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ADVANCED REVIEW

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Advances in quantifying microbial contamination in potable water: Potential of fluorescence-based sensor technology

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Abstract

Improved monitoring of potable water is essential if we are to achieve the UN Sustainable Development Goals (SDGs), specifically SDG6: to make clean water and sanitation available to all. Typically monitoring of potable water requires laboratory analysis to detect indicators of fecal pollution, such as thermotolerant coliforms (TTCs), *Escherichia coli* (*E. coli*), or intestinal enterococci. However, these analyses are time-consuming and expensive, and recent advances in *field deployable* sensing technology offer opportunities to investigate both the spatial and temporal dynamics of microbial pollution in a more resolved and cost-effective manner, thus advancing process-based understanding and practical application for human health. Fluorescence offers a realistic proxy for monitoring coliforms in freshwaters with potential for quantification of potable water contamination in near real-time with no need for costly reagents. Here, we focus on *E. coli* to provide a state-of-the-art review of potential technologies capable of delivering an effective real-time *E. coli* sensor system. We synthesize recent research on the use of fluorescence spectroscopy to quantify microbial contamination and discuss a variety of approaches (and constraints) to relate the raw fluorescence signal to *E. coli* enumerations. Together, these offer an invaluable platform to monitor drinking water quality which is required in situations where the water treatment and distribution infrastructure is degraded, for example in less economically developed countries; and during disaster-relief operations. Overall, our review suggests that the fluorescence of dissolved organic matter is the most viable current method—given recent advances in field-deployable technology—and we highlight the potential for recent developments to enhance approaches to water quality monitoring.

This article is categorized under:
Engineering Water > Water, Health, and Sanitation
Engineering Water > Methods
Human Water > Methods

KEYWORDS
drinking water, *E. coli*, fluorimetry, microbial water quality

1 | INTRODUCTION

Globally approximately 2 billion people currently lack access to safe drinking water and 3.6 billion people do not have access to safe sanitation (WHO & UNICEF, 2021). The limited availability of clean drinking water is one of the major global risk factors for disease and premature death (Fuller et al., 2022). Pathogenic contamination (i.e., bacteria, viruses) of water resources due to inadequate sanitation systems (leaking latrines, septic tanks) and discharge of untreated sewage effluent and wastewater contributes to chronic public health problems. Children aged <5 years are particularly at risk in low and middle-income countries where diarrheal diseases are one of the leading causes of morbidity (GBD 2019, 2020). Of all mortality due to diarrhea, ~60% has been attributed to water quality (Prüss-Ustün et al., 2019), with ingestion of water contaminated by human or animal waste the main cause. Hence, there is a critical need to improve access to safe drinking water—to reduce poverty and improve human health and well-being. Sustainable Development Goal 6 has the aspiration of achieving "universal and equitable access to safe and affordable drinking water" by 2030, but this requires new approaches to water quality monitoring to ensure that public health interventions can be targeted as effectively and efficiently as possible (Hannah et al., 2022).

While there has been limited progress in achieving SDG6, poor health and mortality due to contaminated food and water account for ~8% of all global deaths with most deaths in S Asia and sub-Saharan Africa (UN Inter-agency Group for Child Mortality, 2020). Monitoring of all known waterborne pathogens is unfeasible; but where possible the established approach is to quantify fecal indicators. The presence of heterotrophic bacteria, indicated by laboratory culturing and subsequent colony count, has been widely used since the late 19th Century (Charles et al., 2020) to determine fecal contamination. The WHO recommends enumerations of *Escherichia coli* (*E. coli*) as the primary indicator of fecal contamination in drinking water (UNICEF, 2016) but this requires culturing a point sample for 18–24 h. This, combined with the costs of laboratory analysis, significantly constrains field investigations of drinking water supply which may experience large spikes in contamination during rainfall events, or due to poor sanitary practices. These contamination peaks are transient in nature and can be easily missed when sampling is infrequent or if it relies on an assay that takes several hours by which time the damage from contamination may have already been completed. While fecal contamination may be highly variable over time (e.g., seasonally) and spatially, detection of *E. coli* in water suggests a high probability that the source has been exposed to fecal contamination and therefore the presence of other pathogenic bacteria and viruses can generally be assumed (Edberg et al., 2000). However, the situation may be complicated by factors: there have been suggestions that the survival rates of *E. coli* outside the enteric environment are poor (Odonkor & Ampofo, 2013), and may vary with turbidity (Fluke et al., 2019), physiochemical stressors, and nutrient availability in the water source (Ishii & Sadowsky, 2008).

At present, our understanding of microbial contamination is significantly constrained by the limitations of routine methods, particularly the more traditional laboratory-based culture methods used to detect *E. coli* (Figure 1): these are not amenable to remote locations, or to situations where drinking waters experience episodic contamination. Ideally, microbial contamination would be determined by portable, low-cost devices which require only minimal training, circumventing the need for expensive laboratory analyses or trained technicians. Ideally also, the results would be available in real-time, without the need for intermediate steps such as incubation, which is a requirement of many laboratory-based methods. This provides the motivation for this article in which we review currently available technologies capable of delivering an effective real-time *E. coli* sensor system to analyze potable water. In the following sections, we consider current common and novel technologies and summarize the principles (and practical considerations) underpinning the use of fluorescence-based sensors as an *E. coli* proxy. We conclude by discussing future directions and challenges in seeking to use fluorescence sensors to attain SDG6.

2 | SYNTHESIS OF CURRENT CONVENTIONAL METHODS TO ASSESS MICROBIAL CONTAMINATION

A wide range of technologies exists for detecting microbial contamination, and by extension *E. coli*, in potable waters. These include traditional laboratory-based (e.g., Deshmukh et al., 2016) and molecular detection techniques (Campbell & Kleinheinz, 2020; Esfandiari et al., 2016; Kuo et al., 2021), Biosensors (sub-categorized into electrochemical [Bigham, Dooley, et al., 2019; Thakur et al., 2018; Grossi et al., 2013; Velasquez-Orta et al., 2017] and optical methods), optical detection techniques and fluorescence (Figure 1). These categories are based on the underlying measurement principles for each method illustrating the broad reach of techniques, some of which are widely and routinely used while others are currently at the more novel, proof-of-concept stage. Some overlap between the categories is inevitable given the potential for more hybrid analytical methods. Fluorescence-based techniques constitute an additional category encompassing different measurement approaches reflecting the rate (and complexity) of recent technological development. The selection of a method for testing potable water is highly dependent on the setting and expected quality of the water to be tested.

2.1 | Molecular detection methods

Molecular detection techniques tend to be highly specific and can target individual strains of known pathogens even in samples with low concentrations (Girones et al., 2010). No culturing is required nor isolation of the organism (Gilbride, 2021); however, precursor steps are commonly necessary before analysis which creates potential sources of error. For example, polymerase chain reaction (PCR) relies on amplification and primer stages, with the former allowing pathogenic bacteria to be detected at low concentrations although bias can be acquired at both stages resulting in an overall quantification uncertainty (Gilbride, 2021). False-negative results can occur when investigating environmental samples where matrix components, such as humic acids and metals, inhibit the amplification stage (Girones et al., 2010). The degree to which matrix substances inhibit amplification is unknown; however, droplet digital PCR (ddPCR) has been shown to mitigate their effect through dilution (Ibekwe et al., 2020; Stults et al., 2001).

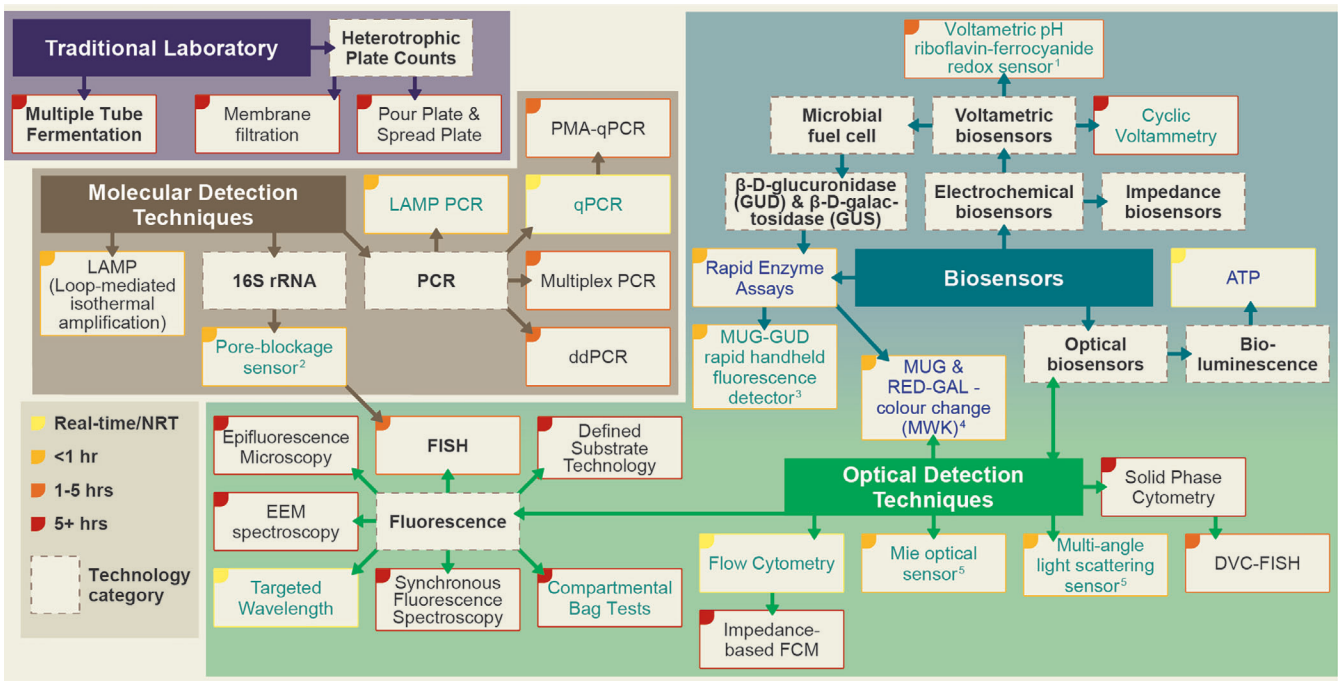

FIGURE 1 The scope and interconnectivity of methods that can be used to measure *E. coli* in water: Text in green denotes methods where their use in the field has been demonstrated in the literature; blue text indicates where potential field use has been identified but not formally demonstrated. Where a method is novel to a particular paper, numbers identify the following references: [1]Bigham, Casimero, et al. (2019); [2]Esfandiari et al. (2016); [3]Wildeboer et al. (2010); [4]Gunda and Mitra (2016); [5]Sherchan et al. (2018).

