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COMMUNICATION

Demonstration of an optical biosensor for the detection of faecal indicator bacteria in freshwater and coastal bathing areas

Ciprian Briciu-Burghina[1] · Brendan Heery[1,2] · Gillian Duffy[1] · Dermot Brabazon[2] · Fiona Regan[1]

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Abstract

ColiSense, an early warning system developed for *Escherichia coli* detection, is assessed using environmental samples. The system relies on the detection of β-glucuronidase (GUS), a biomarker enzyme for *E. coli*. In contrast with other rapid GUS-based methods, ColiSense is the only method that uses 6-chloro-4-methyl-umbelliferyl-β-D-glucuronide (6-CMUG) as a fluorogenic substrate. The system measures a direct kinetic response of extracted GUS, and the detection was carried out in the absence of particles or bacteria. It is necessary to evaluate the system with environmental samples to establish the relationship between faecal indicator bacteria *E. coli* and the response measured by the ColiSense. This paper presents the results of tests carried out with the ColiSense system for 2 trials, one conducted with freshwater samples collected from rivers in the Dublin area and a second conducted with seawater samples from coastal areas collected over the bathing season. A positive linear correlation was found between *E. coli* (MPN 100 mL^{-1}) and ColiSense response ($R^2 = 0.85$, $N = 125$, $p < 0.01$) for the seawater sample. A ColiSense response threshold was identified as 0–1.8 pmol min^{-1} 100 mL^{-1}, equivalent to 0–500 *E. coli* 100 mL^{-1}. Using this threshold, 96.8% of the samples were correctly classified as being above or below 500 *E. coli* 100 mL^{-1} by the ColiSense system. Results presented demonstrate that the ColiSense system can be used as an early warning tool with potential for active management of bathing areas by providing results in 75 min from sample collection.

Keywords Faecal pollution · *E. coli* · β-Glucuronidase · Water · Optical sensor

Introduction

The 2006 European Directive for the management of bathing water quality brings a legal framework to the management of bathing areas and increases the responsibility for monitoring and remediation of such sites [1]. The problem is that there is a 24–72-h delay between sample collection, identifying the presence of high levels of faecal contamination and alerting the users not to bathe at that location. New methods and devices are urgently needed for rapid quantification of faecal indicator bacteria (FIB) [2–4].

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✉ Fiona Regan
fiona.regan@dcu.ie

[1] DCU Water Institute, School of Chemical Sciences, Dublin City University, Glasnevin Campus, Dublin 9, Ireland

[2] School of Mechanical and Manufacturing Engineering, Dublin City University, Dublin 9, Ireland

Enzyme-based methods have shown the greatest potential for recreational water monitoring, mainly due to simplicity in use and equipment required, cost-effectiveness, and short time-to-result [2, 4]. Such methods rely on biomarker enzymes [5] and β-glucuronidase (GUS) is widely accepted as a biomarker for *Escherichia coli* detection [6], with 92.4% of *E. coli* strains found to be GUS positive [7]. Methods relying on GUS can be broadly classified in culture-based methods (selective growth and GUS inducers [8, 9]) and rapid methods which do not use a culturing step, but rather rely on permeabilisation of cellular wall. The rapidity of these methods comes at a cost in specificity. Known interferences are the presence of viable but not culturable *E. coli* cells (VBNC) [10], GUS-positive non-target bacteria [3, 11], or the presence of GUS-positive marine biomass [12]. Despite these known interferences, good correlations were found between log GUS activity and log culturable *E. coli*, with R^2 values ranging from 0.76 up to 0.92 [4, 13–15]. These results have prompted the development of commercially available methods like the Coliplage® [3] and of autonomous systems like the ColiMinder® or BACTcontrol®. The latter have

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shown good performance and correlations with culturable *E. coli*, and proved to be excellent tools for pollution event detection in near-real time [16–19]. Although the autonomous systems offer excellent temporal resolution, the spatial resolution is limited by the relatively high capital cost and site installation requirements [19].

