costs of FCM are usually affordable. Some training for equipment use is required, and expertise is required for gating populations for accurate data interpretation. While no standardised methods are available in the UK, Switzerland has established a national standard to utilise FCM in water quality monitoring.

Low-ranking technologies

Enzymatic (e.g. Colminder) and fluorescent (e.g. tryptophan) sensors scored low compared to the technologies detailed above. The major advantages of sensors are that they enable real-time monitoring and very minimal or training is needed to use the equipment. However, sensors often provide data in units that are unfit for regulatory interpretation, and there is a need for additional validation using different methods. While no standardised methods are available, validated methods are already in use in the water industry. The costs associated with sensors are platform dependent. The price of fluorescent sensors (including the sensor itself, housing, additional hardware and software) vary from £5000 to £100,000. Sensors often come with

added costs (e.g. software subscriptions) which also show high variations. The sensors themselves often need to be replaced regularly which also increases the running costs.

Next-generation sequencing scored the lowest due to the high costs associated with the equipment, maintenance and consumables, the expertise and time needed for sample preparation, sequencing and data analysis, the lack of standardised protocols, and the difficulties with data interpretation. Data derived from sequencing is complex and often ambiguous. Misidentification can also occur, especially when targeted sequencing is utilised (i.e. new/unknown strains can be misidentified due to the lack of reference genomes). Detection limits vary based on approach and targets. Due to these limitations, next-generation sequencing outcomes may be suitable to support existing data, but they are not actionable on their own.

4. Relation to UK legislation and future directions

4.1 Summary

This section explores how faecal indicators and emerging technologies relate to existing UK legislation and outlines potential future directions for regulatory and operational practice. The outcomes of this section combine findings from the literature review, stakeholder engagement, and multi-criteria analysis tasks to provide an actionable and realistic summary of potential future directions for regulatory and operational practice in England and Wales.

Key findings:

- Existing regulatory faecal indicator organisms – *E. coli*, enterococci, and total coliforms – should continue to hold regulatory importance, though flexibility regarding the regulation of total coliforms could be explored. Further work is required to determine how emerging rapid detection methods for these indicators, including sensors, MALDI-TOF MS, and qPCR, can be aligned with current regulatory units and associated human health risks.
- Heterotrophic plate counts (HPC) remain a useful measure of treatment efficiency, but further regulatory guidance on establishing operational baselines and stage-specific thresholds would be valuable. Flow cytometry offers a rapid alternative for assessing microbial loading, though clearer interpretation frameworks and alignment with existing regulatory parameters are still required. Adoption of a proxy viral indicator such as somatic coliphage would be beneficial, but broader accreditation and standardised methods are needed across the industry.
- Faecal pollution source tracking (MST) offers potential to distinguish human versus animal contamination sources, supporting catchment management and outbreak investigations. However, challenges remain around standardisation, interpretation, and integration into compliance frameworks.
- Future research directions include:
    - *Assessment of MST marker prevalence*: Evaluate the geographical distribution and abundance of microbial MST markers across the UK.
    - *Trials on somatic coliphages and MST markers*: Extend knowledge on the usefulness of novel indicators in drinking water monitoring, risk assessment, and mitigation.
    - *Flow cytometry trials*: Collect and collate evidence on the routine use of flow cytometry in drinking water quality surveillance.
    - *HPC bacteria abundance data analysis*: Establish rules for the HPC ‘abnormal change’ definition.

- *Follow-up on industry trials*: Understand the ongoing research in microbial drinking water quality monitoring at UK water companies**.**
- *Establish a Drinking Water Research Framework*: Foster collaboration, reduce duplication of efforts, and optimise resource use for evaluating new techniques and approaches.

4.2 *Escherichia coli*, total coliforms and enterococci

Regulatory limits

In accordance with international guidelines and continuing relevance to sanitary significance within the water industry, enterococci and total coliforms including *E. coli* are faecal indicators that should remain to be of regulatory importance. However, there is contention regarding the relevance of total coliforms in terms of environmental prevalence and risk to human health.

International regulations such as US Revised Total Coliform Rule (2013), suggest a more flexible approach to coliform regulation as it is acknowledged that most coliforms are not harmful to humans. After any total coliform positive (Level 1), the system must collect a defined set of repeat samples from around the site to confirm whether detection persists. The assessment is immediately escalated (Level 2) if any total coliform positive is also detected in the presence of thermo-tolerant coliforms or *E. coli*. This given approach demonstrates that a more pragmatic regulatory framework in the context of risk to human health could be supported.

Methodology

While standardised, culture-based methods are widely available for these indicators, they generally have a turnaround time of >1 working-day, limiting the potential for rapid risk assessment. Now, sensor technologies are available that enable rapid detection of these indicators, however the interpretation of the fluorescence/enzymatic units can be difficult. Further investigation is therefore needed to understand how the units can be implemented as, or complement, existing regulatory guideline values.

Furthermore, methods enabling the relatively rapid species/sub-species level identification of coliforms including *E. coli* (e.g. matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry [MALDI-TOF MS], quantitative polymerase chain reaction [qPCR]) could better inform a targeted and multi-faceted risk assessment procedure for water companies. Effective guidance would require improved understanding of human health risks associated with individual coliform species.

4.3 Assessment of treatment efficacy

Overall treatment efficiency

Heterotrophic plate count (HPC) bacteria do not specifically represent the removal of faecal pathogens through drinking water treatment, however, they have been shown to indicate more general microbiological failures in treatment. Other sanitary indicators such as total coliforms and non-lactose fermenters may also be used to support assessments of treatment efficacy. HPC bacteria are only measured using culture-based methods, and as such their units of detection (CFU) are used as a regulatory compliance parameter, although no acceptable occurrence limit/range within treatment works and distribution systems is given.

Industry stakeholders expressed a lack of clarity regarding how the regulatory term 'no abnormal change' for HPC bacteria should be defined, and a lack of confidence in this indicator was noted, due in part to uncertainty around the appropriate application and interpretation of HPC data. Given the context-specific nature of HPC measurements, fixed regulatory thresholds are not recommended; rather, additional regulatory guidance on best practice for establishing operational baselines and stage-specific thresholds for HPC counts within treatment and distribution would be beneficial.

Flow cytometry has also been shown to be a rapid and reliable method of assessing water quality during treatment and within distribution. Any abnormal increase in particle numbers indicates a deterioration in water quality. Development of an industry standard method is in progress (Standing Committee of Analysts), however a greater understanding of flow cytometry outputs and their relationship with site-specific context and existing regulatory parameters, by both the regulator and industry as a whole, is needed prior to incorporation into regulation.

Virus and protozoa removal

Faecal bacterial indicators may fail to mimic the behaviour of more resistant pathogens, such as enteric viruses and protozoa. Somatic coliphages may also be used to proxy enteric virus removal, however, wider accreditation and adoption of standardised detection techniques in the water industry is needed. In addition, more guidance on remedial actions for example, disinfection strategy, following coliphage detection in treated water is required for effective and meaningful implementation as a regulatory parameter.