PCR-based techniques generally cannot distinguish between alive and dead target DNA, nor the presence of DNA in the environment leading to potential over-estimation (Paul et al., 1989), although there has been some progress through the development of PMA-qPCR and reverse transcription PCR which targets RNA; however, these have had only limited success (Deshmukh et al., 2016; Ju et al., 2016). While qPCR techniques are both sensitive and faster, they are unreliable in detecting 1 cfu/100 ml of *E. coli* or *Enterococcus* although they may be useful for early warning and pre-screening (Krapf et al., 2016; Walker et al., 2017). Fluorescent *in situ* hybridization (FISH) can distinguish between live/dead DNA but is far less sensitive, and increasing the target concentration in samples can lead to increased inhibition and uncertainty (Gilbride, 2021; Girones et al., 2010; Haffar & Gilbride, 2010). While PCR techniques are a robust starting point for molecular detection methods, metabarcoding and metagenomics are extensions of the field that take a sequencing approach to identifying and quantifying microbes, while outside the scope of this review owing to their complexity and specialist nature, more detail can be found in Clark et al. (2018).

While molecular detection techniques produce highly specific results, they also require a significant time investment, training, and technological investment if they are to be used successfully and that produces a significant cumulative cost per analysis, both in terms of finances and time taken to achieve this. There is also a lack of standardization across and between the methods with multiple options for each stage, making cross-comparison difficult (Gilbride, 2021). Hence, despite potential, there are currently no *field deployable* sensors using molecular detection methods.

2.2 | Biosensors

Biosensors are devices that utilize biological material to produce a measurable signal related to the target biological or chemical species. The main feature of biosensors is the transducer element that monitors the rate of biochemical reaction/interactions in the target species such as *E. coli* (as reviewed by: Alonso-Lomillo et al., 2010; Razmi et al., 2020; Rainbow et al., 2020).

Many *E. coli*-focused biosensors work on the principle of detecting β-D-glucuronidase, an enzyme present in most *E. coli* strains. This enzyme is also present in some other members of the Enterobacteriaceae family, however it is generally not present in sufficiently high concentrations to interfere with *E. coli* detection and there are existing mitigation techniques that can be utilized if it is suspected false positive bacteria may become an issue (Tryland & Fiksdal, 1998). Techniques that target either β-D-glucuronidase, or β-D-galactosidase used for coliform detection, generally involve a culture media and optical response such as a color change (Gunda & Mitra, 2016), or fluorogenic product (Hesari et al., 2016). As with other methods, the presence of other bacteria and substances that can provide a response within the criteria of the test, can lead to an erroneous inference (Fiksdall & Tryland, 2008). The rapidity of enzyme-based biosensors makes them an attractive option and an increasing number of methodologies have been developed to use these tests either in the field (Gunda & Mitra, 2016; Hesari et al., 2016) or as an online, autonomous, installation (Burnet et al., 2019; Cazals et al., 2020).

The requirement for easily operated microbiological tests in remote locations and real-time/near real-time data has spurred additional and increasingly novel monitoring approaches. For example, there has been some success with voltammetry pH sensing techniques using coliform-specific enzymes. The b-d-galactosidase enzyme, found in both coliforms and *E. coli* specifically, participates in the conversion of lactose through a process that facilitates a change in pH that can then be measured to indicate the presence of coliforms (Bigham, Casimero, et al., 2019). There has also been the highly selective use of cyclic voltammetry in conjunction with L-cysteine functionalized iron nanoparticles, where the amino group L-cysteine is used due to its ability to target and bind to proteins of pathogenic bacteria (Panhwar et al., 2019). These examples demonstrate current concepts that could potentially be deployed in commercial sensors, given the potential for lower limits of detection and presence/absence monitoring. However, while biosensors have yet to satisfy the required presence/absence monitoring threshold, recent technological developments hold the promise of more successful future field applications.

2.3 | Optical and fluorescence-based approaches

Fluorescence-inclusive assays to detect coliforms and *E. coli* encompass a wide range of approaches. Defined substrate technology (DST) is an emerging technology in which a simplified laboratory assay is created to quantify *E. coli* and

coliforms, based on the fluorogenic reaction between β-D-glucuronidase possessing *E. coli* and 4-methyl-umbelliferone (MUG) (IDEXX, 22022; Tiwari et al., 2016). Despite the requirement for an incubation stage, results from DST systems tend to be easier to interpret, and are often displayed through a color change compared with traditional culture-based techniques. Furthermore, the 24 h turnaround time and the lack of any requirement for laboratory training have made them attractive options for field campaigns, with the investigation into off-grid methods of incubation (Bernardes et al., 2020).

Flow cytometry, combining microfluidics and fluorescence, enables targeting of coliforms based on characteristics, that is, alive or dead (Cheswick et al., 2019; Hammes et al., 2012), bacterial species and individual strains, for example, *E. coli* O157 via the use of fluorescent probes (McCarthy & Culloty, 2011; Safford & Bischel, 2019; Vital et al., 2012). Direct enumeration of samples is possible via staining with fluorometric dyes, however, while FCM provides rapid results during analysis, sample pre-processing is time-consuming and several incubation and filtration steps may be required. Hence, in most cases, this approach is normally used in the laboratory (Ou et al., 2017; Yu et al., 2015), although online systems have been recently used in drinking water treatment plants (Favere et al., 2020). While this seems feasible at specific sites, FCM is currently unsuited to field use or as a roving method.

Fluorescence can also be utilized as a standalone method for laboratory and field-based measurements. Traditional excitation-emission matrix (EEM) fluorescence spectroscopy entails the scanning of a range of excitation and emission wavelengths to construct a 3D "EEM" (Figure 3). This approach can target specific organic matter groups which are excited in the UV and emit in the UV-blue range. The wavelengths can then be displayed on a 3D matrix which also provides information on the fluorescence intensity (Hudson et al., 2007). More selective fluorescence techniques include synchronous fluorescence spectroscopy (SFS), in which excitation and emission wavelengths are scanned over a fixed wavelength interval (Hur et al., 2010). Recently, fluorescence investigations have been further refined using targeted wavelength fluorescence *field deployable*, by targeting known excitation/emission pairs, such as tryptophan-like fluorescence (Khamis et al., 2017; Sorensen, Vivanco, et al., 2018). While more novel experiments can be done using reagents to trigger a fluorescence response (e.g., Wang et al., 2022), field deployable fluorescence can capitalize on the intrinsic fluorescence of different molecules of organic matter (OM) to identify different functional groups present such as proteinaceous compounds and humic and fulvic acids. In situ fluorescence can also yield instantaneous data, at a fine temporal resolution.

Currently, non-fluorescence-based field identification of *E. coli* is generally limited to proof-of-concept designs (e.g., Esfandiari et al., 2016; Gunda & Mitra, 2016; Thakur et al., 2018) owing to a lack of suitable methods. However, several fluorescence-based studies have specifically targeted *E. coli* in the field (Baker et al., 2015; Bridgeman et al., 2015; Cumberland et al., 2012; Nowicki et al., 2019; Simoes et al., 2021; Sorensen, Vivanco, et al., 2018; Ward et al., 2021). Herein, we focus on fluorescence in the context of field-based identification and quantification of coliforms in potable water.

3 | RECENT DEVELOPMENTS IN FLUORESCENCE SPECTROSCOPY

3.1 | Specific wavelengths of interest

The tryptophan-like fluorophore has excitation/emission pairs at 280/350 nm and 230/350 nm, respectively. Tryptophan fluorescence is facilitated by the indole group attached to the molecule and is often referred to as "Tryptophan-like fluorescence (TLF)" which encompasses both the amino acid identifier and other compounds, such as free indole, that fluoresce at the same wavelength (Aiken, 2014). In environmental fluorescence spectroscopy, the focus is typically on the wavelengths $280_{ex}/350–60_{em}$ nm as this has been shown to be a proxy for biological activity in water (Box 1) (Baker, 2001; Bedell et al., 2020; Bridgeman et al., 2013; Carstea et al., 2010; Cumberland et al., 2012; Sorensen, Baker, et al., 2018).

In recent years, attempts have been made to use TLF as a proxy for specific parameters such as biochemical oxygen demand (BOD; Khamis et al., 2017, 2021), total or fecal coliform counts, (Sorensen, Diaw, et al., 2020; Ward et al., 2020) and *E. coli* (Baker et al., 2015; Fox et al., 2017; Sorensen, Baker, et al., 2018; Ward et al., 2021). This has been facilitated by the development of in situ sensors that target specific wavelength ex/em pairs, as opposed to scanning a full EEM in a laboratory that requires a bench-top spectrophotometer. Other wavelength pairs have been used in a complementary way, to target different humic peaks.

3.2 | Common interference with fluorescence signals

Fluorescence in both freshwater and marine environment is susceptible to a range of interferences which can affect the fluorescence signal (Table 1). Some interferences can be mitigated, such as via temperature correction algorithms, but others are increasingly complex and reliable correction in real-time has not yet been achieved. For a *field deployable* sensor, it is advantageous to process statistical corrections instantaneously, but for some scenarios a static coefficient would be ineffective if thresholds are reached requiring a greater or lesser level of compensation.

While methods of temperature compensation have been suggested (Watras et al., 2011), complications arise as the level of compensation required differs between fluorophores and can also be influenced by the environmental setting (Carstea et al., 2014). Even once a compensation coefficient has been established, this can still vary between different sensors (Khamis et al., 2017). The discussion of universal correction factors is interesting; however, for interferences which have multiple complicating facets, the likelihood of a reliable correction factor is currently small. For example, two water samples can have the same turbidity value but very different effects on scattering and absorption when the particle size distribution varies (Khamis et al., 2015). At the very least, a set of correction factors using additional sampling data from the full range of conditions would be required to have confidence in the data output.

The development of such correction and compensation factors is also challenging, not least because the results tend to differ between laboratory and field samples, as noted for both pH, turbidity, and inner filter effect (IFE) (Downing et al., 2012; de Oliveira et al., 2018; Baker et al., 2007; Table 1). In terms of potable water, IFE is probably less of an issue as organic loads are generally low, particularly when compared with wastewater. However, in cases of drinking water contamination, IFE should still be considered when using fluorescence-based methods because of the risk of IFE from the contaminant. While research is advancing, fluorophores themselves can differ in terms of their reaction to interference and therefore all fluorescence peaks need individual consideration; for turbidity, Peak C is the main fluorescent peak of interest whereas Peak T has comparatively less data available (Carstea et al., 2020; Lee et al., 2015).