The ColiSense system described previously was designed to be portable, user friendly, and rapid, providing results in 75 min [20]. The system distinguishes from the rapid GUS-based methods and systems as it relies on cell lysis and GUS recovery via elution in detection buffer. In terms of enzyme kinetics, the signal is recorded as a function of extracted GUS, in the absence of *E. coli* and other bacteria and particles—although it is possible that cell debris is present [21]. Other rapid GUS-based methods like the Coliplage® or the autonomous systems rely on substrate permeabilisation or uptake, and GUS detection is carried out in the presence of *E. coli* and other particles present. ColiSense relies on the use of 6-chloro-4-methyl-umbelliferyl-β-D-glucuronide (6-CMUG) as substrate which although has a slightly lower K_{cat} value than 4-methyl-umbelliferyl-β-D-glucuronide (4-MUG) has the benefit of generating higher fluorescent signals at physiological pHs [22].

The objective of this study was to demonstrate the performance of the ColiSense system with real environmental samples (not spiked) and to establish the relationship between the ColiSense response and *E. coli* numbers.

Materials and methods

Materials

Sodium phosphate monobasic and dibasic and 1,4-dithiothreitol (DTT) were all purchased from Sigma-Aldrich, Ireland. The fluorogenic substrate 6-chloro-4-methyl-umbelliferyl-β-D-glucuronide (6-CMUG) (97%) was ordered from Glycosynth Limited (UK); 6-chloro-4-methyl-umbelliferone (6-CMU) (97%) was ordered from Carbosynth Limited (UK). Water was passed through a Milli-Q water purification system (18 MΩ). Stock solutions of fluorophores and substrates (100 mM) were prepared in 1 mL DMSO (99.5%) and kept at 4 °C. Further dilutions were prepared daily in buffer or deionised water with a final DMSO concentration in the working solution, ranging from 0.01 to 1%. Bacterial PE LB™ (PELB) with PE LB™ lysozyme was ordered from VWR. Corning® syringe filters with cellulose acetate surfactant-free membrane (SFCA) with pore sizes of 0.45 μm were ordered from Sigma-Aldrich, Ireland. Syringe filter caps and Combi-Cap male/female Luer were ordered from VWR, Ireland. The Colilert-18®/Quanti-Tray 2000® system (IDEXX Laboratories) was used for the enumeration of coliforms and *Escherichia coli*, and the Enterolert®/Quanti-Tray 2000® system (IDEXX Laboratories) was used for the enumeration of enterococci (Techno-Path, Ireland). Positive controls of *E. coli* ATCC 11775 and *Enterococcus faecalis* ATCC 19433 were ordered from Techno-Path, Ireland.

Methods

Sample collection

Fresh water samples were collected over a 5-day period from five rivers in the Dublin area (Liffey, Tolka, Camac, Poddle, and Dodder); a more in-depth description is provided in [23]. Coastal water samples were collected during the 2016 Water Bathing Season from 11 designated bathing areas and 2 non-designated bathing areas known to experience faecal pollution events.

Microbial analysis of water samples

Freshwater samples Water samples were collected in sterile 1-L high-density polypropylene (HDPP) bottles. Samples were transported to the lab on ice within 4 h and inoculated within 8 h after collection. The Colilert-18®/Quanti-Tray 2000® system (IDEXX Laboratories) was used for the enumeration of coliforms and *Escherichia coli*.

Seawater samples Water samples were collected at each site by Fingal City Council personnel. For every official bathing water sample taken by environmental health officers during the 2016 season, a second, duplicate sample was taken simultaneously at the same location. This was to ensure that both samples would have similar levels of *E. coli*. One sample (1 L) was delivered to DCU for analysis using the ColiSense system, while the other sample (100 mL) was delivered to the Central Laboratory for microbiological analysis using the Colilert® method.