4.4 Faecal pollution source tracking

Microbial source tracking (MST) has been shown to be an effective approach to determine the source and quantify the magnitude of faecal contamination. MST monitoring is an emerging faecal source diagnostics approach used by select authorities and water companies in the UK and internationally. As multiple MST markers are available, the most common faecal sources (i.e. human, agriculture, wildlife) can be determined accurately and rapidly using PCR based techniques. For a deeper, less targeted assessment, 16S RNA gene sequencing can also be utilised.

However, further research is required to establish the sensitivity and specificity of MST markers to host faecal sources in the UK. Faecal taxon libraries would need to be compiled in the UK to benefit the accuracy of deeper library-dependent gene sequencing analysis. Standardised procedure of MST methodology (including data interpretation) is also required to enable acceptance within the industry and regulation. It should be noted that MST markers are not necessarily an indication of pathogen presence or viability and therefore are not typically used in the context of sanitary indicators in water quality assessments.

Chemical markers and bacterial typing using MALDI-TOF MS have also been trialled for faecal pollution source tracking, however, to a lesser extent than MST markers, as reviewed in Section

2. When evidence has been collated on utilising these approaches, guidance on their use for source tracking should be established prior to regulatory use.

4.5 Future research directions

This section outlines priority areas for future investigation, focusing on the validation and optimisation of novel indicators, the development of robust analytical methods, and the generation of evidence to inform regulatory and operational decision-making. By targeting these research directions, the water sector can support the safe integration of emerging technologies and multi-indicator approaches, ultimately strengthening public health protection and regulatory compliance.

1. Assessment of MST marker prevalence	
Aim:	Evaluation of the geographical distribution and abundance of microbial MST markers across the UK.
Scope:	○ Conduct a spatio-temporal study evaluating the abundance of the most promising human and animal MST markers among the populations and in drinking water sources across the UK. ○ Collect both faecal and water samples regularly at representative sites and utilise both targeted (PCR-based) and untargeted (sequencing-based) approaches to explore the prevalence of MST markers in relation to pathogen and conservative indicator (e.g. *E. coli*) presence.
Deliverables:	Evidence on the prevalence and distribution of MST markers across the UK with recommendations on their utilisation in UK drinking water regulations.

2. Trials on somatic coliphages and MST markers	
Aim:	Extend knowledge on the usefulness of novel indicators in drinking water monitoring, risk assessment, and mitigation.
Scope:	○ Conduct a series of trials involving water sampling at different stages of drinking water treatment.

	○ Test for somatic coliphages and MST markers and evaluate their removal in relation to conservative water quality indicators and pathogens.
Deliverables:	Comprehensive report on the applicability and utilisation of somatic coliphages and MST markers for water treatment efficiency testing.

3. Flow cytometry trials	
Aim:	Collect and collate evidence on the routine use of flow cytometry in drinking water quality surveillance.
Scope:	○ Conduct further trials on the use of flow cytometry in drinking water quality testing, focus on implementation into routine monitoring and regulation.
Deliverables:	Evidence of actionable data production and detailed guidance on day-to-day use, including SOPs, threshold settings, and reference libraries.

4. HPC bacteria abundance data analysis	
Aim:	Establish rules for HPC ‘abnormal change’ definition.
Scope:	○ Collect and analyse existing data on HPC counts at different water treatment sites/treatment stages and utilise statistical approaches to establish levels for normal distribution and abnormal changes under different conditions (i.e. water chemistry, treatment methods, etc.).
Deliverables:	Guidance document on the interpretation of HOC ‘abnormal changes’ for monitoring and regulatory purposes.

5. HPC bacteria abundance data analysis

Aim:	Establish rules for HPC ‘abnormal change’ definition.
Scope:	○ Collect and analyse existing data on HPC counts at different water treatment sites/treatment stages and utilise statistical approaches to establish levels for normal distribution and abnormal changes under different conditions (i.e. water chemistry, treatment methods, etc.).
Deliverables:	Guidance document on the interpretation of HOC ‘abnormal changes’ for monitoring and regulatory purposes.

6. Follow-up on industry trials	
Aim:	Understand the ongoing research in microbial drinking water quality monitoring at UK water companies**.**
Scope:	○ Engage with UK water companies to evaluate outcomes of ongoing trials assessing new indicators and equipment (e.g., sensors, MALDI-TOF MS, chemical indicators). ○ Assess applicability of these approaches in drinking water quality monitoring.
Deliverables:	Report on the utilisation potential of emerging microbial indicators and technologies in routine drinking water quality monitoring.

7. Establish a Drinking Water Research Framework	
Aim:	Foster collaboration, reduce duplication of efforts, and optimise resource use for evaluating new techniques and approaches.
Scope:	○ Collect data on innovations trialled in the UK water industry. ○ Include logging of ongoing and completed case studies and trials to share outcomes with Defra/DWI and the industry.

Deliverables:	Framework for tracking drinking water quality monitoring research efforts in the UK.

5. Conclusions

This review has comprehensively evaluated both established and emerging microbial indicators and analytical technologies for detecting faecal contamination in drinking water. The evidence demonstrates that traditional indicators, particularly *Escherichia coli* and enterococci, remain the most reliable and actionable tools for routine monitoring, supported by robust standard methods and clear regulatory frameworks. Their continued use is essential for safeguarding public health, given their sensitivity, specificity, and widespread acceptance in national and international regulations.

However, this review confirmed that no single indicator or method provides a complete measure of microbial water quality and faecal contamination. The limitations of traditional indicators, especially in detecting non-bacterial pathogens such as viruses and protozoa, highlight the need for a multi-barrier, risk-based approach that integrates multiple lines of evidence. Emerging indicators, such as somatic coliphages and microbial source tracking (MST) markers, offer valuable supplementary information, particularly for viral risk assessment and source attribution. Their broader adoption is currently constrained by the lack of evidence on their resistance the water treatment, limited laboratory capacity and the lack of standardised protocols.

Technological innovations, such as matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS), polymerase chain reaction (PCR)-based methods and flow cytometry, are the most promising emerging tools for delivering rapid, high-resolution and actionable results. These methods are increasingly being trialled or adopted for operational and investigative purposes, though challenges remain around standardisation, training, and initial investment. Sensor-based and next-generation sequencing approaches also offer potential for real-time and high-resolution monitoring, respectively, but face barriers related to data complexity hindering actions.

Stakeholder insights reveal a growing momentum both in the UK and internationally toward flexible, risk-based regulatory frameworks that can accommodate new technologies and multi-indicator strategies. There is a strong demand for evidence-based guidance to support the integration of advanced methods into routine practice, ensuring that innovations translate into practical improvements in water safety.

In summary, the optimal approach to monitoring faecal contamination in drinking water is a multi-indicator, multi-technology strategy. Traditional indicators should remain the foundation of compliance monitoring, while the gradual and evidence-based adoption of advanced methods can enhance responsiveness, specificity, and overall risk management. Continued research, standardisation, and collaboration across the water industry will be essential to realise the full potential of emerging indicators and technologies, ultimately ensuring the highest standards of public health protection.