4 | FLUORESCENCE FOR REAL-TIME DETECTION OF *E. COLI*

4.1 | Relationship between tryptophan-like fluorescence and *E. coli*

Protein-like fluorescence can be used as a proxy for fecal coliforms/thermotolerant coliforms, and there has been interest in using fluorescence to detect *E. coli* in potable water. *E. coli* can be identified using TLF wavelength pairs, specifically the higher excitation pair $270–280_{ex}/350–360_{em}$. A raw fluorescence signal at these wavelengths can be correlated with *E. coli* counts due to the presence and use of the aromatic amino acid tryptophan in the bacterial membrane (Fox et al., 1990). However, when considering the use of fluorescence data as a direct proxy for *E. coli*, the components that

BOX 1 The principles of fluorescence spectroscopy

Fluorescence is one of several components that make up the sub-group photoluminescence; the emission of light occurs due to de-excitation of an electron from an electronically excited state (Valuer, 2001). Fluorescence was first recognized by George Stokes in 1852 and refers to an electron that has been excited via irradiation returning to the ground state via the emission of a photon (Figure 2; Reynolds, 2014; Lakowicz, 2006). A fluorophore, sometimes referred to as a fluorochrome, is a chemical compound, either part of a molecule or independent, that can fluoresce following excitation with a light source.

Within aquatic dissolved organic matter, several intrinsic fluorophores can be identified with known excitation and emission wavelength pairs (Figure 3). Early characterization studies identified five fluorescent DOM peaks in sea water, two protein-like and three humic-like (Coble, 1996). Protein-like fluorescence has three intrinsically fluorescent amino acids; tryptophan, tyrosine, and phenylalanine (Lakowicz, 2006). Of these, tryptophan is the dominant fluorophore, with the longest extinction coefficient and the strongest fluorescence intensity (Aiken, 2014; Lakowicz, 2006).

TABLE 1 Summary of the main principles and compensation approaches for common interferences for fluorescence-based methods

Interference	Underlying principle	Effect and compensation	Key references
Temperature	Increasing water temperature increases collisional quenching of electrons, facilitating a non-radiative return to the ground state.	Increase in temperature can suppress fluorescence intensity. Development of algorithms can compensate in real-time, with prior calibration.	Baker (2005) Carstea et al. (2014) Khamis et al. (2015) Watras et al. (2011)
Turbidity	Suspended sediment and particulate matter within water can alter how light within the water is scattered and absorbed.	Complex to quantify scattering and absorbance independently and cumulatively. Empirically-based corrections must consider sediment composition and particle size distribution. General algorithms are inefficient due to differences between fluorometers and turbidity sensor construction.	de Oliveira et al. (2018) Downing et al., 2012 Khamis et al., 2015
pH	Higher pH introduces conformational change resulting in increased exposure of the molecule and therefore a higher fluorescence intensity.	Results differ between laboratory and field samples. Suggested main peak intensity remains stable over "normal" pH range (5.5–7.5).	Baker et al. (2007) Hudson et al. (2007) Reynolds, (2003) Groeneveld et al. (2022)
Inner filter effect (IFE)	Inner filtering occurs due to absorption causing attenuation of either the excitation (primary) or emission (secondary) light.	Correction can be achieved using absorbance measurements, e.g., UV254 or a broader spectrum. Thresholds for correction are debated depending on the water matrix and fluorophores of interest.	Valuer (2001) Ohno (2002) Lakowicz (2006) Murphy et al. (2010) Kothawala et al. (2013) Goffin et al. (2020)

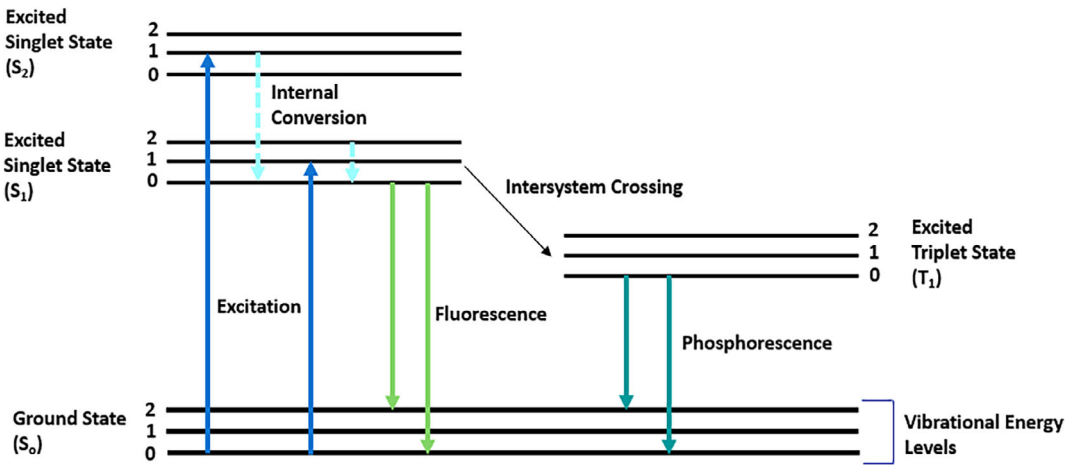

FIGURE 2 Simplified Jablonski diagram showing the electron movement between different energy levels in response to excitation by a photon.

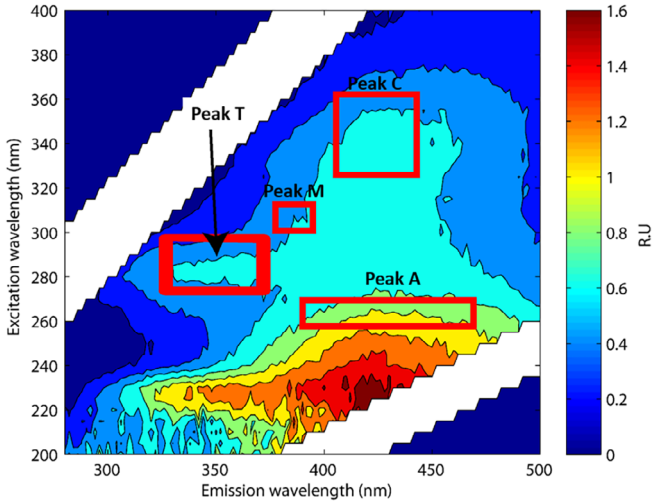

FIGURE 3 Excitation emission matrix (EEM) showing common aquatic OM fluorophores in a wastewater impacted river sample. Peaks derived from Coble (1996), intensity and range can vary betwen sample source. RU: Raman units.

make up the detectable TLF signal must be considered carefully. The term "fluorescence fingerprint" has typically been used when describing the fluorescence characteristics of DOM in a water sample in the context of the entire EEM; however, individual peaks within the EEM could also be considered using the same term.

A TLF "fingerprint" or peak can have a very different composition of compounds and intensity both between and within water samples/sources (Coble et al., 2014). For *E. coli*, the two main related fluorescent molecules are tryptophan and indole; *E. coli* produces indole from lactose and tryptophan (Cumberland et al., 2012). However, some of the TLF signal may be unrelated to the presence of *E. coli* cells (Coble, 1996; Sorensen, Baker, et al., 2018) and as such, fluorescence data requires careful validation using a secondary method. It is also important to consider the fluorescence fingerprint as the total *E. coli* related portion of the signal can vary in its fluorophore composition between different samples (Baker, 2001). For example, indole fluoresces at a 33% greater intensity on its own than when it forms part of the tryptophan molecule (Sorensen, Baker, et al., 2018). Indole is produced via the tryptophanase enzyme (Li & Young, 2013) and is utilized as both an intracellular and extracellular signal within bacteria (Gaimster et al., 2014; Lee & Lee, 2010; Wang et al., 2001). The production of indole by *E. coli* can vary depending on a range of internal and external stressors (Li & Young, 2013; Sorensen, Baker, et al., 2018) highlighting how complex just one element of the TLF signal can be, if trying to relate it to an accurate number of *E. coli*.

Understanding the cellular nature of TLF in water, and whether there is a predominance toward either intracellular or extracellular material can yield insight on the likelihood that the detected signal relates to *E. coli*; and therefore, the potential occurrence of false positive readings (Sorensen, Carr, et al., 2020). In the case of laboratory-cultured *E. coli*, it has been shown the majority of TLF was intracellular (Fox et al., 2017) whereas Sorensen, Diaw, et al. (2020) demonstrated that groundwater TLF was predominantly extracellular owing to filtration through the aquifer matrix. Indeed, the size fractionation of fluorescent DOM has been an area of interest in the field for a while (Baker et al., 2007; Bridgeman et al., 2013; Carstea et al., 2018; Sgroi et al., 2020) and is something that deserves continued attention going forward. In addition, the environmental conditions (i.e., hydrology and geology) at the point of sample can influence the observed fluorescent organic matter fraction, a complex issue which is beyond the scope of this review.

4.2 | Field-deployable, in situ fluorometers

Given the current lack of technology for commercially available real-time coliform and *E. coli* monitoring in the field, *field deployable* fluorescence has some useful features. Targeted wavelength fluorometers are increasingly compact making them highly portable, either as standalone sensors or as part of a multiparameter monitoring platform. Data can be acquired reagent-less with sensors either deployed directly into the water source/sample of interest or with water collected in a small cuvette. The ease of operation at the point of measurement is one of the most attractive features of

field deployable fluorometry as it allows the sensors to be operated by anyone, which is important when considering scenarios following a natural disaster where relief efforts are led by volunteers and aid workers, not laboratory technicians.

4.2.1 | Calibration

Fluorometers are normally calibrated using a synthetic fluorescence standard of known concentration. LED-based fluorometers experience minimal drift from their initial baseline, and consume less energy than their deuterium lamp counterparts, meaning the sensor should not require re-calibration before every deployment. However, output is not necessarily easily interpreted as fluorescence data can take the form of either a relative fluorescence unit (QSU or Raman units R.U.) or parts per billion (PPB), which requires a calibration to a known standard for each peak. None of the options have a quantitative relationship to thermotolerant coliforms / *E. coli* without additional investigation. A growing body of work is considering categories based on coliform abundance in relation to the raw fluorescence reading (Nowicki et al., 2019; Sorensen, Baker, et al., 2018); however, the relationship changes between sites according to water source and associated matrix components. The relationship is currently based on testing for *E. coli* using at least one other method and then correlating the results with simultaneous fluorescence intensity readings. For accurate calibration, samples need to be taken over a wide range of expected conditions which is not always feasible. Once a calibration is established, this can be applied to some monitoring platforms so that the coliform count is added to the output in real-time. While there is a substantial discussion around method selected, the whole process can be a source of error and introduces an element of laboratory work, at least in the initial phase, which limits the use of this technology in remote locations. While there is an argument for "global" calibrations based on prior work, there has been some success in quantifying the TLF–BOD relationship (Khamis et al., 2021). An attempt at a global calibration would not be robust without prior knowledge of the water source given the potential effect of factors such as IFE; global calibrations may never be applicable to a wide range of environments and may only suit specific applications.

4.2.2 | Limit of detection

Drinking water quality standards tend to use methods that work on a simple presence/absence monitoring principle and the ability to differentiate between 0 and 1 coliforms of a fecal indicator bacteria present in a sample, commonly *E. coli* and *I. enterococci* (Martins, 1991; Rice et al., 1989). As such, all new methods that seek authentication with legislation must meet the same standard, usually with a defined positive and negative error percentage threshold.