GUS activity measurement using the ColiSense system

The ColiSense system comprises a sample preparation protocol and the ColiSense detector (Fig. 1). A sample preparation protocol described elsewhere was used for *E. coli* recovery and GUS extraction from environmental samples [21]. For the detection of GUS activity in a continuous setup, a miniaturised fluorimeter (ColiSense) was designed and built *in-house* [20]. The specifications of the system were tailored to target the requirements of the GUS-based method described previously [22]. For both trials, samples were analysed on the system within 24 h from sample collection in the laboratory.

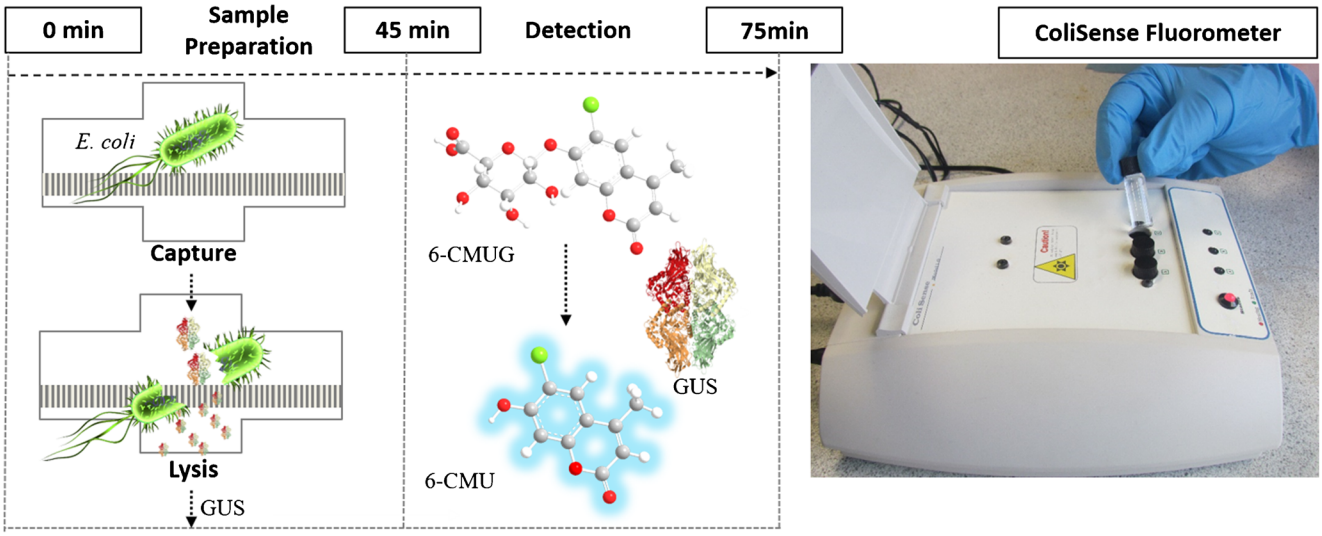


Fig. 1 Overview of the ColiSense system outlining the main sample preparation and detection steps [20, 21]

Results and discussion

Relationship between GUS activity and *E. coli*

Methods based on rapid GUS assay are not limited by the detection system, in this case the ColiSense, but mainly by the *E. coli* populations present in the sample and by interferences—GUS-positive organisms of non-faecal origin [2, 6]. The ColiSense method shows an almost perfect linear response (in terms of R^2) when dilutions come from the same *E. coli* population (Fig. 2a). However, deviations from linearity are observed when samples are collected from different water bodies due to a myriad of factors, including pollution source (agricultural runoff, sewer overflow), *E. coli* strain population and abundance, and the age of the pollution event (Fig. 2b–d).

