Showcasing a recent innovation in water quality monitoring by Dr. Sushanta K. Mitra and his team at the Micro & Nanoscale Transport Laboratory, York University, Canada - partner in Innovation York’s spin-off Glacierclean Technologies Inc.

A hydrogel based rapid test method for detection of *Escherichia coli* (E. coli) in contaminated water samples

Mitra and his team have developed a novel *E. coli* detection tool for potable water using a porous hydrogel matrix to cage specific enzymatic substrates and two other reagents, the matrix was incorporated inside a plunger-tube assembly as an integrated platform for pre-concentration and detection. This is a significant improvement over their previous breakthrough on water quality monitoring - the Mobile Water Kit (MWK).

As featured in:

A hydrogel based rapid test method for detection of *Escherichia coli* (*E. coli*) in contaminated water samples†

Naga Siva Kumar Gunda, Ravi Chavali and Sushanta K. Mitra*

We have formulated a new chemical composition for rapid detection of *Escherichia coli* (*E. coli*) with currently available enzymatic substrates. We have evaluated the performance of the new chemical composition with different kinds of bacteria, and metallic and ionic interferences and optimized the chemical composition for rapid and specific detection of *E. coli*. We used a novel hydrogel based porous matrix to encapsulate the optimized chemical compounds and incorporated it within a readily available plunger-tube assembly. This overall system allows efficient, field deployable, rapid testing of water samples by simultaneously pre-concentrating and detecting *E. coli* within one integrated unit. We were able to detect *E. coli* concentrations of $4 \times 10^6$ CFU mL$^{-1}$ to $4 \times 10^5$ CFU mL$^{-1}$ within 5 min and $4 \times 10^4$ CFU mL$^{-1}$ to 400 CFU mL$^{-1}$ within 60 min using the integrated plunger-tube assembly containing the hydrogel matrix.

1. Introduction

Microbial contamination of water sources is a huge problem in the world and researchers have been looking at different ways for easy, rapid, specific and sensitive detection of bacteria in contaminated water samples. It is well known that traditional microbiological testing methods are time consuming, since bacteria have to be isolated, cultured and require a series of biochemical tests for identification and confirmation. In recent years, there have been several approaches that have been pursued towards the development of methods for the detection and quantification of bacteria in water. The conventional approach for the detection and enumeration of bacteria involves filtering the water samples through a membrane filter, followed by counting the number of bacteria colonies of the filtrate sample using the plate counting method.†–‡ The counted colonies can be related to the number of cells based on which the quality of water is determined. However, these methods take 24 to 48 hours to produce results, often requiring transportation of water samples to a central laboratory and trained personnel to perform the tests. Alternatively, several rapid detection methods have also been developed using advanced techniques such as quantum dots,§ magnetic beads,∥ flow cytometry,¶ polymerase chain reaction (PCR),†⁰–†² DNA microarrays,†³–†⁶ enzyme linked immunosorbent assays (ELISA),†⁷ Fluorescent *In situ* Hybridization (FISH),†⁸ surface-enhanced Raman scattering (SERS),†⁹,‡⁰ electrochemical,‡¹,‡² impedance,‡³,‡⁴ microfluidics,‡⁵ lateral flow assays,‡⁶ personal glucose meters,‡⁷ microwells,‡⁸ and paper microfluidics.‡⁹,‡¹⁰ However, these methods require the use of sophisticated equipment to perform the tests thereby necessitating the establishment of well-equipped laboratories, which may not be feasible in poorly resourced communities. Hence, extensive testing of water quality on a regular basis would require simple, rapid and field deployable tests that can be performed at the point of use by any unskilled individual.

One of the most common and best approaches to assess the extent of microbial pollution in water is to estimate the density of certain indicator bacteria (*e.g.* coliforms, fecal coliforms and *E. coli*) in the water samples to be tested.†¹–†³ The presence of *E. coli* bacteria in water samples is an indicator of the presence of water-borne pathogens and precautionary measures are to be taken in these cases to avoid any outbreaks. The focus of the present work is the development of a low-cost, field deployable and rapid method for the detection of *E. coli* in potable water, which is particularly relevant for limited resource communities and developing economies. One of the most promising approaches to achieve the same, is to identify certain biomarkers (*e.g.*, enzymes) secreted by bacteria using a specific chromogenic or fluorogenic enzymatic substrate.‡⁴–‡⁸ The resulting color/fluorescence intensity from the enzyme-substrate interaction can be correlated to the bacterial concentration in the water sample. The detection of enzymes using a defined substrate technique is well known, robust and cost effective.
effective. The most commonly used marker enzymes for *E. coli* detection are β-D-galactosidase (GAL) and β-D-glucuronidase (GUD).