Currently, commercial fluorescence technology cannot reliably detect the difference between 0 and 1 coliforms due to false positives and negatives. However, not all products need a definitive 0–1 differentiation with threshold minimums being set instead; a UNICEF (2016) procurement profile set out the threshold at 10 cfu/100 ml or less with a false negative and positive boundary of <10%. Defining lower limits of detection is an area of considerable interest given the possibility to use fluorescence for bacterial enumeration. Studies focus on different fluorometer designs to lower the detection limit, Bedell et al. (2020) achieved a LOD of 4 cfu/ml of *E. coli* and Simoes et al. (2021) achieved 0.1 $\mu g\ L^{-1}$ of synthetic L-tryptophan. However, where experimental methods use ultrapure deionized water instead of more representative matrix waters, the LOD has to be challenged if it were applied to a field water sample with a more complex fluorescent fingerprint. Studies that collect information on the wider EEM show that the intensity of the different fluorescence peaks vary according to water source (Baker, 2001; Carstea et al., 2010; Wells et al., 2017). Targeted wavelength *field deployable* fluorometry is fluorophore specific which does not allow the same quantity of information to be collected, although multiple fluorometers can be utilized that target a range of wavelengths.

It is well recognized that a wide range of compounds fluoresce in the optical space covered by most field deployable fluorometers. This can, in some cases, lead to overlapping fluorescence signals. For example, polycyclic aromatic hydrocarbons (PAHs) can fluoresce in the TLF emission region and can be a problem when monitoring urban rivers (Carstea et al., 2010), although less of an issue for drinking water samples. There can also be overlap from some humic-like fluorescence (HLF) wavelengths, particularly when concentrations are elevated. This is more common with environmental

(riverine) samples but can be accounted for using an instrument set-up that captures multiple peaks (Sorensen, Vivanco, et al., 2018). In some instances, the overlap from HLF wavelengths can be related to *E. coli* and coliform activity as it is produced in different stages of bacterial cell growth (Fox et al., 2017). Studies have found that under certain conditions HLF could raise the TLF baseline making the signal less sensitive to changes under low concentrations which will affect the LOD (Ward et al., 2020) and therefore should be considered when using fluorescence sensing in environmental water samples.

Studies which evaluate the signal of laboratory-grown bacteria at a single, usually stationary, stage of the growth curve do not necessarily reflect the wider context of fluorescence associated with *E. coli*. Tryptophan is used in basic metabolic growth processes throughout the growth and development of *E. coli* coliforms, linking it to both cell number and cell activity (Fox et al., 2017). An increasing body of research also questions whether TLF is better at indicating the presence of *E. coli* rather than enumerating it, because of the link to activity where tryptophan and cellular exudates vary at different stages of growth (i.e., the highest fluorescence intensity might not correlate with the highest number of cells). With regard to potable water testing, consideration should be given to the likely growth status of *E. coli* within the sample. Studies conclude that *E. coli* survival outside the intestinal tract varies depending upon several features including temperature, strain variation, nutrient availability, and predation (Chekabab et al., 2013; Ishii & Sadowsky, 2008; Suzuki et al., 2019). The potential for growth and regrowth of *E. coli* in the environment is lower than straight survival, yet there is a suggestion that it could be possible particularly where biofilms or communities of periphyton form (Abberton et al., 2016; Suzuki et al., 2019). If using *field deployable* TLF sensors for *E. coli* detection, while the activity portion of the signal should not be dismissed, this particular effect may have little practical impact on the resulting data.

4.2.3 | Tryptophan-like fluorescence and *E. coli* relationship

The relationship between the validation method and the raw fluorescence signal can then be used to "calibrate" the instrument and data outputted (Khamis et al., 2021). Within the literature, several different techniques have been trialed to validate fluorescence data (Table 2). The most common are traditional laboratory culture-based techniques followed by the use of flow cytometry. At present, no single method has been identified as "best," leaving an interesting challenge to the accuracy of fluorescence data when used as a specific proxy for *E. coli*.

4.2.4 | Culture-based methods

Typically, bacterial enumeration in water quality assessments will use a culture-based method, such as membrane filtration (MF) which is used as a standard across the water industry (ISO, 2014). Consequently, MF is commonly used to validate fluorescence data; however, direct comparison and correlation of fluorescence data with culture data from MF have had mixed results. Some studies have shown a consistently high correlation across all sites surveyed (Cumberland et al., 2012) whereas others have demonstrated a good overall correlation but shown inconsistency between individual sites (Baker et al., 2015).

Using MF as a method for fluorescence data validation is not straightforward. There are a variety of different culture mediums available for use in MF methods (Maheux et al., 2008), and the basis on which they work also varies; that is, enzyme detection, lactose confirmation, or the selective inhibition of certain features/organism processes (Maheux et al., 2008). However, the inability to detect viable but non-culturable bacteria (VBNC) is a well-recognized disadvantage (Garcia-Armisen & Servais, 2004; Oliver, 2005) that requires additional methods or steps to circumnavigate (e.g., Guo et al., 2021; Thulsiraj et al., 2017).

Conversely, fluorescence in its raw form is primarily defined as a measure of microbial activity and as such might not be a suitable proxy for a direct enumeration method (Fox et al., 2019). This was illustrated by Bridgeman et al. (2015) in a low-contamination environment, where a correlation between the fluorescence signal and MF could not be achieved at any sites as the cultures were returned with no colonies grown. Filtration at 0.45 μm could also be a complicating factor in equating MF with targeted wavelength fluorescence, as filtration can remove the fluorescent matter bound in microbial cells. The degree to which this occurs is inconsistent between different wavelength pairs, with TLF being more affected than HLF (Baker et al., 2007; Khamis et al., 2017).

TABLE 2 Summary of methods currently used to validate fluorescence–*E. coli* relationship

Method	Principle	Time to results	Results format	Studies
Lab culturing plate count via membrane filtration	Filtering of samples through a membrane filer, usually 0.45-μm pore size, to capture coliforms on agar. Agar will isolate bacterial species by identifying unique factors, e.g., lactose positive.	>12 h	CFU/ml	Bedell et al. (2020) Bridgeman et al. (2015) Cumberland et al. (2012) Simoes and Dong (2018) Sorensen, Carr, et al. (2020), Sorensen, Diaw, et al. (2020), Sorensen et al. (2015); Sorensen, Baker, et al. (2018) Ward et al. (2020), Ward et al., (2021) Nowicki et al. (2019)
Flow cytometry	Microfluidics whereby fluorescent dyes are used to tag both alive and dead cells for enumeration.	<1 h (including staining and incubation as FCM is instantaneous)	Total bacterial cells (TBCs)	Bridgeman et al. (2015) Sorensen, Carr, et al. (2020), Sorensen, Diaw, et al. (2020)
Colilert	Defined substrate technology using OPNG and MUG. Incubate at 35°C. *E. coli* metabolize MUG and create a fluorogenic product. Coliforms metabolize OPNG turning it yellow.	24 h	Presence/absence, MPN/100 ml	Baker et al. (2015) Cumberland et al. (2012)
Compartmental Bag Tests	X-Gluc substrate metabolized by *E. coli* causing color change of water to blue. Incubate at 25°C–44.5°C. Additional growth medium MUGal which can be metabolized by coliforms and turns red under a 365 nm UV light.	20–48 h	Presence/absence, MPN/100 ml	Ward et al. (2021)

4.2.5 | Flow cytometry

Flow cytometry (FCM) takes an entirely different approach to bacteria enumeration than MF as a cultivation-independent method (Hammes & Egli, 2010). FCM utilizes different fluorescent dyes to identify bacteria, be it a total enumeration (e.g., Hammes et al., 2008), an assessment of bacterial viability (e.g., Ou et al., 2017), or targeting specific strains through antigen binding FITC (e.g., McCarthy & Culloty, 2011; Vital et al., 2012). As a cultivation-independent method, it avoids the traditional problems of VBNC detection and the "great plate count anomaly" (Gillespie, 2016; Van Nevel et al., 2017); which makes it an interesting method to validate fluorescence-based *E. coli* data.

While MF failed to correlate with fluorescence, due to the low contamination (e.g., Bridgeman et al., 2015), FCM-derived total cell counts did correlate with fluorescence. A similar positive correlation between fluorescence data and FCM has been subsequently found in other studies (Sorensen, Baker, et al., 2018; Sorensen, Diaw, et al., 2020). The fact that these examples use "total cell counts" and are not only detecting *E. coli* is likely a reason for the more robust correlation in comparison to MF.

As previously mentioned, TLF signals can be associated with a wide range of matter both related and unrelated to *E. coli*. FCM is, in principle, able to detect a wider range of bacteria than culture-based methods, and in this case the techniques share more similarities than fluorescence, with a culture-based MF method. The broader range of bacteria captured by FCM is likely responsible for the stronger correlation between the two. However, FCM is not shown to be a definitively "better" method of validation for fluorescence data than MF as it does not always return the stronger correlation; Sorensen, Baker, et al. (2018) found little difference in the correlation between fluorescence and both validation methods.

The clear potential issue in the use of FCM for validation of fluorescence data when pertaining to *E. coli* is that, if using a non-selective bacterial stain, it will capture *E. coli* and any other bacterial cells present. This can cause correlations which are unrelated to the presence of *E. coli* when accounting for something both methods have detected. Interestingly, Sorensen, Carr, et al. (2020) found that when comparing thermotolerant coliforms (TTCs) enumerated via MF and total bacterial cell (TBC) counts from FCM in groundwater, TLF offered a better predictor of TBCs. There was also a close correlation between TBCs and humic-like fluorescence, suggesting the source of the TLF in those samples was unrelated to *E. coli* or other fecal indicator bacteria and indicating the potential to use fluorescence to identify the organic matter signals which are most likely to be associated with a high abundance of *E. coli*.

5 | REALIZING THE POTENTIAL OF SENSOR-BASED TECHNOLOGY: WAYS FORWARD

Poor water quality remains a substantial public health issue and there are considerable challenges if SDG6 is to be achieved by 2030 (Hannah et al., 2022). A reorientation of approach in the way we conduct water quality monitoring is required to overcome existing challenges along with a wider consideration of the standards used to evaluate new technology where this has the potential to improve water quality. Accurate detection of *E. coli* as a fecal indicator bacteria (FIB) using portable and time-efficient methods must be a priority; however, culture-based assays remain enshrined in legislation. While assay-based methods still have an important contribution, there are wider questions concerning their accuracy and the technical requirements which mean that heterotrophic plate counts (HPCs) are unsuited as a roving, real-time water quality assessment method. Despite the known accuracy issues, HPCs have become the standard against which new methods are judged, without regard for any differences in the monitoring approach. In contrast, fluorescence provides a more thorough assessment of OM content. Fluorescence is also versatile and not limited to detection of *E. coli* but can also provide surrogates for BOD, TOC alongside more general microbial activity and pollution indication. The current state of fluorescence technology renders it unsuitable for use as a direct quantification method. However, it has considerable potential as a rapid assessment tool that can function without the need for reagents, specialist training, and other constraints of pre-existing methods. Field deployable fluorescence spectroscopy can thus be an important component of a wider suite of monitoring tools that together can optimize microbial water quality monitoring to establish (immediately) the risk of contamination. While the potential of other approaches using molecular detection and biosensors has been highlighted in this review, and by others, factors including cost, technical expertise required for operation, and the technology readiness level prevents them from being viable alternatives at this point in time for a field deployable, real-time sensor. Fluorescence sensor technology is at a pivotal stage in development, already commercially available in a field-deployable form however moving forward with *E. coli* detection is not without its own challenges.