Results collected from environmental water samples show a significant linear correlation between *E. coli* and GUS activity in log units ($R^2 = 0.53$, $N = 45$, $p < 0.001$, fresh water trial) (Fig. 2b), and a significant linear correlation between *E. coli* and ColiSense response (GUS activity) ($R^2 = 0.85$, $N = 125$, $p < 0.01$, seawater trial) (Fig. 2c, d). Similar relationships were found previously between *E. coli* and GUS activities and are summarised in Table 1. The slope reported for this study in the freshwater trial is found to be in the same range as the slopes reported in the literature for river and wastewater samples, although better R^2 values were obtained in the literature. Samples analysed for the freshwater trial contained a selection of five rivers with both freshwater and brackish water (3 sampling points—River Liffey). Samples were collected during both heavy rainfall and sunny conditions but also at various locations on the river course. This accounts for different faecal pollution sources (continuous or intermittent inputs) and various *E. coli* populations. Given this complex contamination pattern, a weaker correlation between *E. coli* and GUS activities is expected.

For the seawater trial, a relationship between ColiSense response and culturable *E. coli* numbers was observed ($R^2 = 0.85$, $N = 125$, $p < 0.01$) with a slope 0.0011 pmol $min^{-1} E. coli^{-1}$. In log-transformed data plots, the slope of the relationship was 0.19 (Table 1).

A lower slope suggests the GUS activity is higher/culturable cell when *E. coli* number in the sample is lower. As the data set collected contains a high number of samples within 0–500 *E. coli* ($N = 108$ out of $N = 125$), the relationship is mostly driven by the ColiSense response recorded for this range. Further expansion of the data set to include more contaminated samples should increase the slope but also the R^2 of the relationship. Coastal area samples contain mixtures of *E. coli* populations originating from sources within the catchment while samples collected from tributaries contain specific pollution source-driven populations. This suggests coastal waters contain a more homogenous mixture of *E. coli* populations and that measured GUS activities are not as strongly impacted by the pollution source.

Application of the ColiSense for *E. coli* monitoring

E. coli prediction

For GUS activity to be used as a reliable *E. coli* surrogate, a background model which can accurately predict *E. coli* levels is required. The current seawater trial was designed to investigate this relationship and to determine such a model for bathing sites in Dublin area. A ColiSense response threshold was identified as 0–1.8 pmol min^{-1} 100 mL^{-1} for the 0–500 *E. coli* 100 mL^{-1}. The current data set offers a reliable prediction for the 0–500 *E. coli* thresholds. Using this threshold, 96.8% out of the 125