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Appendix A International regulatory requirements

Table A.1 Microbiological compliance and operational monitoring parameters in national and international regulations

Regulations	Country/region	Microbiological compliance parameters[1]	Parametric value (CFU/100 mL)	Compliance point	Operational monitoring parameters[2]	Parametric value (CFU/100 mL)	Compliance point	Notes on operational parameters
Water Supply (Water Quality) Regulations 2016	England	*E. coli*	0	CT, SR, WTW	*C. perfringens* (including spores)	0	SP	*C. perfringens* monitoring required only for supplies fed by surface waters[3] or if risk assessment (RA) indicates that it is appropriate
		Enterococci	0	CT	Coliform bacteria	0	CT	
		Coliform bacteria	0	SR, WTW	HPC (colony counts at 22°C)	No abnormal change	CT, SR, WTW	
Water Supply (Water Quality) Regulations (Wales) 2018	Wales	*E. coli*	0	CT, SR, WTW	*C. perfringens* (including spores)	0	SP	
		Enterococci	0	CT	Coliform bacteria	0	CT	
		Coliform bacteria	0	SR, WTW	HPC (colony counts at 22°C)	No abnormal change	CT, SR, WTW	
The Public Water Supplies (Scotland) Regulations 2014	Scotland	*E. coli*	0	CT	*C. perfringens* (including spores)	0	SP	*C. perfringens* monitoring required only if RA indicates that it is appropriate
		Enterococci	0	CT	*E. coli*	0	SR, WTW	

					Coliform bacteria	0	SR, WTW, CT	
					HPC (colony counts at 22°C)	No abnormal change	CT, SR, WTW	
					Somatic coliphage	50 (PFU/100 mL)	RW	Somatic coliphage monitoring is only required if RA indicates that it is appropriate. In the event of an exceedance, risk of viral breakthrough throughout treatment train should be evaluated.
The Water Supply (Water Quality) Regulations (Northern Ireland) 2017	Northern Ireland	*E. coli*	0	CT, SR, WTW	*C. perfringens* (including spores)	0	SP	
		Enterococci	0	CT	Coliform bacteria	0	CT	
		Coliform bacteria	0	SR, WTW	HPC (colony counts at 22°C and 37°C)	No abnormal change	CT, SR, WTW	
EU Drinking Water Directive 2020/2184	European Union	*E. coli*	0	CT	*C. perfringens* (including spores)	0	n.g.	*C. perfringens* monitoring required only if RA indicates that it is appropriate
		Enterococci	0	CT	Coliform bacteria	0	n.g.	
					HPC (colony counts at 22°C)	No abnormal change	n.g.	
					Somatic coliphage	50	RW	Somatic coliphage monitoring is only required if RA

						(PFU/100 mL)		indicates that it is appropriate. In the event of an exceedance, risk of viral breakthrough throughout treatment train should be evaluated.
Drinking water Regulations S.I. No. 99/2023	Ireland	*E. coli*	0	CT	*C. perfringens* (including spores)	0	n.g.	*C. perfringens* monitoring required only if RA indicates that it is appropriate
		Enterococci	0	CT	HPC (colony counts at 22°C)	No abnormal change	n.g.	
					Coliform bacteria	0	n.g.	
					Somatic coliphage	50 (PFU/100 mL)	RW	Somatic coliphage monitoring is only required if RA indicates that it is appropriate. In the event of an exceedance, risk of viral breakthrough throughout treatment train should be evaluated.
Dutch Drinking Water Decree	The Netherlands	*E. coli*	0	CT	*Cryptosporidium*	n.g.	RW	RW monitoring and log removal data informs QMRA process for surface water and vulnerable groundwater sites,
		Enterococci	0	CT	Enteroviruses	n.g.	RW	
					Giardia	n.g.	RW	
					Campylobacter	n.g.	RW	

					Coliphage (Somatic and F-specific)	n.g.	RW	where risk of infection must be <1 infection/10,000 persons/year
					Aeromonas (30°C)	1	n.g	
					Coliform bacteria	0	n.g	
					C. perfringens (including spores)	0	n.g	
					HPC (colony counts at 22°C)	100 (CFU/mL)	n.g	
Regulations on water supply and water intended for human consumption (Drinking Water Regulations)	Norway	*E. coli*	0	CT	*E. coli*	n.g.	RW	
		Enterococci	0	CT	Enterococci	n.g.	RW	RW monitoring only required for >10 m^3/day supplies
					Coliform bacteria	0, n.g.	RW	RW monitoring only required for >10 m^3/day supplies
					C. perfringens (including spores)	0	n.g.	*C. perfringens* monitoring required only for supplies fed by surface waters*
					Cryptosporidium/protozoa	Presence/ absence	n.g.	Only required following *C. perfringens* detection
					HPC (colony counts at 22°C)	100 (CFU/mL) and no abnormal change	n.g.	

201/2001. (X. 25.) edict	Hungary	*E. coli*	0	CT/SP/Water tank	*E. coli*	0	RW/WTW	In water tanks, the limit is 0 CFU/250 mL.
		Enterococci	0	CT/SP/Water tank	Enterococci	0	RW/WTW	In water tanks, the limit is 0 CFU/250 mL.
		Pseudomonas aeruginosa	0 (CFU/250mL)	Water tank	*Pseudomonas aeruginosa*	0	WTW	Monitored at WTW after filter backwash
		Colony count at 22°C	100 (CFU/mL)	Water tank	Colony count at 22°C	100 (CFU/mL)	RW/WTW	Monitored at WTW after filter backwash
		Colony count at 37°C	20 (CFU/mL)	Water tank	Colony count at 37°C	20 (CFU/mL)	RW/WTW	Monitored at WTW after filter backwash
		Contamination indicator bacteria identified via light microscopy	0/L	CT/SP/Water tank				Refers to *Spirillum, Spirochaeta, Sarcina, Zoogloea, Beggiatoa.*
					C. perfringens	0	RW/WTW	*C. perfringens* monitoring required only for supplies fed by surface waters.
Water Services (Drinking Water Standards for New Zealand) Regulations 2022	New Zealand	*E. coli*	<1	CT	*E. coli*[4]	ng[4]	RW, WTW, DZ[4]	
		Total pathogenic protozoa	<1 (infectious (oo)cyst/100 L)	CT	Total coliforms[4]	ng[4]	RW, WTW, DZ[4]	

Guidelines for Canadian Drinking Water Quality	Canada	*E. coli*	0	CT, WTW, GW, SR, DZ				
		Total coliforms	0	WTW, GW, SR, DZ				
		Enteric viruses	≥4 LRV	RW/WTW				
		Enteric protozoa	≥3 LRV	RW/WTW				
National Primary Drinking Water Regulations	USA	*E. coli*	0	CT, DZ, GW[6]				
		Total coliforms	≤5% positive/month	CT, DZ				
		Cryptosporidium	0[5] or 99% removal	CT/WTW				
		Giardia lamblia	0[5] or 99% removal	CT/WTW				
		Enteric viruses	99% removal	CT/WTW				
		HPC	500 (CFU/mL)	CT				
		Enterococci	0	GW[6]				
		Coliphage (Somatic and F-specific)	0	GW[6]				
Guideline values (non-mandatory)								
	Australia	*E. coli*	0	CT	Thermotolerant coliforms	0	CT	