5.1 | Challenges to validation

The limited number of studies using fluorescence to detect *E. coli* complicates attempts to determine the most effective method of validation. The raw fluorescence signal is complex but comparing it to a method like MF/heterotrophic plate counts, which has well-established limitations that do not mirror fluorescence data, potentially weakens it's use as a tool to detect microbial contamination and *E. coli*, more in some environments than others. This has the potential to create confusion and false negatives when the fluorescence data directly contradicts the culture data, as observed by Bridgeman et al. (2015). Due to the nature of fluorescence as a proxy, this could report as a genuine negative with fluorescence yielding from other TLF-producing components within the sample or as a false negative, whereby the *E. coli* present were in a viable but non-culturable state and therefore still posing a risk. There is, therefore, a question regarding the use of MF methods for direct validation via enumeration when fluorescence is technically more able to capture an additional level of information than MF. There could be a risk of underestimating the usefulness of fluorescence as a metric for *E. coli*, TTCs, and so on, if the method it is being compared with is ill-suited for comparison.

From published studies, FCM presents an interesting alternative however it is a technical method which requires specialized equipment. Culture-based methods are also to some extent commercially available in easy-to-use kits (Nowicki et al., 2019; Ward et al., 2021). The topic of filtration can confound all three methods as, with fluorescence, filtering is a fine balance between removing optical interferences and removing fluorescent organic matter (Baker et al., 2007; Khamis et al., 2017). MF requires filtering at 0.45 μm whereas microfluidic flow cytometry requires a

smaller mechanical filter to avoid instrument clogging (Cheswick et al., 2019). This poses challenges to validation as variability between methods will depend on the proportion of the fluorescence that is extracellular versus intracellular. While these could be significant complicating factors, they do not render validation impossible but rather indicate that further work is needed to assess best practice and ensure that a robust relationship can be established across a range of environmental conditions.

Considering the potential future use of a real-time, *field deployable* fluorometer if validation needs to take place then the method must be something that can be carried out easily, or it will severely limit the application of such a sensor. This issue of validation is perhaps one of the biggest issues facing fluorescence as a method of *E. coli* detection moving forward. Current best practice involves two independent methods to verify the fluorescence data which, although laborious in many ways, should provide mitigation to the inconsistencies between *E. coli* measurement techniques.

5.2 | Future directions

The field of water quality monitoring is being transformed by the availability of new technology which requires continual re-evaluation and questioning of our pre-existing knowledge and methodological techniques to ensure that monitoring goals are achieved. Moreover, there have been recent questions over the suitability of various FIB in water treatment plants or in situations of rapid environmental change where there may be inconsistent correlations, for example, with viruses (Teixeira et al., 2020). While *E. coli* remains a robust indicator of fecal contamination, fluorescence is providing alternative approaches to monitoring which are not just limited to *E. coli* and BOD as considered in this review. The potential to use fluorescence for virus monitoring has yet to be explored in detail, although there has been some exploratory work in this area (Alimova et al., 2007; Owoicho et al., 2021).

Technological developments are advancing applications of *field deployable* fluorescence spectroscopy given improvements in both the detection limit and in quantifying interferences to improve the minimum detection limit. In terms of the "field deployable" aspect, fluorescence technology is still relatively young but recent progress highlights its potential as a practical and useful technique. Moreover, field deployable fluorescence targeting multiple wavelength pairs has the potential to yield far more information about water quality than a simple culture assay, thus aiding quick diagnosis of potable water safety. At a time when water policies are constantly evolving, with laws becoming more stringent for pollution detection (e.g., EPA, 2013) and the threshold for "good" water quality being pushed ever higher (Hannah et al., 2022), the need for spatiotemporally resolved information on water quality is paramount. The "invisible water crisis" can only be tackled with increasing information and monitoring capabilities to understand every facet of pollution from occurrence to legacy and how multipollutant cocktails interact with the environment and the implications for human health (Damania et al., 2019). To facilitate these advances a paradigm-shift is required, there needs to be more incentives for water managers and governmental agencies to explore the potential new technologies and equally for technological innovation that can drive down the unit cost of field-deployable instrumentation. We also need to recognize the limitations of current water quality monitoring programs in many parts of the world. This partly reflects the costs of instrumentation and laboratory analyses, and highlights the wider benefits associated with field-deployable sensor technologies provided sensor output can be appropriately validated.

AUTHOR CONTRIBUTIONS

Hannah Gunter: Conceptualization (lead); writing – original draft (lead). **Chris Bradley:** Conceptualization (equal); funding acquisition (equal); writing – original draft (equal); writing – review and editing (equal). **David M. Hannah:** Conceptualization (equal); methodology (equal); writing – review and editing (equal). **Semira Manaseki-Holland:** Conceptualization (supporting); methodology (supporting). **Rob Stevens:** Funding acquisition (supporting); methodology (supporting); writing – review and editing (supporting). **Kieran Khamis:** Conceptualization (equal); methodology (equal); writing – review and editing (equal).

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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REFERENCES

Abberton, C. L., Bereschenko, L., Van Der Wielen, P. W. J. J., & Smith, C. J. (2016). Survival, biofilm formation, and growth potential of environmental and enteric Escherichia coli strains in drinking water microcosms. *Applied and Environmental Microbiology*, *82*(17), 5320–5331. https://doi.org/10.1128/aem.01569-16

Aiken, G. (2014). Fluorescence and dissolved organic matter: A chemist's perspective. In P. Coble, J. Lead, A. Baker, D. Reynolds, & R. Spencer (Eds.), *Aquatic organic matter fluorescence (Cambridge environmental chemistry series)* (pp. 35–74). Cambridge University Press.

Alimova, A., Katz, A., Podder, R., Minko, G., Wei, H., Alfano, R. R., & Gottlieb, P. (2007). Virus particles monitored by fluorescence spectroscopy: A potential detection assay for macromolecular assembly. *Photochemistry and Photobiology*, *80*(1), 41–46. https://doi.org/10.1111/j.1751-1097.2004.tb00047.x

Alonso-Lomillo, M. A., Dominguez-Renedo, O., & Arcos-Martinez, M. J. (2010). Screen-printed biosensors in microbiology; a review. *Talanta*, *82*(5), 1629–1636. https://doi.org/10.1016/j.talanta.2010.08.033

Baker, A. (2001). Fluorescence excitation–emission matrix characterization of some sewage-impacted rivers. *Environmental Science & Technology*, *35*(5), 948–953. https://doi.org/10.1021/es000177t

Baker, A. (2005). Thermal fluorescence quenching properties of dissolved organic matter. *Water Research*, *39*(18), 4405–4412. https://doi.org/10.1016/j.watres.2005.08.023

Baker, A., Cumberland, S. A., Bradley, C., Buckley, C., & Bridgeman, J. (2015). To what extent can portable fluorescence spectroscopy be used in the real-time assessment of microbial water quality? *Science of the Total Environment*, *532*, 14–19. https://doi.org/10.1016/j.scitotenv.2015.05.114

Baker, A., Elliott, S., & Lead, J. R. (2007). Effects of filtration and pH perturbation on freshwater organic matter fluorescence. *Chemosphere*, *67*(10), 2035–2043. https://doi.org/10.1016/j.chemosphere.2006.11.024

Bedell, E., Sharpe, T., Purvis, T., Brown, J., & Thomas, E. (2020). Demonstration of tryptophan-like fluorescence sensor concepts for fecal exposure detection in drinking water in remote and resource constrained settings. *Sustainability*, *12*(9), 3768. https://doi.org/10.3390/su12093768

Bernardes, C., Bernardes, R., Zimmer, C., & Dorea, C. C. (2020). A simple off-grid incubator for microbiological water quality analysis. *Water*, *12*(1), 240. https://doi.org/10.3390/w12010240

Bigham, T., Casimero, C., Dooley, J. S. G., Ternan, N. G., Snelling, W. J., & Davis, J. (2019). Microbial water quality: Voltammetric detection of coliforms based on riboflavin-ferrocyanide redox couples. *Electrochemistry Communications*, *101*, 99–103. https://doi.org/10.1016/j.elecom.2019.02.022

Bigham, T., Dooley, J. S. G., Ternan, N. G., Snelling, W. J., Castelan, M. C. H., & Davis, J. (2019). Assessing microbial water quality: Electroanalytical approaches to the detection of coliforms [review]. *TrAC-Trends in Analytical Chemistry*, *121*, 115670. https://doi.org/10.1016/j.trac.2019.115670

Bridgeman, J., Baker, A., Brown, D., & Boxall, J. B. (2015). Portable LED fluorescence instrumentation for the rapid assessment of potable water quality. *Science of the Total Environment*, *524-525*, 338–346. https://doi.org/10.1016/j.scitotenv.2015.04.050

Bridgeman, J., Baker, A., Carliell-Marquet, C., & Carstea, E. (2013). Determination of changes in wastewater quality through a treatment works using fluorescence spectroscopy. *Environmental Technology*, *34*(21-24), 3069–3077. https://doi.org/10.1080/09593330.2013.803131

Burnet, J. B., Dinh, Q. T., Imbeault, S., Servais, P., Dorner, S., & Prevost, M. (2019). Autonomous online measurement of beta-D-glucuronidase activity in surface water: Is it suitable for rapid *E. coli* monitoring? *Water Research*, *152*, 241–250. https://doi.org/10.1016/j.watres.2018.12.060

Campbell, A., & Kleinheinz, G. (2020). Comparison of qPCR and traditional beach monitoring methods at select Great Lakes beaches and the impact on beach management. *Lake and Reservoir Management*, *36*(2), 155–168. https://doi.org/10.1080/10402381.2020.1745338

Carstea, E. M., Baker, A., Bieroza, M., & Reynolds, D. (2010). Continuous fluorescence excitation-emission matrix monitoring of river organic matter. *Water Research*, *44*(18), 5356–5366. https://doi.org/10.1016/j.watres.2010.06.036

Carstea, E. M., Baker, A., Bieroza, M., Reynolds, D. M., & Bridgeman, J. (2014). Characterisation of dissolved organic matter fluorescence properties by PARAFAC analysis and thermal quenching. *Water Research*, *61*, 152–161. https://doi.org/10.1016/j.watres.2014.05.013