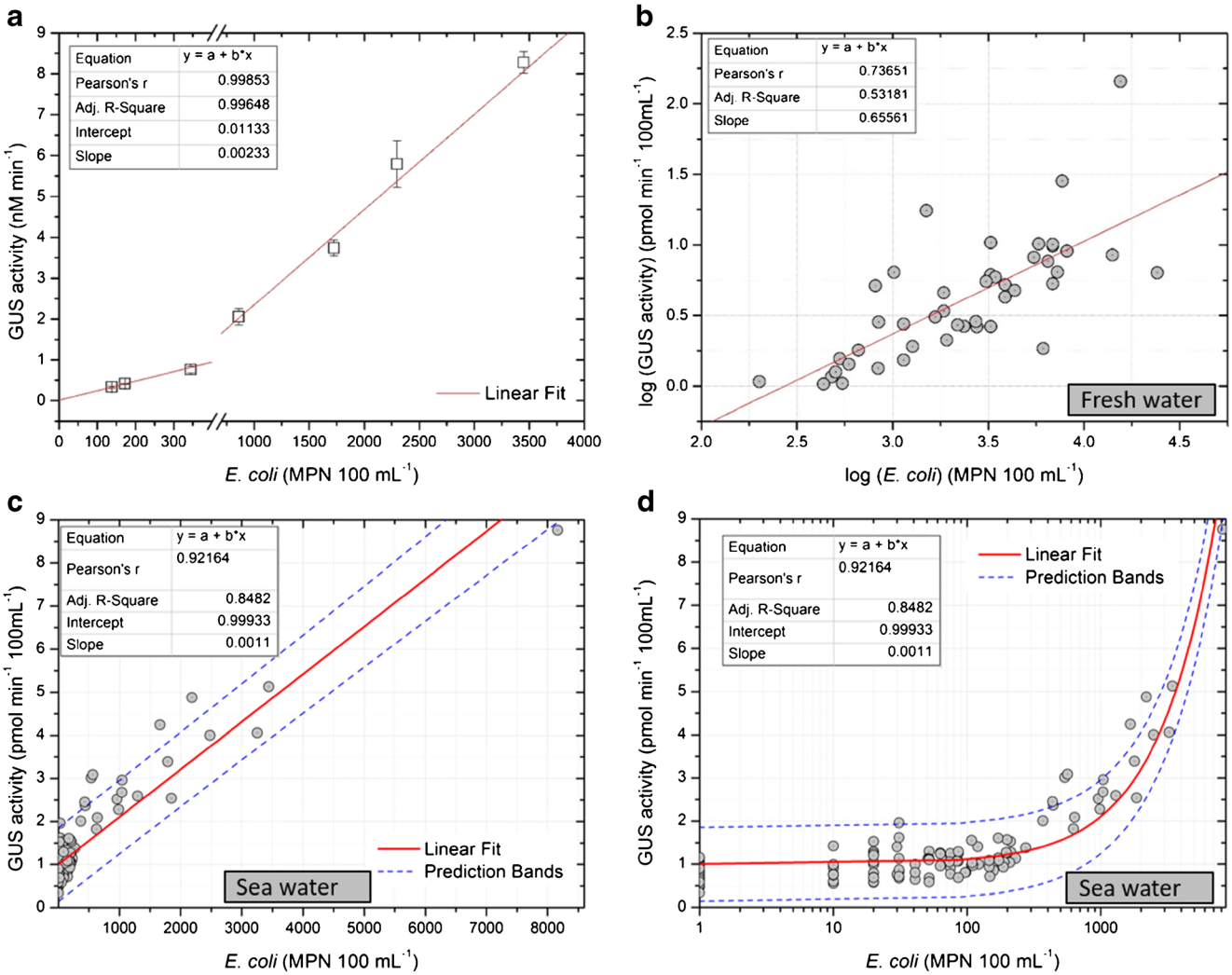


Fig. 2 Relationship between ColiSense response as GUS activity and culturable *E. coli* counts. GUS activities recorded from dilutions of 1 freshwater sample in **a**, GUS activities vs *E. coli* in freshwater samples ($N = 45$) in **b**, and GUS activities vs *E. coli* in seawater samples ($N = 125$) in **c** and **d**. In **d**, the *X*-axis is set in logarithmic scale to highlight the lower range of the data. Axis break inserted between 400 and 700 in **a**. Insets show R^2 and slope values (pmol min^{-1} *E. coli*$^{-1}$). Experimental details are described in "Methods." Error bars representing the standard deviation of three measurements were removed in **b**, **c**, and **d** for clarity. Prediction bands represent 95% confidence intervals in **c** and **d**

samples analysed were correctly classified as being above or below 500 *E. coli* 100 mL^{-1} by the ColiSense. However, 4 of the 125 samples (3.2%) were wrongly classified as being above 500 *E. coli* (Fig. 3). These 4 samples had 31, 368, 441, and 435 *E. coli* 100 mL^{-1}. It is possible that the reason for this misclassification is a high population of VBNC bacteria (which are detected by the ColiSense but missed by the standard method), the presence of GUS-positive non-target bacteria [3, 11], or/and the presence of GUS-positive marine biomass [12]. A previous study has reported that in environmental samples containing between 10^2 and 10^3 *E. coli* 100 mL^{-1}, the ratio of VBNC to VC cells can be as high as 100:1 [10]. This ratio was found to decrease to 10:1 in the 10^5–10^6 range.