There are several chromogenic and fluorogenic substrates available in the literature for the detection of these enzymes. Chromogenic substrates are complex chemical compounds that react with enzymes (GAL or GUD) and produce colored molecules. For example, a chromogenic compound Red-Gal (6-chloro-3-indolyl-β-D-galactopyranoside) contains two components: 6-chloro-3-indolyl and β-D-galactopyranoside. The GAL enzyme produced by bacteria hydrolyses this complex Red-Gal molecule resulting in the release of the red color producing dimerized 6-chloro-3-indolyl compound. Fluorogenic substrates are also complex chemical compounds that react with enzymes (GAL or GUD) and produce fluorescent molecules. For example, a fluorogenic substrate MUG (4-methylumbelliferyl-β-D-glucuronide, trihydrate) contains two components: 4-methylumbelliferyl (4-MU) and β-D-glucuronic acid. The GUD enzyme produced by bacteria hydrolyses this complex MUG molecule resulting in the release of the fluorogenic compound 4-MU.

There are several examples of such enzyme based detection techniques available in the literature. Though these enzyme-based techniques are quite robust, complexities arise in simplifying these test methods for field use as well as for rapid detection.

A rapid method with detection time less than one hour can be achieved by adding certain inducers and promoters to the above mentioned enzymatic substrates. The detection time can be reduced significantly by rapid extraction of enzymes (GAL and/or GUD) from *E. coli*. This can be achieved by lysing the cells using thermal, electrical, mechanical, acoustic and chemical methods. Chemicals like Triton X-100, chloroform, sodium dodecyl sulfate (SDS) and bacteria protein extraction reagent (B-PER) can be used for lysing the cells. B-PER is the most prominent one used for extracting enzymes from bacteria. In the present work, we formulated a chemical composition with a combination of enzymatic substrate, growth medium and cell lysing agent that can detect *E. coli* in contaminated potable water samples within one hour. A comprehensive study has been conducted to evaluate the performance of this new composition with different enzymatic substrates for detecting *E. coli* bacteria in the presence of several non-*E. coli* bacteria, metals and ions. Later, we impregnated the optimized chemical composition inside a hydrogel matrix and created a novel method of detecting *E. coli* using a very simplified plunger-tube assembly. This plunger-tube assembly allows the simultaneous pre-concentration and detection of bacteria from contaminated water samples.

Gunda *et al.* have developed a low-cost and extremely efficient testing method known as the Mobile Water Kit (MWK) that has greatly simplified the process of *E. coli* detection in the field, which was not feasible until recently. The MWK can accurately detect extremely low concentrations of *E. coli* (2 CFU per 100 mL) under an hour and is faster than the current conventional laboratory methods, which require 24 to 48 hours to produce proper results. However, the MWK may require a trained technician to perform the test as there are a large number of steps involved, including the collection of a water sample to the broadcasting of test results. Moreover, the MWK contains the chemical reagents in liquid form to be stored and handled in a careful and manageable way.

The plunger-tube assembly developed in this work is much simpler that it will eventually replace syringes, filters, centrifuge tubes, pipette tips, etc., which were essential components for the MWK. Also, this new method has eliminated the use of additional reagents (i.e., FeCl₃ and MUG used in MWK). The plunger-tube assembly would allow sample concentration and detection in an integrated fashion. This plunger-tube assembly simplifies the testing method and make it amenable for regular field use without the help of a trained technician. We have filed a patent for the design of the plunger-tube assembly to concentrate any bacteria (like *E. coli*) for efficient detection. Details on this plunger-tube assembly technology can be found in recent patent applications filed by the research group.