Carstea, E. M., Popa, C. L., Baker, A., & Bridgeman, J. (2020). In situ fluorescence measurements of dissolved organic matter: A review. *Science of the Total Environment*, *699*, 16. https://doi.org/10.1016/j.scitotenv.2019.134361

Carstea, E. M., Zakharova, Y. S., & Bridgeman, J. (2018). Online fluorescence monitoring of effluent organic matter in wastewater treatment plants. *Journal of Environmental Engineering*, *144*(5), 1–9. https://doi.org/10.1061/(asce)ee.1943-7870.0001360

Cazals, M., Stott, R., Fleury, C., Proulx, F., Prévost, M., Servais, P., Dorner, S., & Burnet, J.-B. (2020). Near real-time notification of water quality impairments in recreational freshwaters using rapid online detection of β-D-glucuronidase activity as a surrogate for *Escherichia coli* monitoring. *Science of the Total Environment*, *720*, 137303. https://doi.org/10.1016/j.scitotenv.2020.137303

Charles, K. J., Nowicki, S., & Bartram, J. J. (2020). A framework for monitoring the safety of water services: From measurements to security. *npj Clean Water*, *3*, 36. https://doi.org/10.1038/s41545-020-00083-1

Chekababb, S. M., Paquin-Veillette, J., Dozois, C. M., & Harel, J. (2013). The ecological habitat and transmission of *Escherichia coli* O157:H7. *FEMS Microbiology Letters*, *341*(1), 1–12. https://doi.org/10.1111/1574-6968.12078

Cheswick, R., Moore, G., Nocker, A., Hassard, F., Jefferson, B., & Jarvis, P. (2019). Chlorine disinfection of drinking water assessed by flow cytometry: New insights. *Environmental Technology and Innovation*, *19*, 101032. https://doi.org/10.1016/j.eti.2020.101032

Clark, D. R., Ferguson, R. M., Harris, D. N., Matthews Nicholass, K. J., Prentice, H. J., Randall, K. C., Randall, L., Warren, S. L., & Dumbrell, A. J. (2018). Streams of data from drops of water: 21st century molecular microbial ecology. *Wiley Interdisciplinary Reviews: Water*, *5*(4), e1280.

Coble, P. G. (1996). Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry*, *51*, 325–346.

Coble, P., Spencer, R., Baker, A., & Reynolds, D. (2014). Aquatic organic matter fluorescence. In P. Coble, J. Lead, A. Baker, D. Reynolds, & R. Spencer (Eds.), *Aquatic organic matter fluorescence (Cambridge environmental chemistry series)* (pp. 75–122). Cambridge University Press.

Cumberland, S., Bridgeman, J., Baker, A., Sterling, M., & Ward, D. (2012). Fluorescence spectroscopy as a tool for determining microbial quality in potable water applications. *Environmental Technology*, *33*(6), 687–693. https://doi.org/10.1080/09593330.2011.588401

Damania, R., Desbureaux, S., Rodella, A. S., Russ, J., & Zaveri, E. (2019). *Quality unknown: The invisible water crisis*. World Bank. https://doi.org/10.1596/978-1-4648-1459-4

de Oliveira, G. F., Bertone, E., Stewart, R. A., Awad, J., Holland, A., O'Halloran, K., & Bird, S. (2018). Multi parameter compensation method for accurate In situ fluorescent dissolved organic matter monitoring and properties characterization. *Water*, *10*(9), 1146. https://doi.org/10.3390/w10091146

Deshmukh, R. A., Joshi, K., Bhand, S., & Roy, U. (2016). Recent developments in detection and enumeration of waterborne bacteria: A retrospective minireview. *MicrobiologyOpen*, *5*(6), 901–922. https://doi.org/10.1002/mbo3.383

Downing, B. D., Pellerin, B. A., Bergamaschi, B. A., Saraceno, J. F., & Kraus, T. E. C. (2012). Seeing the light: The effects of particles, dissolved materials, and temperature on in situ measurements of DOM fluorescence in rivers and streams. *Limnology and Oceanography: Methods*, *10*(10), 767–775. https://doi.org/10.4319/lom.2012.10.767

Edberg, S. C. L., Rice, E. W., Karlin, R. J., & Allen, M. J. (2000). *Escherichia coli*: The best biological drinking water indicator for public health protection. *Journal of Applied Microbiology*, *88*(S1), 106S–116S. https://doi.org/10.1111/j/1365-2672.2000.tb05338.x

EPA. (2013). National primary drinking water regulations: Revisions to the total coliform rule. Final rule. https://www.govinfo.gov/content/pkg/FR-2013-02-13/pdf/2012-31205.pdf

Esfandiari, L., Wang, S., Wang, S., Banda, A., Lorenzini, M., Kocharyan, G., Monbouquette, H. G., & Schmidt, J. J. (2016). PCR-independent detection of bacterial species-specific 16 S rRNA at 10 fM by a pore-blockage sensor. *Biosensors*, *6*(3), 37. https://doi.org/10.3390/bios6030037

Favere, J., Buysschaert, B., Boon, N., & De Gusseme, B. (2020). Online microbial fingerprinting for quality management of drinking water: Full-scale event detection. *Water Research*, *170*, 115353. https://doi.org/10.1016/j.watres.2019.115353

Fiksdall, L., & Tryland, I. (2008). Application of rapid enzyme assay techniques for monitoring of microbial water quality [review]. *Current Opinion in Biotechnology*, *19*(3), 289–294. https://doi.org/10.1016/j.copbio.2008.03.004

Fluke, J., González-Pinzón, R., & Thomson, B. (2019). Riverbed sediments control the spatiotemporal variability of *E. coli* in a highly managed, Arid River. *Frontiers in Water*, *1*, 4. https://doi.org/10.3389/frwa.2019.00004

Fox, A., Ueda, K., & Morgan, S. L. (1990). Analysis of bacterial amino acids. In A. Fox, S. L. Morgan, L. Larsson, & G. Odham (Eds.), *Analytical microbiology methods*. Springer.

Fox, B. G., Thorn, R. M. S., Anesio, A. M., & Reynolds, D. M. (2017). The in situ bacterial production of fluorescent organic matter; an investigation at a species level. *Water Research*, *125*, 350–359. https://doi.org/10.1016/j.watres.2017.08.040

Fox, B. G., Thorn, R. M. S., Anesio, A. M., Cox, T., Attridge, J. W., & Reynolds, D. M. (2019). Microbial processing and production of aquatic fluorescent organic matter in a model freshwater system. *Water*, *11*(1), 10. https://doi.org/10.3390/w11010010

Fuller, R., Landrigan, P. J., Balakrishnan, K., Bathan, G., Bose-O'Reilly, S., Brauer, M., Caravanos, J., Chiles, T., Cohen, A., Corra, L., Cropper, M., Ferraro, G., Hanna, J., Hanrahan, D., Hu, H., Hunter, D., Janata, G., Kupka, R., Lanphear, B., ... Yan, C. (2022). Pollution and health: A progress update. *Lancet Planetary Health*, *6*(6), 535–547.

Gaimster, H., Cama, J., Hernandez-Ainsa, S., Keyser, U. F., & Summers, D. K. (2014). The indole pulse: A new perspective on indole signalling in *Escherichia coli*. *PLoS One*, *9*(4), 93168. https://doi.org/10.1371/journal.pone.0093168

Garcia-Armisen, T., & Servais, P. (2004). Enumeration of viable *E. coli* in rivers and wastewaters by fluorescent in situ hybridization. *Journal of Microbiological Methods*, *58*(2), 269–279. https://doi.org/10.1016/j.mimet.2004.04.014

GBD 2019. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: A systematic analysis for the global burden of disease study. *The Lancet*, *396*(10258), 1204–1222. https://doi.org/10.1016/S0140-6736(20)30925-9

Gilbride, K. (2021). Molecular methods for the detection of waterborne pathogens. In H. Bridle (Ed.), *Waterborne pathogens detection methods and applications* (pp. 237–292). Elsevier.

Gillespie, S. (2016). Current status of molecular microbiological techniques for the analysis of drinking water. In N. Cook, M. D'Agostino, & K. Clive Thompson (Eds.), *Molecular microbial diagnostic methods* (pp. 39–58). Academic Press.

Girones, R., Ferrus, M. A., Alonso, J. L., Rodriguez-Manzano, J., Calgua, B., Correa Ade, A., Hundesa, A., Carratala, A., & Bofill-Mas, S. (2010). Molecular detection of pathogens in water--the pros and cons of molecular techniques. *Water Research*, *44*(15), 4325–4339. https://doi.org/10.1016/j.watres.2010.06.030

Goffin, A., Vasquez-Vergara, L. A., Guerin-Rechdaoui, S., Rocher, V., & Varrault, G. (2020). Temperature, turbidity, and the inner filter effect correction methodology for analyzing fluorescent dissolved organic matter in urban sewage. *Environmental Science and Pollution Research International*, *27*(28), 35712–35723. https://doi.org/10.1007/s11356-020-09889-5

Groeneveld, M., Catalán, N., Einarsdottir, K., Bravo, A. G., & Kothawala, D. N. (2022). The influence of pH on dissolved organic matter fluorescence in inland waters. *Analytical Methods*, *14*(13), 1351–1360. https://doi.org/10.1039/d1ay01702k

Grossi, M., Lazzarini, R., Lanzoni, M., Pompei, A., Matteuzzi, D., & Ricco, B. (2013). A portable sensor with disposable electrodes for water bacterial quality assessment. *IEEE Sensors Journal*, *13*(5), 1775–1782. https://doi.org/10.1109/jsen.2013.2243142

Gunda, N. S. K., & Mitra, S. K. (2016). Rapid water quality monitoring for microbial contamination. *Electrochemical Society Interface*, *25*(4), 73–78. https://doi.org/10.1149/2.F06164if

Guo, L., Wan, K., Zhu, J., Ye, C., Chabi, K., & Yu, X. (2021). Detection and distribution of vbnc/viable pathogenic bacteria in full-scale drinking water treatment plants. *Journal of Hazardous Materials*, *406*, 124335. https://doi.org/10.1016/j.jhazmat.2020.124335

Haffar, M., & Gilbride, K. A. (2010). The utility and application of real-time PCR and FISH in the detection of single-copy gene targets in *Escherichia coli* O157:H7 and *Salmonella typhimurium*. *Canadian Journal of Microbiology*, *56*(3), 254–262. https://doi.org/10.1139/w09-126

Hammes, F., & Egli, T. (2010). Cytometric methods for measuring bacteria in water: Advantages, pitfalls and applications. *Analytical and Bioanalytical Chemistry*, *397*(3), 1083–1095. https://doi.org/10.1007/s00216-010-3646-3

Hammes, F., Berney, M., Wang, Y., Vital, M., Koster, O., & Egli, T. (2008). Flow-cytometric total bacterial cell counts as a descriptive microbiological parameter for drinking water treatment processes. *Water Research*, *42*(1-2), 269–277. https://doi.org/10.1016/j.watres.2007.07.009