Data collected in this trial suggests that the performance of the ColiSense is limited by the presence of VBNC bacteria, similar to other rapid GUS-based systems [18, 19]. The effect is stronger in samples containing less 500 culturable *E. coli* 100 mL^{-1} which present a higher variability of VBNC populations. This variability generates a high signal noise which in turn limits the resolution and capabilities of the system in the lower range to ≥ or ≤ 500 *E. coli* 100 mL^{-1}. While this limits the use for drinking water quality, the system is well suited for monitoring bathing water quality. Also, while false positive results were noted in 4 samples, none of the studies identified a false negative for the 500 *E. coli* thresholds. The 0% false negative confirms that the sensor shows promise for risk assessment and is very important in screening/alerting systems, where such performance is critical. The system can successfully predict if culturable *E. coli* are ≥ or ≤ 500 and with an extended data set to cover the higher range, it is estimated the system can differentiate between the other action thresholds presented in Table 2.

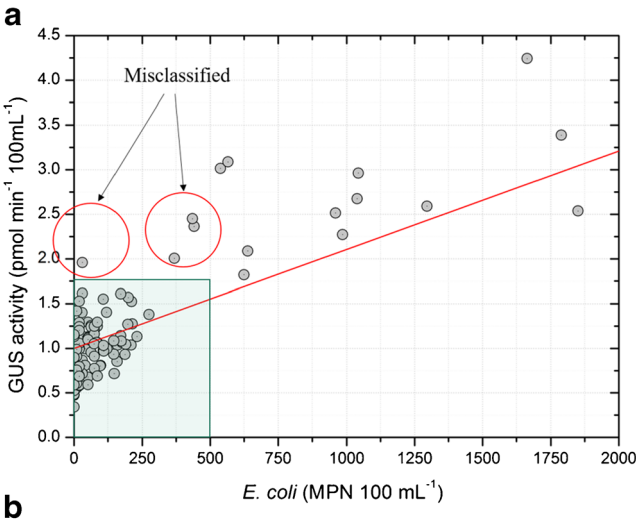
Table 1 Slope of the regression straight lines between log-transformed GUS activity and log-transformed concentrations of *E. coli* and faecal coliforms (FC) published in the literature. The culture-based methods used for the enumeration of culturable *E. coli* and FC are also mentioned

Organism detected	Methods	Type of water	Sample size (*N*)	R^2	Slope	References
FC	MacConkey agar	Estuarine and wastewater	24	0.88	0.68	[24]
FC	Tergitol TCC agar	River	163	0.83	0.61	[15]
FC	Tergitol TCC agar	River	132	0.56	0.55	[25]
FC	m-FC-agar	Seawater	34	0.34	0.31	[13]
FC	m-FC-agar	River	98	0.90	0.83	[14]
FC	Tergitol TCC agar	River and wastewater	152	0.72	0.48	[26]
E. coli	m-FC-agar	River	98	0.83	0.82	[27]
E. coli	Chromocult coliform agar	River	98	0.87	0.82	[14]
E. coli	MPN microplates	River and wastewater	41	0.92	0.73	[10]
E. coli	MPN microplates	Seawater	256	0.81	0.44	[28]
E. coli	MPN microplates	River and wastewater	166	0.76	0.52	[26]
E. coli	MPN Colilert-18®	River	45	0.53	0.66	This study
E. coli	MPN Colilert-18®	Seawater	125	0.57	0.19	This study
E. coli	MPN Colilert-18®	Seawater*	125*	0.85*	0.001*	This study

*Values reported for normal plots, not log-transformed data

Implications for the use of the ColiSense system

Given the urgent requirement for rapid testing of microbiological water quality, the ColiSense system shows real potential for early warning. Rapid monitoring of bathing water quality using GUS-based systems can potentially lead to a disruptive technological advance. Systems that can provide results within short time frames allow for reactive measures to be taken within the same day to protect human health. Field portable kits, or tests, capable of processing grab samples concomitantly represent an attractive resource for bathing water management. Data presented herein shows that the ColiSense can successfully respond to pollution events, and it can predict if *E. coli* are present at higher than 500 bacteria 100 mL^{-1} in coastal bathing water samples. The main limitation of the system arises from interferences associated with the presence of VBNC which are known to interfere with most autonomous



b

Correctly classified (%)	96.8 %, N=121
Misclassified as >500 (%)	3.2 % , N=4 (31, 368, 441, 435 *E. coli*)
Misclassified as <500 (%)	0 %
Total Misclassified (%)	3.2 % N=4 (31,368, 441, 435 *E. coli*)