Australian Drinking Water Guidelines					HPC	No abnormal change	WTW/DZ/CT	
					Coliphage (Somatic and F-specific)	0 (PFU/100 mL)	CT	
					Enterococci	0	CT	
					E. coli	n/a	RW	For QMRA purposes (source water vulnerability assessment)
WHO Guidelines for Drinking Water Quality	n/a	*E. coli* OR thermotolerant coliforms	0	CT, WTW, DZ				

CT=Customer Tap, SR=Service Reservoir, WTW=Water Treatment Works, SP=Supply Point, RW=Raw Water, DZ=Distribution Zone, GW=Ground Water (non-disinfected), ng=not given, LRV=Log Removal Value, HPC=Heterotrophic Plate Count

1. Defined as a microbiological water quality parameter for which compliance is required under Regulation 4 (Wholesome water) of the Water Supply (Water Quality) Regulations 2016, or national/international equivalent

2. Defined as a microbiological water quality parameter for which compliance is required or recommended outside of Regulation 4 (e.g. for supply zone monitoring), or national/international equivalent.

3. Includes groundwaters influenced by surface waters

4. As per Drinking Water Quality Assurance Rules 2022 (Taumata Arowai, New Zealand)

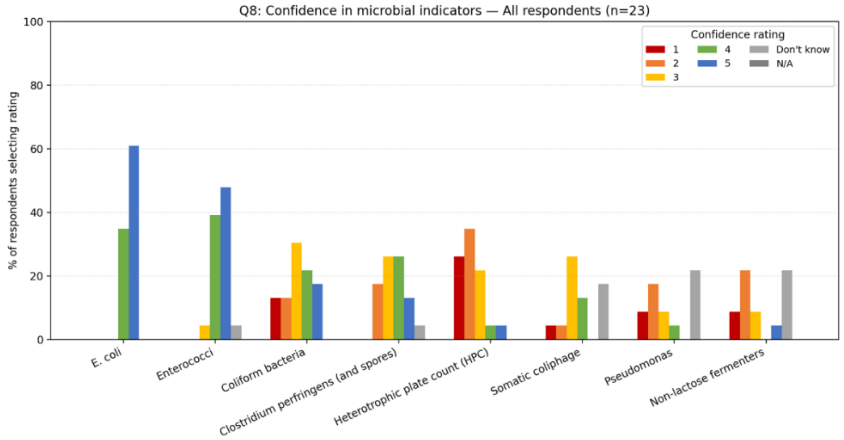
5. Non-enforceable maximum contaminant goal

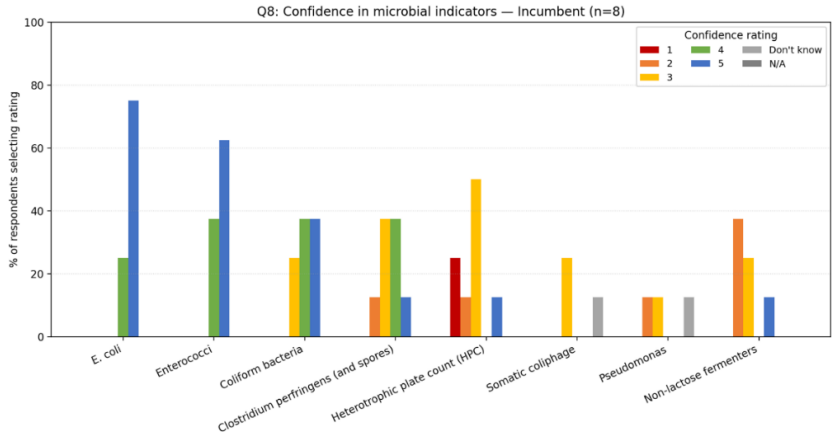
6. As per Groundwater Rule (2008), monitoring of *E. coli* OR enterococci OR coliphage is required for non-surface water influenced groundwater sources

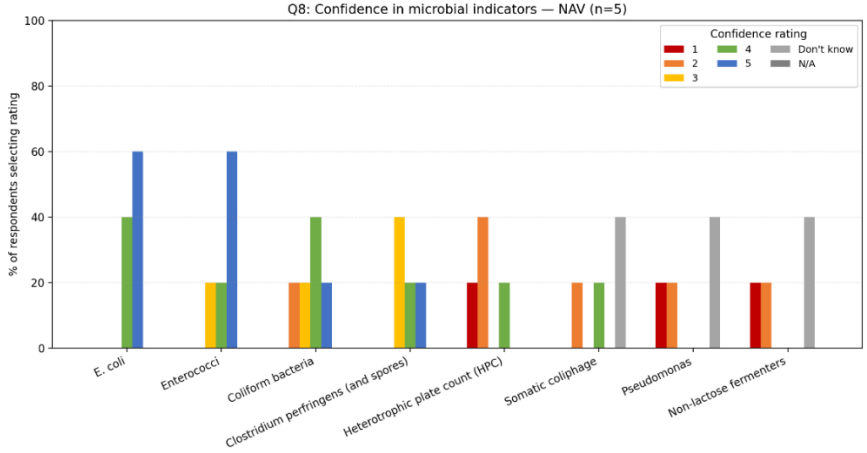
Appendix B Online survey outcomes

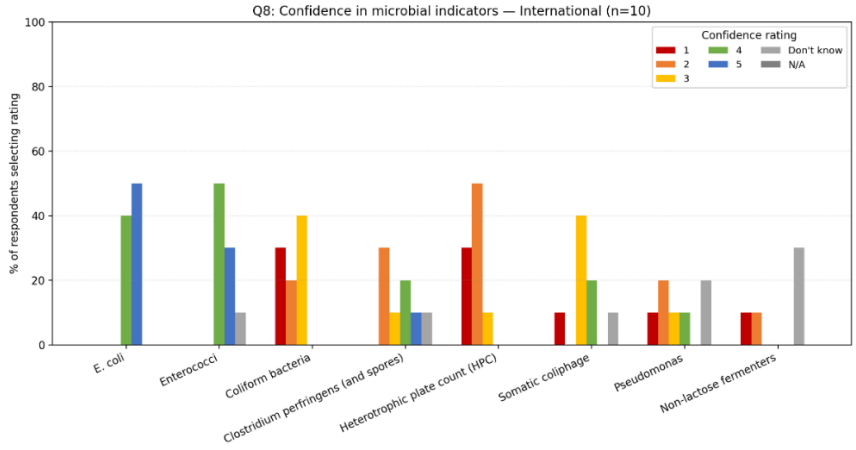
Table B.1 Additional technical survey questions and responses