Hammes, F., Broger, T., Weilenmann, H. U., Vital, M., Helbing, J., Bosshart, U., Huber, P., Peter Odermatt, R., & Sonnleitner, B. (2012). Development and laboratory-scale testing of a fully automated online flow cytometer for drinking water analysis. *Cytometry Part A*, *81*(6), 508–516. https://doi.org/10.1002/cyto.a.22048

Hannah, D. M., Abbott, B. W., Khamis, K., Kelleher, C., Lynch, I., Krause, S., & Ward, A. S. (2022). Illuminating the 'invisible water crisis' to address global water pollution challenges. *Hydrological Processes*, *36*(3), e14525. https://doi.org/10.1002/hyp.14525

Hesari, N., Alum, A., Elzein, M., & Abbaszadegan, M. (2016). A biosensor platform for rapid detection of *E. coli* in drinking water. *Enzyme and Microbial Technology*, *83*, 22–28. https://doi.org/10.1016/j.enzmictec.2015.11.007

Hudson, N., Baker, A., & Reynolds, D. (2007). Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters—A review. *River Research and Applications*, *23*(6), 631–649. https://doi.org/10.1002/rra.1005

Hur, J., Lee, B. M., Lee, T. H., & Park, D. H. (2010). Estimation of biological oxygen demand and chemical oxygen demand for combined sewer systems using synchronous fluorescence spectra. *Sensors (Basel)*, *10*(4), 2460–2471. https://doi.org/10.3390/s100402460

Ibekwe, M. A., Murinda, S. E., Park, S., Obayiuwana, A., Murry, M. A., Schwartz, G., & Lundquist, T. (2020). Comparative use of quantitative PCR (qPCR), droplet digital PCR (ddPCR), and recombinase polymerase amplification (RPA) in the detection of Shiga toxin-producing *E. coli* (STEC) in environmental samples. *Water*, *12*(12), 3507. https://doi.org/10.3390/w12123507

IDEXX. (2022). *Colilert [online]*. IDEXX https://www.idexx.co.uk/en-gb/water/water-products-services/colilert/ [Last Accessed November 10, 2022]

Ishii, S., & Sadowsky, M. J. (2008). *Escherichia coli* in the environment: Implications for water quality and human health. *Microbes and Environments*, *23*(2), 101–108.

ISO. (2014). Water quality—Enumeration of *Escherichia coli* and coliform bacteria—Part 1: Membrane filtration method for waters with low bacterial background flora (ISO 9308-1:2014). https://www.iso.org/standard/55832.html

Ju, W., Moyne, A.-L., & Marco, M. L. (2016). RNA-based detection does not accurately enumerate living *Escherichia coli* 157:H7 cells on plants. *Frontiers in Microbiology*, *223*(7), 1–9. https://doi.org/10.3389/fmicb.2016.00223

Khamis, K., Bradley, C., Gunter, H. J., Basevi, G., Stevens, R., & Hannah, D. M. (2021). Calibration of an in-situ fluorescence based sensor platform for reliable BOD5 measurement in wastewater [article; early access]. *Water Science and Technology*, *17*, 3075–3091. https://doi.org/10.2166/wst.2021.197

Khamis, K., Bradley, C., Stevens, R., & Hannah, D. M. (2017). Continuous field estimation of dissolved organic carbon concentration and biochemical oxygen demand using dual-wavelength fluorescence, turbidity and temperature. *Hydrological Processes*, *31*(3), 540–555. https://doi.org/10.1002/hyp.11040

Khamis, K., Sorensen, J. P. R., Bradley, C., Hannah, D. M., Lapworth, D. J., & Stevens, R. (2015). In situ tryptophan-like fluorometers: Assessing turbidity and temperature effects for freshwater applications. *Environmental Science: Processes & Impacts*, *17*(4), 740–752. https://doi.org/10.1039/c5em00030k

Kothawala, D. N., Murphy, K. R., Stedmon, C. A., Weyhenmeyer, G. A., & Tranvik, L. J. (2013). Inner filter correction of dissolved organic matter fluorescence. *Limnology and Oceanography: Methods*, *11*(12), 616–630. https://doi.org/10.4319/lom.2013.11.616

Krapf, T., Kuhn, R. M., Kauf, P., Gantenbein-Demarchi, C. H., & Fieseler, L. (2016). Quantitative real-time PCR does not reliably detect single fecal indicator bacteria in drinking water. *Water Supply*, *16*(6), 1674–1682. https://doi.org/10.2166/ws.2016.052

Kuo, J. T., Chang, L. L., Yen, C. Y., Tsai, T. H., Chang, Y. C., Huang, Y. T., & Chung, Y. C. (2021). Development of fluorescence in situ hybridization as a rapid, accurate method for detecting coliforms in water samples. *Biosensors-Basel*, *11*(1), 13. https://doi.org/10.3390/bios11010008

Lakowicz, J. R. (2006). *Principles of fluorescence spectroscopy* (3rd ed., pp. 529–575). Springer.

Lee, E. J., Yoo, G. Y., Jeong, Y., Kim, K. U., Park, J. H., & Oh, N. H. (2015). Comparison of UV–VIS and FDOM sensors for in situ monitoring of stream DOC concentrations. *Biogeosciences*, *12*(10), 3109–3118. https://doi.org/10.5194/bg-12-3109-2015

Lee, J. H., & Lee, J. (2010). Indole as an intercellular signal in microbial communities. *FEMS Microbiology Reviews*, *34*(4), 426–444. https://doi.org/10.1111/j.1574-6976.2009.00204.x

Li, G., & Young, K. D. (2013). Indole production by the tryptophanase TnaA in *Escherichia coli* is determined by the amount of exogenous tryptophan. *Microbiology*, *159*(Pt 2), 402–410. https://doi.org/10.1099/mic.0.064139-0

Maheux, A. F., Huppé, V., Boissinot, M., Picard, F. J., Bissonnette, L., Bernier, J.-L. T., & Bergeron, M. G. (2008). Analytical limits of four β-glucuronidase and β-galactosidase-based commercial culture methods used to detect *Escherichia coli* and total coliforms. *Journal of Microbiological Methods*, *75*(3), 506–514. https://doi.org/10.1016/j.mimet.2008.08.001

Martins, M. (1991). Comparison of the presence-absence (P-A) test and conventional methods for detection of bacteriological water quality indicators. *Water Research*, *25*(10), 1279–1283. https://doi.org/10.1016/0043-1354(91)90068.2

McCarthy, M., & Culloty, S. C. (2011). Optimization of two immunofluorescent antibodies for the detection of *Escherichia coli* using immunofluorescent microscopy and flow cytometry. *Current Microbiology*, *62*(2), 402–408. https://doi.org/10.1007/s00284-010-9721-3

Murphy, K. R., Butler, K. D., Spencer, R. G. M., Stedmon, C. A., Boehme, J. R., & Aiken, G. R. (2010). Measurement of dissolved organic matter fluorescence in aquatic environments: An interlaboratory comparison. *Environmental Science & Technology*, *44*(24), 9405–9412. https://doi.org/10.1021/es102362t

Nowicki, S., Lapworth, D. J., Ward, J. S. T., Thomson, P., & Charles, K. (2019). Tryptophan-like fluorescence as a measure of microbial contamination risk in groundwater. *Science of the Total Environment*, *646*, 782–791. https://doi.org/10.1016/j.scitotenv.2018.07.274

Odonkor, S. T., & Ampofo, J. K. (2013). *Escherichia coli* as an indicator of bacteriological quality of water: An overview. *Microbiology Research*, *4*(e2), 5–11.

Ohno, T. (2002). Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environmental Science & Technology*, *36*(4), 742–746. https://doi.org/10.1021/es0155276

Oliver, J. D. (2005). The viable but nonculturable state in bacteria. *Journal of Microbiology*, *43*, 93–100.

Ou, F., McGoverin, C., Swift, S., & Vanholsbeeck, F. (2017). Absolute bacterial cell enumeration using flow cytometry. *Journal of Applied Microbiology*, *123*(2), 464–477. https://doi.org/10.1111/jam.13508

Owoicho, O., Olwal, C. O., & Quaye, O. (2021). Potential of laser-induced fluorescence-light detection and ranging for future stand-off virus surveillance. *Microbial Biotechnology*, *14*(1), 126–135. https://doi.org/10.1111/1751-7915.13698

Panhwar, S., Hassan, S. S., Mahar, R. B., Carlson, K., Rajput, M. H., & Talpur, M. Y. (2019). Highly sensitive and selective electrochemical sensor for detection of *Escherichia coli* by using L-cysteine functionalized iron nanoparticles. *Journal of the Electrochemical Society*, *166*(4), B227–B235. https://doi.org/10.1149/2.0691904jes

Paul, J. H., Jeffrey, W. H., David, A. W., Deflaun, M. F., & Cazares, L. H. (1989). Turnover of extracellular DNA in eutrophic and oligotrophic freshwater environments of Southwest Florida. *Applied and Environmental Microbiology*, *55*, 1823–1828.

Prüss-Ustün, A., Wolf, J., Bartram, J., Clasen, T., Cumming, O., Freeman, M. C., Gordon, B., Hunter, P. R., Medlicott, K., & Johnston, R. (2019). Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: An updated analysis with a focus on low- and middle-income countries. *International Journal of Hygiene and Environmental Health*, *222*(5), 765–777. https://doi.org/10.1016/j.ijheh.2019.05.004

Rainbow, J., Sedlackova, E., Jiang, S., Maxted, G., Moschou, D., Richtera, L., & Estrela, P. (2020). Integrated electrochemical biosensors for detection of waterborne pathogens in low-resource settings. *Biosensors*, *10*(4), 36. https://doi.org/10.3390/bios10040036

Razmi, N., Hasanzadeh, M., Willander, M., & Nur, O. (2020). Recent progress on the electrochemical biosensing of *Escherichia coli* O157:H7: Material and methods overview. *Biosensors-Basel*, *10*(5), 18. https://doi.org/10.3390/bios10050054

Reynolds, D. (2014). The principles of fluorescence. In P. G. Coble, J. Lead, A. Baker, D. M. Reynolds, & R. Spencer (Eds.), *Aquatic organic matter fluorescence* (pp. 3–34). Cambridge University Press.

Reynolds, D. M. (2003). Rapid and direct determination of tryptophan in water using synchronous fluorescence spectroscopy. *Water Research*, *37*(13), 3055–3060. https://doi.org/10.1016/s0043-1354(03)00153-2

Rice, E. W., Geldreich, E. E., & Read, E. J. (1989). The presence-absence coliform test for monitoring drinking water quality. *Public Health Reports*, *104*(1), 54–58.