2. Materials and methods

2.1. Materials

Molecular biology grade agarose hydrogel and Tris/borate/EDTA (TBE) buffer were obtained from Biorad, Canada. Bacteria protein extraction reagent (B-PER), Difco Lauryl Tryptose Broth (LTB) (BD 224150), Difco Nutrient Broth (BD 234000), BBL Brain Heart Infusion Broth (BD 237500), Difco Veal Infusion Broth (BD 234420) and Bacto Yeast Extract (BD 212750) were obtained from Fisher Scientific, Canada. 6-Chloro-3-indolyl-β-D-galactopyranoside (also called Rose Gal, Salmon Gal or Red-Gal), 2-nitrophenyl-β-D-galactopyranoside (ONPG), 4-methylumbelliferyl-β-D-glucuronide trihydrate (4-MUG), 5-bromo-6-chloro-3-indolyl-β-D-glucopyranoside (Magenta Gluc), 4-methylumbelliferyl-β-D-glucopyranoside (MUGal), 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal), 4-methylumbelliferyl-β-D-glucuronide, dehydrate (MUG), and 5-bromo-6-chloro-3-indolyl-β-D-galactopyranoside (Magenta Gal) enzymatic substrates were procured from Sigma Aldrich, Canada. Plunger-tube assemblies of 5 mL volume with a built-in filter (0.45 μm pore size) were procured from VWR, Canada. Nunc-Immuno MicroWell 96-well solid plates were obtained from Sigma Aldrich, Canada.

*E. coli* K-12 strains were obtained from New England Biolabs, Ipswich, Massachusetts, USA. *E. coli* Castellani and Chalmers (American Type Culture Collection (ATCC) 11229), Enterococcus faecalis (*E. faecalis*) (Andrewes and Horder) Schleifer and Kilpper-Balz (ATCC 19433), Salmonella enterica subsp. enterica (*S. enterica*) (ex Kauffmann and Edwards) Le Minor and Popoff serovar Typhimurium (ATCC 14028) and Bacillus subtilis (*B. subtilis*) (Ehrenberg) Cohn (ATCC 33712, MI112 strain) were purchased from Cedarlane, Burlington, ON, Canada. N,N-Dimethylformamide (DMF) and anhydrous ferric chloride (FeCl₃) were obtained from Sigma-Aldrich, Canada. Deionized (DI) water was used to prepare most of the solu-
tions. Materials were sterilized whenever needed in an autoclave.

E. coli K-12 and E. coli ATCC 11229 strains were cultured in LTB medium at 37 °C in a microbiological incubator (Lab Companion SI-300 Benchtop Incubator and Shaker, GMI, Ramsey, Minnesota, USA) for 24 hours. Similarly, S. enterica and E. faecalis were grown in a Nutrient Broth and Brain Heart Infusion Broth, respectively. B. subtilis was grown in a medium consisting of Veal Infusion Broth and Yeast Extract in 5:1 ratio at 30 °C in an incubator for 24 h. Unless otherwise stated, serial dilutions were made in DI water to produce bacteria concentrations in the range of 400–4 × 10⁶ CFU mL⁻¹. These known bacteria concentration water samples were used to check the performance of the newly formulated chemical.

2.2. Methods

2.2.1. Preparation of chemical composition. The combination of chemicals required for the detection of E. coli consists of three key ingredients, henceforth referred to, as reagents X, Y, and Z. Chemical reagent X was prepared by dissolving a mixture of LTB in DI water. Chemical reagent Y was B-PER. Chemical reagent Z was prepared by dissolving an enzymatic substrate (30 mg) in 1 mL of a 1:1 mixture of DMF and DI water. All the chemical reagents were maintained at pH 7.

2.2.2. Method of detection in 96-well plates. The first step of the test method involves concentrating the bacteria samples in small volumes, by filtering 100 mL contaminated water samples with a syringe filter or a steri-cup filter. 100 μL of the pre-concentrated water samples containing bacteria was added to the wells of 96-well plates. A volume of 100 μL of reagent X, 25 μL of reagent Y, and 50 μL of reagent Z was then added sequentially to the wells. The plates were then incubated for one hour at 37 °C. The appearance of color/fluorescence in the wells containing E. coli was observed. This test was conducted with different combinations of enzymatic substrates with different kinds of bacteria, metals and ions to find the optimized chemical composition for rapid and specific detection of E. coli.