Microbial indicators
Q7 How confident are you in the sensitivity and reliability of these indicators for detecting microbial and/or faecal contamination in operational settings? (1 = Not confident at all, 5 = Very confident)







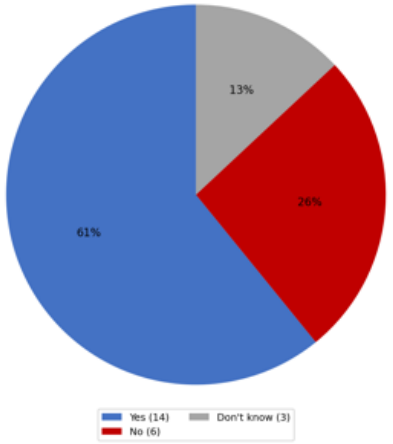


Q8 If you selected 'Other' for any microbial indicators or purposes, please provide further details. */* **Q9 If you selected 'Other' in Q6/7, please indicate your *confidence (1-5)* in the additional indicators you have listed.**	
Respondent	Indicator (confidence score)
UK Water Company	• Customer complaints • *Cryptosporidium* (5)
NAV	No response
International	• Enteric pathogens such as viruses, *Cryptosporidium*, *Giardia* (5) • *Campylobacter* • *Aeromonas* • *Legionella* • Aerobic spore-forming bacteria for treatment process monitoring • Human and animal-associated MST markers (5) • *Bacteroides* (4)

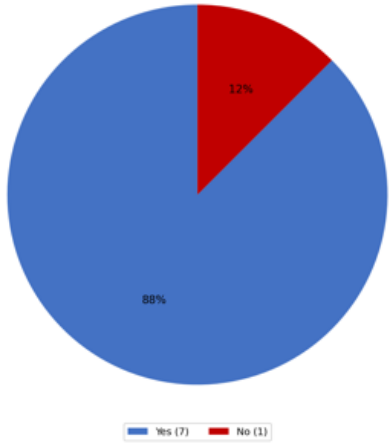
Analytical techniques

Q10 Do you use any alternative methodologies or techniques to assess microbiological water quality outside of those listed in Schedule 5 of the Regulations?

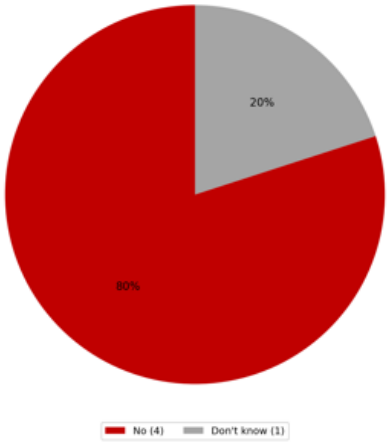
Q10: Use of alternative methodologies outside Schedule 5 — All respondents (n=23)



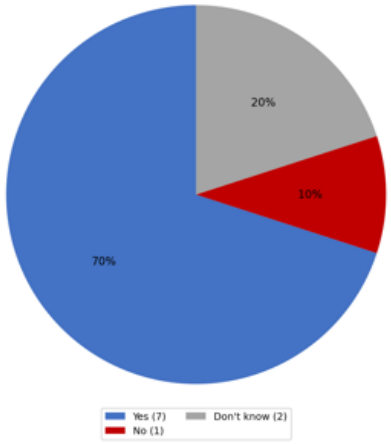
Q10: Use of alternative methodologies outside Schedule 5 — Incumbent (n=8)



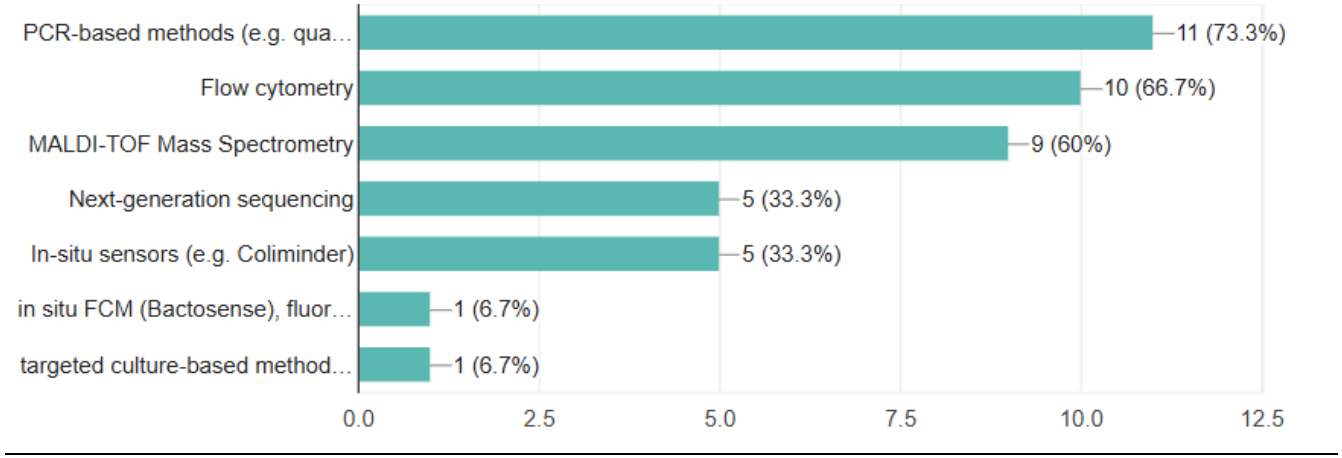
Q10: Use of alternative methodologies outside Schedule 5 — NAV (n=5)



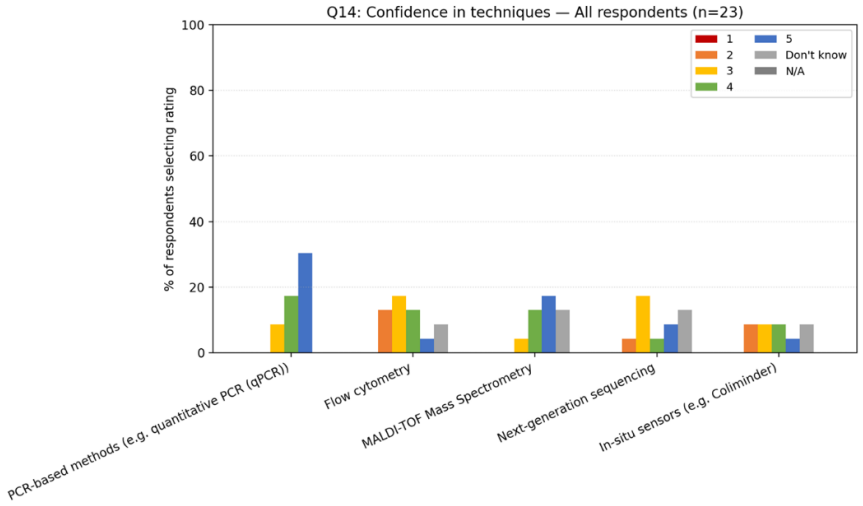
Q10: Use of alternative methodologies outside Schedule 5 — International (n=10)