Safford, H. R., & Bischel, H. N. (2019). Flow cytometry applications in water treatment, distribution, and reuse: A review. *Water Research*, *151*, 110–133. https://doi.org/10.1016/j.watres.2018.12.016

Sgroi, M., Gagliano, E., Vagliasindi, F. G. A., & Roccaro, P. (2020). Inner filter effect, suspended solids and nitrite/nitrate interferences in fluorescence measurements of wastewater organic matter. *Science of the Total Environment*, *711*, 14. https://doi.org/10.1016/j.scitotenv.2019.134663

Sherchan, S., Miles, S., Ikner, L., Yu, H.-W., Snyder, S. A., & Pepper, I. L. (2018). Near Real-Time Detection of E. coli in Reclaimed Water. *Sensors*, *18*(7), 2303.

Simoes, J., & Dong, T. (2018). Continuous and real-time detection of drinking-water pathogens with a low-cost fluorescent optofluidic sensor. *Sensors*, *18*(7), 14. https://doi.org/10.3390/s18072210

Simoes, J., Yang, Z. C., & Dong, T. (2021). An ultrasensitive fluorimetric sensor for pre-screening of water microbial contamination risk. *Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy*, *258*, 119805. https://doi.org/10.1016/j.saa.2021.119805

Sorensen, J. P. R., Baker, A., Cumberland, S. A., Lapworth, D. J., MacDonald, A. M., Pedley, S., Taylor, R. G., & Ward, J. S. T. (2018). Real-time detection of faecally contaminated drinking water with tryptophan-like fluorescence: Defining threshold values. *Science of the Total Environment*, *622*, 1250–1257. https://doi.org/10.1016/j.scitotenv.2017.11.162

Sorensen, J. P. R., Carr, A. F., Nayebare, J., Diongue, D. M. L., Pouye, A., Roffo, R., Gwengweya, G., Ward, J. S. T., Kanoti, J., Okotto-Okotto, J., van der Marel, L., Ciric, L., Faye, S. C., Gaye, C. B., Goodall, T., Kulabako, R., Lapworth, D. J., MacDonald, A. M., Monjerezi, M., ... Taylor, R. G. (2020). Tryptophan-like and humic-like fluorophores are extracellular in groundwater: Implications as real-time faecal indicators. *Scientific Reports*, *10*(1), 9. https://doi.org/10.1038/s41598-020-72258-2

Sorensen, J. P. R., Diaw, M. T., Pouye, A., Roffo, R., Diongue, D. M. L., Faye, S. C., Gaye, C. B., Fox, B. G., Goodall, T., Lapworth, D. J., MacDonald, A. M., Read, D. S., Ciric, L., & Taylor, R. G. (2020). In-situ fluorescence spectroscopy indicates total bacterial abundance and dissolved organic carbon. *Science of the Total Environment*, *738*, 10. https://doi.org/10.1016/j.scitotenv.2020.139419.

Sorensen, J. P. R., Lapworth, D. J., Marchant, B. P., Nkhuwa, D. C. W., Pedley, S., Stuart, M. E., Bell, R. A., Chirwa, M., Kabika, J., Liemisa, M., & Chibesa, M. (2015). In-situ tryptophan-like fluorescence: A real-time indicator of faecal contamination in drinking water supplies. *Water Research*, *81*, 38–46. https://doi.org/10.1016/j.watres.2015.05.035

Sorensen, J. P. R., Vivanco, A., Ascott, M. J., Gooddy, D. C., Lapworth, D. J., Read, D. S., Rushworth, C. M., Bucknall, J., Herbert, K., Karapanos, I., Gumm, L. P., & Taylor, R. G. (2018). Online fluorescence spectroscopy for the real-time evaluation of the microbial quality of drinking water. *Water Research*, *137*, 301–309. https://doi.org/10.1016/j.watres.2018.03.001

Stults, J. R., Snoeyenbos-West, O., Methe, B., Lovley, D. R., & Chandler, D. P. (2001). Application of the 5′ fluorogenic exonuclease assay (TaqMan) for quantitative ribosomal DNA and rRNA analysis in sediments. *Applied and Environmental Microbiology*, *67*(6), 2781–2789. https://doi.org/10.1128/AEM.67.6.2781-2789.2001

Suzuki, Y., Hashimoto, R., Xie, H., Nishimura, E., Nishiyama, M., Nukazawa, K., & Ishii, S. (2019). Growth and antibiotic resistance acquisition of Escherichia coli in a river that receives treated sewage effluent. *Science of the Total Environment*, *690*, 696–704. https://doi.org/10.1016/j.scitotenv.2019.07.050

Teixeira, P., Salvador, D., Brandão, J., Ahmed, W., Sadowsky, M. J., & Valério, E. (2020). Environmental and adaptive changes necessitate a paradigm shift for indicators of fecal contamination. *Microbiology Spectrum*, *8*(2), 8.2.1. https://doi.org/10.1128/microbiolspec.ERV-0001-2019

Thakur, B., Zhou, G., Chang, J., Pu, H., Jin, B., Sui, X., Yuan, X., Yang, C.-H., Magruder, M., & Chen, J. (2018). Rapid detection of single E. coli bacteria using a graphene-based field-effect transistor device. *Biosensors and Bioelectronics*, *110*, 16–22. https://doi.org/10.1016/j.bios.2018.03.014

Thulsiraj, V., Zimmer-Faust, A. G., & Jay, J. A. (2017). Use of viability-based methods for improved detection of recent fecal contamination in a microbial source tracking study near Tijuana, Mexico. *Water, Air, & Soil Pollution*, *228*(2), 17. https://doi.org/10.1007/s11270-016-3204-5

Tiwari, A., Niemela, S. I., Vepsalainen, A., Rapala, J., Kalso, S., & Pitkanen, T. (2016). Comparison of Colilert-18 with miniaturised most probable number method for monitoring of Escherichia coli in bathing water. *Journal of Water and Health*, *14*(1), 121–131. https://doi.org/10.2166/wh.2015.071

Tryland, I., & Fiksdal, L. (1998). Enzyme characteristics of beta-D-galactosidase- and beta-D-glucuronidase-positive bacteria and their interference in rapid methods for detection of waterborne coliforms and *Escherichia coli*. *Applied and Environmental Microbiology*, *64*(3), 1018–1023. https://doi.org/10.1128/AEM.64.3.1018-1023.1998

UN Inter-agency Group for Child Mortality. (2020). *Levels & Trends in child mortality: Report 2020*. UNICEF. https://www.unicef.org/media/79371/file/UN-IGME-child-mortality-report-2020.pdf.pdf

UNICEF. (2016). UNICEF target product profile rapid E. coli detection tests. Version 3.0. 16 pp. https://www.unicef.org/supply/media/2511/file/Rapid-coli-detection-TPP-2019.pdf

Valuer, B. (2001). *Molecular fluorescence, principles and applications* (pp. 3–19). Federal Republic of Germany: Wiley-VCH Verlag GmbH.

Van Nevel, S., Koetzsch, S., Proctor, C. R., Besmer, M. D., Prest, E. I., Vrouwenvelder, J. S., Knezev, A., Boon, N., & Hammes, F. (2017). Flow cytometric bacterial cell counts challenge conventional heterotrophic plate counts for routine microbiological drinking water monitoring. *Water Research*, *113*, 191–206. https://doi.org/10.1016/j.watres.2017.01.065

Velasquez-Orta, S. B., Werner, D., Varia, J. C., & Mgana, S. (2017). Microbial fuel cells for inexpensive continuous in-situ monitoring of groundwater quality. *Water Research*, *117*, 9–17. https://doi.org/10.1016/j.watres.2017.03.040

Vital, M., Hammes, F., & Egli, T. (2012). Competition of *Escherichia coli* O157 with a drinking water bacterial community at low nutrient concentrations. *Water Research*, *46*(19), 6279–6290. https://doi.org/10.1016/j.watres.2012.08.043

Walker, D. I., McQuillan, J., Taiwo, M., Parks, R., Stenton, C. A., Morgan, H., Mowlem, M. C., & Lees, D. N. (2017). A highly specific *Escherichia coli* qPCR and its comparison with existing methods for environmental waters. *Water Research*, *126*, 101–110. https://doi.org/10.1016/j.watres.2017.08.032

Wang, D., Ding, X., & Rather, P. N. (2001). Indole can act as an extracellular signal in *Escherichia coli*. *Journal of Bacteriology*, *183*(14), 4210–4216. https://doi.org/10.1128/JB.183.14.4210-4216.2001

Wang, M., Ateia, M., Hatano, Y., Miyanaga, K., & Yoshimura, C. (2022). Novel fluorescence-based method for rapid quantification of live bacteria in river water and treated wastewater. *Environmental Science: Advances.*, *1*, 30–36. https://doi.org/10.1039/d1va00017a

Ward, J. S. T., Lapworth, D. J., Read, D. S., Pedley, S., Banda, S. T., Monjerezi, M., Gwengweya, G., & MacDonald, A. M. (2020). Large-scale survey of seasonal drinking water quality in Malawi using in situ tryptophan-like fluorescence and conventional water quality indicators. *Science of the Total Environment*, *744*, 12. https://doi.org/10.1016/j.scitotenv.2020.140674

Ward, J. S. T., Lapworth, D. J., Read, D. S., Pedley, S., Banda, S. T., Monjerezi, M., Gwengweya, G., & MacDonald, A. M. (2021). Tryptophan-like fluorescence as a high-level screening tool for detecting microbial contamination in drinking water. *Science of the Total Environment*, *750*, 12. https://doi.org/10.1016/j.scitotenv.2020.141284

Watras, C. J., Hanson, P. C., Stacy, T. L., Morrison, K. M., Mather, J., Hu, Y. H., & Milewski, P. (2011). A temperature compensation method for CDOM fluorescence sensors in freshwater. *Limnology and Oceanography: Methods*, *9*(7), 296–301. https://doi.org/10.4319/lom.2011.9.296

Wells, M. J. M., Mullins, G. A., Bell, K. Y., Da Silva, A. K., & Navarrete, E. M. (2017). Fluorescence and quenching assessment (EEM-PARAFAC) of de facto potable reuse in the Neuse River, North Carolina, United States. *Environmental Science & Technology*, *51*(23), 13592–13602. https://doi.org/10.1021/acs.est.7b03766

WHO & UNICEF. (2021). *Progress on household drinking water, sanitation and hygiene 2000–2020: Five years into the SDGs*. World Health Organisation (WHO) and the United Nation's Children's Fund (UNICEF).

Wildeboer, D., Amirat, L., Price, R. G., & Abuknesha, R. A. (2010). Rapid detection of Escherichia coli in water using a hand-held fluorescence detector, *Water Research*, *44*(8), 2621–2628. https://doi.org/10.1016/j.watres.2010.01.020

Yu, M., Wu, L., Huang, T., Wang, S., & Yan, X. (2015). Rapid detection and enumeration of total bacteria in drinking water and tea beverages using a laboratory-built high-sensitivity flow cytometer. *Analytical Methods*, *7*(7), 3072–3079. https://doi.org/10.1039/c4ay02919d

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