2.2.3. Impregnating chemical compounds in a hydrogel matrix. A hydrogel solution was prepared by dissolving 100 mg of agarose hydrogel in 10 mL of TBE buffer. The optimized chemical composition (LTB, B-PER and Red-Gal), obtained through systematic testing using 96-well plates, was then added to 100 μL of hydrogel solution kept inside the tube of the plunger-tube assembly and the entire hydrogel matrix with impregnated chemical compounds was allowed to solidify. The tubes were then dried under vacuum for 24 hours to obtain a thin layer of dried hydrogel, which occupied the bottom portion of the plunger-tube assembly.

2.2.4. Method of detection in plunger-tube assemblies. The ease in method of detection was achieved by using a standard plunger-tube assembly with a built-in filter (0.45 μm pore size), as shown in Fig. 1. The plunger-tube assembly allowed E. coli present in the contaminated water to be concentrated in the head-space between the filter (integrated with the plunger) and the bottom of the tube. The filter blocked the E. coli, if present in water, allowing only contaminant free water to be collected inside the plunger. Secondly, all the chemical reagents (i.e., LTB, B-PER and Red-Gal) were impregnated inside a hydrogel (Agarose), which was dried and placed inside the head-space, whereby E. coli got trapped within the hydrogel matrix by the downward movement of the plunger inside the plunger-tube assembly. Once E. coli is in contact with the coated hydrogel, indicator enzymes were released in response to the enzymatic substrates within the hydrogel. This immediately led to the release of colored molecules within the head-space, indicating the presence of E. coli in contaminated water. It is important to note that such color indicators are meant for quick qualitative measurements only. The quantitative measurements would require well calibrated look-up tables containing the color intensity with known concentrations of E. coli. The time of detection was also greatly reduced due to the increased surface area to volume ratio provided by the hydrogel and also the reagent combination involved in promoting the reaction.

2.2.5. Water quality monitoring with plunger-tube assemblies. The color produced at the head-space of the plunger-tube assemblies was captured using a mobile device sensor (i.e., camera) and analyzed using the custom mobile software application. The data can be further transmitted over a telecommunication network to a cloud-based data server, as shown in Fig. 1(a). The software can also access location-based services of the mobile device and thereby identify the test location and create a searchable water quality map for the community. The software can also send messages to all the end users including individual consumers of water, healthcare professionals, and regulators about the quality of the water sources. This early warning system will help the appropriate regulatory agencies, municipalities and individuals to take the appropriate measures for treating the source water (i.e., the cheapest solution being chlorination). The entire procedure to use the plunger-tube assembly for water quality monitoring is schematically depicted in Fig. 1(b). We refrain from discussing in more detail such a water monitoring platform here, as the main focus of this work is to evaluate the newly formulated chemical composition and newly developed method of detection with plunger-tube assemblies.

3. Results and discussion

After a comprehensive review of the literature, detailed studies were conducted to test and select an appropriate combination of reagents to detect E. coli in contaminated water samples. The chemical composition used in this work for detecting E. coli contains three components: (1) growth media to provide nutrients for the growth of E. coli; (2) a lysing agent to extract the enzymes from E. coli; and (3) enzymatic substrate to target specific enzymes (GAL or GUD) of E. coli. We used LTB as the growth media to provide nutrients to E. coli. We used lysing detergent (B-PER) to extract the enzymes associated with the E. coli bacteria. The lysing method employed here can extract
the enzymes from \textit{E. coli} within seconds and at the same time enhance the interaction between the enzymes and the chemicals to immediately release the colored or fluorescent chemicals for visualization and quantification. We have selected several enzymatic substrates for the enzymes GAL and GUD thereby enabling detection of \textit{E. coli}. Enzymatic substrates with $\beta$-D-galactopyranoside were used to target the GAL enzyme produced by \textit{E. coli} in order to yield some color or fluorescent derivatives. Similarly, enzymatic substrates with $\beta$-D-glucuronic acid were used to target the GUD enzyme produced by \textit{E. coli} in order to yield some color or fluorescent derivatives.
3.1. 96-Well plate method