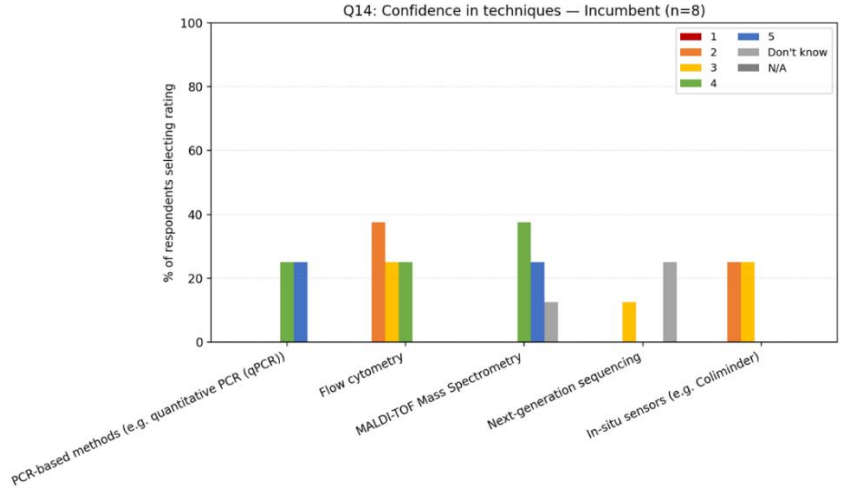


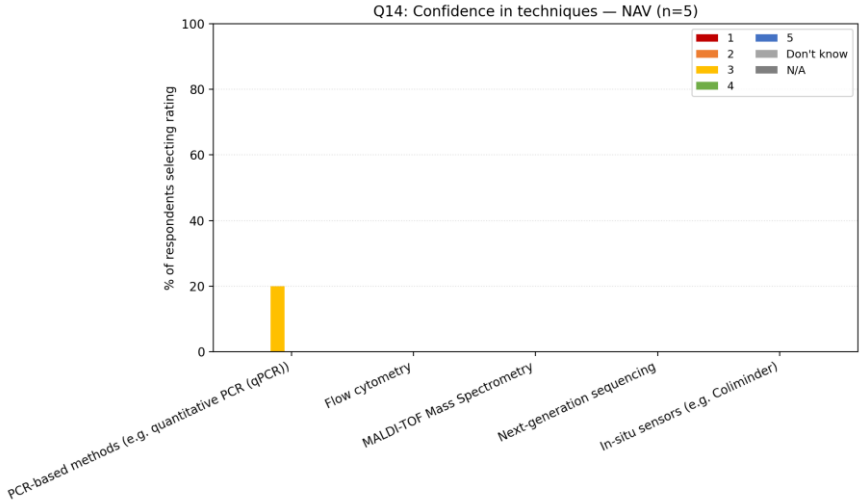
Q11 Outside of those listed in Schedule 5 of the Regulations, what alternative analytical techniques do you use to assess microbiological water quality?

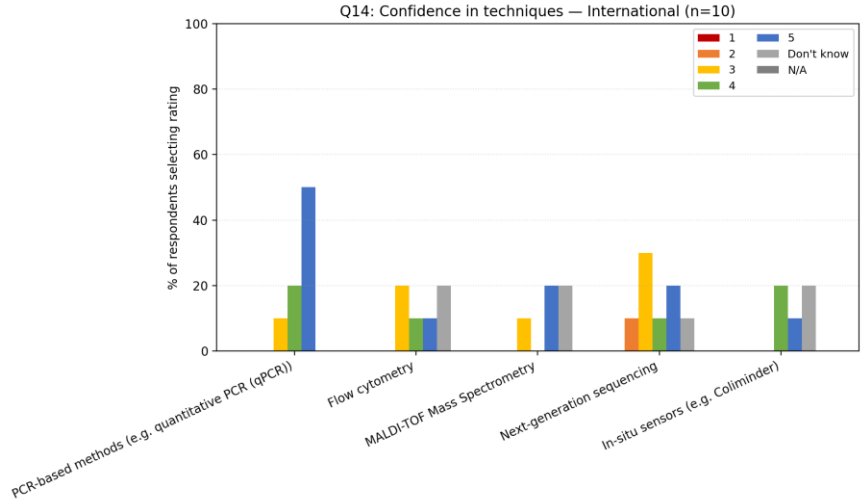


Q14 How confident are you in the sensitivity and reliability of these methods for assessing microbiological water quality in operational settings? (1 = Not confident at all, 5 = Very confident)