Fig. 3 **a** Relationship between ColiSense response as GUS activity and *E. coli*. Highlighted region shows the 0–1.8 pmol min^{-1} 100 mL^{-1} threshold corresponding to 0–500 *E. coli* 100 mL^{-1} and the 4 misclassified samples. **b** Classification results for the 0–1.8 pmol min^{-1} 100 mL^{-1} threshold corresponding to 0–500 *E. coli* 100 mL^{-1}

Table 2 Action levels in response to microbiological sample results, Irish Health and Safety Executive (HSE)

E. coli	Intestinal enterococci (IE)	Recommended action
> 2000 *E. coli*	OR > 250 IE	Issue a bathing water prohibition
≥ 1000–≤ 2000 *E. coli*	AND > 200 IE	Issue a bathing water prohibition
≥ 1000–≤ 2000 *E. coli*	BUT < 200 IE	Issue an advisory notice and re-sample immediately. If re-sample is still ≥ 1000 *E. coli*, issue a bathing water prohibition
≥ 500–≤ 1000 *E. coli*	AND ≥ 100–≤ 250 IE	Monitor situation and re-sample. Decision based on evidence available/details of pollution event

Based on Irish HSE risk assessment taking into account the beach profile, previous sampling history, probable source of contamination, evidence of human illness, etc.

based systems [18, 19, 29]. Other known interferences are GUS-positive non-target bacteria [3, 11] or the presence of GUS-positive marine biomass [12]. As the ColiSense relies on a cell lysis step optimised for gram-negative bacteria, the presence of GUS-positive marine biomass should not be problematic. The goal of monitoring culturable *E. coli* counts is to detect faecal pollution events which increases the likelihood of hazardous pathogen occurrence. In this context, it can been argued whether GUS activity could be an equivalent or better predictor of exposure to microbial hazards [19] [2, 4] and if GUS activity correlates better with waterborne pathogens known to persist longer than culturable *E. coli* in water [30]. Such future work could provide validity to GUS-based methods and increase confidence in GUS-based technologies.

Conclusions

ColiSense, an early warning sensor system, was assessed for the rapid detection of FIB, *E. coli* in environmental samples. A positive linear correlation was found between *E. coli* (MPN 100 mL^{-1}) and ColiSense response ($R^2 = 0.85$, $N = 125$, $p < 0.01$) for seawater samples collected during the bathing season. Furthermore, a ColiSense response threshold was identified as 0–1.8 pmol min^{-1} 100 mL^{-1} equivalent to 0–500 *E. coli* 100 mL^{-1}.Using this threshold, 96.8% out of the 125 samples analysed were correctly classified as being above or below 500 *E. coli* 100 mL^{-1} by the ColiSense while 4 of the 125 samples (3.2%) were wrongly classified as being above 500 *E. coli*. For the freshwater trial, the system successfully detected *E. coli* in all of the 45 grab samples tested and a positive correlation was found between the log-transformed GUS activities and the log-transformed *E. coli* counts ($R^2 = 0.53$).

The data presented demonstrates that the ColiSense system shows potential as a solution for active management of coastal bathing areas. It can successfully detect pollution events containing more than 500 *E. coli* 100 mL^{-1} in 75 min and allows for reactive measures to be taken within the same day. Future work should extend the predictive capabilities of the system to the ≥ 1000 *E. coli* 100 mL^{-1} and ≥ 2000 *E. coli* 100 mL^{-1} thresholds, by building on the current data set. This would allow managers to react in a timely fashion by issuing immediate bathing prohibition notices where contamination events are found breach these thresholds.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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