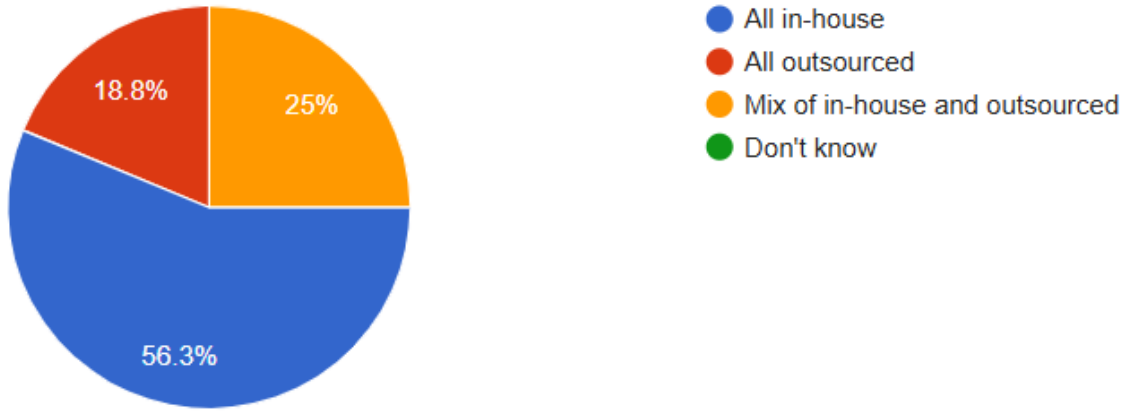




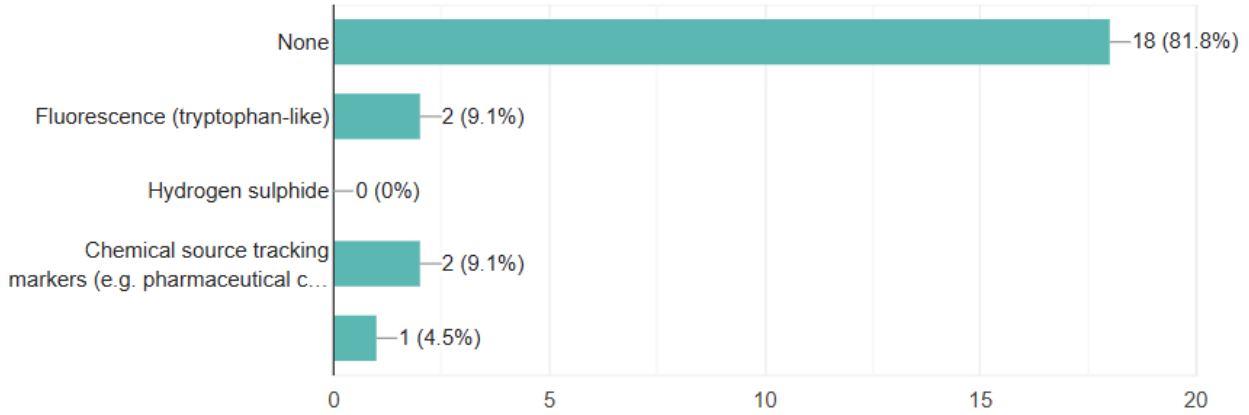


Q13 If you selected 'Other' for any techniques or purposes, please provide further details. / Q15 If you selected 'Other' in Q12/13, please indicate your confidence (1-5) in the additional techniques you have listed.	
Respondent	Technique (confidence score)
UK water utilities	no response
UK NAVs	no response
International organisations:	• MALDI-TOF is used as a complementary confirmation method for standard culture methods (3). • PCR-based methods are used for research purposes, and both PCR and targeted culture based methods are used for source investigation of potentially waterborne infections (3)

Q 16 Are these techniques used in-house or outsourced to external laboratories?



Q17 Do you use any non-regulatory chemical parameters as indicators/proxies for faecal pollution?



Q18 Please specify how your above selections are used in operational risk assessment and regulatory compliance.

UK water utilities/incumbents:

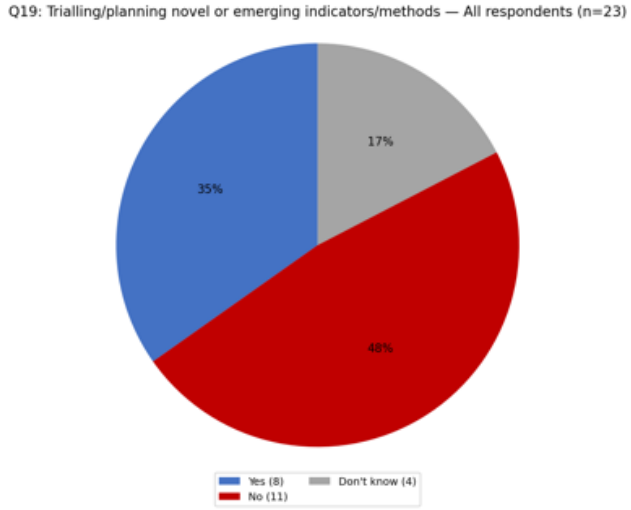
- (Flourescence) Ad hoc, mainly in the catchments
- (Chemical source tracking) Have been used very infrequently to inform catchment assessments with poor microbiological quality and likelihood of that contamination being linked to sewer contamination

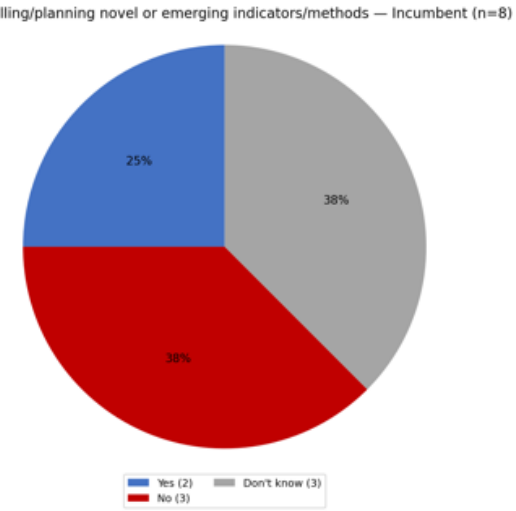
UK NAVs: n/a

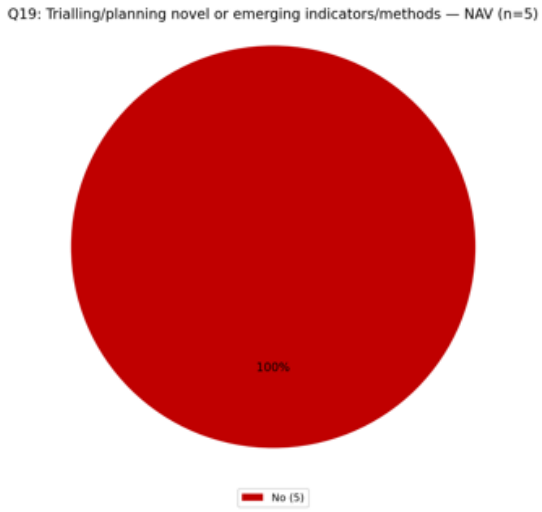
International organisations:

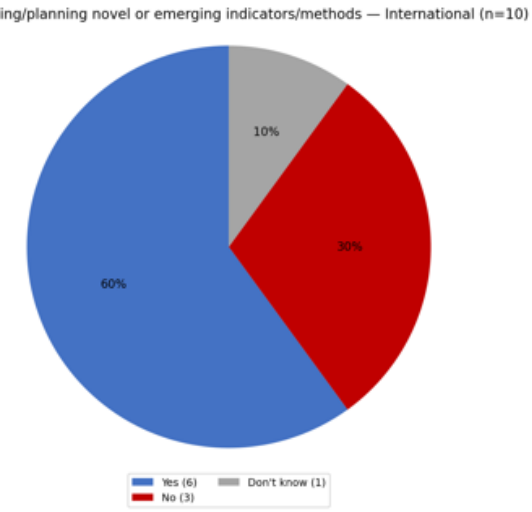
- Turbidity is used as an indicator for pollution in vulnerable catchments, e.g. open karstic systems
- (Chemical source tracking) We use targeted and untargeted methods (HPLC Quadrupole Time-of-Flight or Liquid Chromatography Tandem Mass Spectrometry) to describe the contamination of our surface or underground resources, including pharmaceutical compounds). This results could be use to confirm human pollution, and/or monitor our treatment (especially granular activated carbon or polymer)
- (Chemical source tracking) maybe used in source water risk management assessments (groundwater/surfacewater) to support primary microbiological techniques as indicators of faecal contamination
- (Fluorescence) We are currently comparing the indicator value of different parameters measured by in situ sensors to assess fecal pollution in vulnerable groundwater supplies.

Q19 Is your company trialling or planning to trail any novel or emerging microbial indicators or methods?









Appendix C Scoring methods implemented for indicator and technology ranking

Table C.1 Scoring method used for indicator ranking

ID	Criterium title	Description	Criteria type	Weight	Justification	5	4	3	2	1
IC1	Sensitivity	How common is the indicator in the faecal matter of warm-blooded animals (birds and mammals)	Qualitative or semi-quantitative	0.2	A faecal indicator should be abundant in the intestines of humans and other warm-blooded animals and consistently present in their faeces.	**Most abundant** – The indicator is readily detectable in the faeces of all warm blooded animals throughout the year	**Abundant** – The indicator is detectable in the faeces of all / most warm blooded animals with minor variations throughout the year	**Present** – The indicator is less detectable in the faeces of most warm blooded animals and/or show some seasonal variations	**Rare** – The indicator is less detectable in the faeces of a limited number of species/groups and/or detected in only one season	**Not present** – The indicator is not detectable in the faeces of any warm blooded animals
IC2	Specificity	How specific is the indicator to faecal contamination in water	Qualitative or semi-quantitative	0.2	A faecal indicator should not be found in environments that are absent of faecal contamination from warm-blooded animals to avoid false positive detections.	**Very specific** – Exclusively indicates faecal contamination	**Specific** – Strongly associated with faecal contamination with minimal overlap with non-contaminated environments	**Moderately specific** – Indicates faecal contamination with some overlap	**Non-specific** – Equally abundant in faecal-contaminated and non-contaminated environments	**Indeterminate** – Mostly present in non-contaminated environments
IC3a	Indicator for enteric bacteria	How well the indicator correlates with enteric bacteria in water	Qualitative or semi-quantitative	0.07	A faecal indicator's presence should indicate a high likelihood of faecal bacteria being present in water.	**Highly predictive** – Strongly correlates with the presence of enteric pathogens; reliable indicator	**Predictive** – Often associated with enteric pathogens, though not exclusively	**Moderately predictive** – Sometimes present with enteric pathogens, but correlation is inconsistent	**Weakly predictive** – Rarely correlates with enteric pathogens; limited reliability	**Not predictive** – No meaningful correlation with enteric pathogens; unsuitable as an indicator
IC3b	Indicator for enteric viruses	How well the indicator correlates with enteric viruses in water	Qualitative or semi-quantitative	0.07	A faecal indicator's presence should indicate a high likelihood of faecal viruses being present in water.					

IC3c	Indicator for enteric protozoa	How well the indicator correlates with enteric protozoa in water	Qualitative or semi-quantitative	0.07	A faecal indicator's presence should indicate a high likelihood of faecal protozoa being present in water.					
IC4	Indicator for operation	How well the indicator correlates with the removal of enteric pathogens during water treatment	Qualitative or semi-quantitative	0.2	A faecal indicator should have a survival rate and response to water treatment (such as chlorine resistance) similar to most resistant enteric pathogens.	**Highly representative** – The indicator's removal/inactivation closely correlates with the reduction of enteric pathogens and is consistent across different treatment technologies	**Representative –** The indicator reflects pathogen reduction with some variability depending on the treatment method or environmental conditions	**Moderately representative** – The indicator's removal does not consistently align with pathogen reduction	**Weakly representative** – The indicator is removed differently than pathogens or show inconsistent behaviour across treatment processes	**Not representative –** The indicator behave independently of pathogen removal
IC5	Detection	How easy and reliable the detection of the indicator is, i.e. are standard methods available	Qualitative or semi-quantitative	0.2	The indicator should be easy to detect and quantify using simple, reliable and cost-effective laboratory methods.	**Very easy** – Standard (ISO, BSI, SCA) analytical methods are well established, internationally and nationally recognised, and routinely applied. Methods are widely available and require no adaptation	**Easy** - Validated standard methods are available but only in certain non-UK regions or organisations. Methods are available and require minimal adaptation	**Moderately easy** - Standardised methods developed for other matrices (e.g. environmental waters) are available and can be adapted for drinking water. Some validation may be required	**Complex** - Only non-standardised methods are available. Some validation and specialist expertise may be required	**Very complex** - Methods are not well developed and need full validation and specialist expertise prior to application

Table C.2 Scoring method used for technology ranking

ID	Criterium title	Description	Criteria type	Weight	Justification	5	4	3	2	1
TC 1	Cost-efficiency	How expensive is to set up and run tests (i.e. equipment and consumables costs)	Qualitative or semi-quantitative	0.15	The technology should be affordable to set up and maintain.	**Very affordable** – The equipment and consumables/maintenance needed are affordable	**Affordable** – The equipment is somewhat expensive but the consumables/maintenance needed are affordable	**Moderately affordable** – The equipment and consumables/maintenance needed are somewhat affordable	**Expensive** – The equipment is expensive and the consumables/maintenance are affordable	**Very expensive** – The equipment and the consumables/maintenance are expensive
TC 2	Training needs	How complicated are the tests to learn	Qualitative or semi-quantitative	0.1	The technology should be easy to learn with limited troubleshooting between runs.	**Very easy** – The technology only requires a few days of training and troubleshooting is rarely necessary	**Easy** – The technology only requires a few days of training but troubleshooting is often necessary	**Moderately easy** – The technology requires extensive training but troubleshooting is rarely necessary	**Complex** – The technology requires extensive training and troubleshooting	**Very complex** – The technology requires experts to run
TC 3	Time-efficiency	How long does the analysis take	Qualitative or semi-quantitative	0.15	The test should be rapid enabling timely response to potential contamination events.	**Very rapid** – Results available real-time or near real-time	**Rapid** – Results available the same day as sampling	**Moderately rapid** – Results available in a few days following sampling	**Slow** – Results available in 1-2 weeks following sampling	**Very slow** – Results available in months following sampling
TC 4	Data interpretation	How easy is to achieve actionable results	Qualitative or semi-quantitative	0.25	The technology should provide data that is easy to interpret and representative of water quality, and hence actionable.	**Highly actionable** – Data interpretation is clear, timely, and directly supports decision-making with minimal effort	**Actionable** – Results are useful and interpretable, though may require some contextual understanding or minor processing	**Moderately actionable** – Data provides insights, but requires further analysis or expert input to inform decisions	**Impractical** – Interpretation is difficult or unclear; further data is necessary for decision-making	**Not actionable** – Data is too complex, ambiguous, or irrelevant to support decision–making

TC 5	Reliability	How accurate and sensitive the test is	Qualitative or semi-quantitative	0.25	The technology should provide highly accurate data with no false negative or false positive results even at low target concentrations.	**Highly reliable** – Very low occurrence of false positives and false negatives; low detection limits	**Reliable** – Very low occurrence of false positives and false negatives; somewhat high detection limits	**Moderately reliable** – Noticeable rate of false positives or negatives that require further testing; somewhat high detection limits	**Impractical** – Frequent false results and somewhat high detection limits	**Not reliable** – High rate of false results and high detection limits; not suitable decision-making
TC 6	Standardisation/ industry recognition	How well recognised is the technique in the water industry, and how standardised are the methods	Qualitative or semi-quantitative	0.1	The technology should be validated and suited for water industry applications.	**Highly standardised/recognised** - Standard methods exist and are nationally (UK) or internationally recognised (ISO, SCA)	**Standardised/recognised** - Methods have been standardised in specific non-UK regions or countries (e.g. USEPA)	**Recognised/not standardised** - Methods are recommended in technical protocols/guidance documents but not formally standardised	**Limited recognition/Not standardised** - No formal standardisation exists; used in the water industry in relevant applications	**Not recognised/not standardised** - No standard methods exist, and the technology has only been used in research