Initial experiments were conducted in 96-well plates using several enzymatic substrates in the presence of LTB and B-PER. Table S1 (see ESI†) shows the list of enzymatic substrates used in this work. Enzymatic substrates # A, B, F and G are chromogenic compounds, which produce color when they react with the GAL enzyme whereas enzymatic substrate # E is a fluorogenic compound, which produces blue fluorescence when it reacts with the GAL enzyme. The enzymatic substrate # A (Red-Gal) is an off-white to pale pink solid, which develops pinkish red to dark red color producing a dimerized 6-chloro-3-indolyl compound when it interacts with the GAL enzyme. Enzymatic substrate # B (ONPG) is a white to off-white solid, which can be hydrolyzed by the GAL enzyme to produce a yellowish ortho-nitrophenol compound. Enzymatic substrate # F (X-Gal) is a white to off-white solid, which interacts with the GAL enzyme and produces a blue compound (5,5′-dibromo-4,4′-dichloro indigo). The GAL enzyme produced by *E. coli* hydrolyses the white/off-white enzymatic substrate # G (Magenta Gal) resulting in the release of a magenta color producing compound. Enzymatic substrate # E (MUGal) is a white solid, which can be hydrolyzed by the β-D-galactosidase enzyme to release the blue fluorescence producing (4-MU) compound. Enzymatic substrates # C (4-MUG) and H (MUG) are fluorogenic compounds which produce a blue fluorescent molecule when they interact with the GUD (β-D-glucuronidase) enzyme. Enzymatic substrate # D (Magenta Gluc) is a colorimetric compound which reacts with the GUD enzyme to release a magenta color producing compound.

*E. coli* K-12 and ATCC 11229 were used as model *E. coli* bacteria for testing. In order to test the interference from other species of bacteria commonly found in water samples, experiments were conducted with different water samples containing several kinds of bacteria strains, metals and ions. Table S2 (see ESI†) provides the list of water samples used in this work. *E. faecalis*, *S. enterica*, and *B. subtilis* were used as interference bacteria whereas cadmium, lead, sodium fluoride and sodium chloride were used as metallic and/or ionic contamination interferences. In total 21 different water samples were used to optimize the chemical composition.

3.1.1. Using colorimetric substrates. Enzymatic substrates # A, B, D, F, and G are colorimetric substrates. Fig. 2 shows the color development in several combinations of water samples containing *E. coli* and different interference bacteria, metals and/or ions after reacting with enzymatic substrates. It also depicts the comparison of colors at the start of the process, *i.e.*, at *t* = 0 min and after *t* = 60 min. It is observed that the

![Fig. 2](https://example.com/fig2.png)
row with enzymatic substrate # D did not produce any color after reacting with water samples containing E. coli, which indicates that the model E. coli K-12 or ATCC 11229 does not have the GUD enzyme. Enzymatic substrates # A, B, F and G produce color after reacting with water samples containing E. coli (i.e., water samples # 1–4). It is found that E. coli ATCC 11229 and E. coli K-12 grown in LTB release more intense color compared to E. coli ATCC 11229 and E. coli K-12 grown in nutrient broth.

Effect of interfering bacteria. Enzymatic substrates # A, B, F and G produce color after reacting with water samples containing E. coli (i.e., water samples # 1–4 and 10–16), whereas no notable color production is observed for water samples containing bacteria other than E. coli only (i.e., water samples # 5–8). It is clearly indicated that the interference bacteria have no effect on the detection of E. coli with these enzymatic substrates (A, B, F and G). Water samples # 10, 11 and 12 contain E. coli bacteria and the other interference bacteria including E. faecalis, S. enterica, and B. subtilis. It is observed that these water samples produce color with the enzymatic substrates (A, B, F and G), although they contain a mixture of interference bacteria.

Effect of interfering metal ions. Enzymatic substrates # A, B, F and G produce color after reacting with water samples containing E. coli (i.e., water samples # 1–4 and 10–16). The metallic ions cadmium and lead produced less color intensity after reacting with water samples containing E. coli whereas the other samples produced more color intensity. Water samples # 13, 14, 15 and 16 contain E. coli along with cadmium, lead, sodium fluoride and sodium chloride, respectively. However, these metallic and ionic solutions did not affect the production of color when they interacted with enzymatic substrates # A, B and G. Enzymatic substrate # F does not produce any color when it reacted with water samples containing E. coli along with cadmium and lead. Water samples # 17 and 18 did not show any color though they have E. coli bacteria along with all the interference bacteria, metals and ions. The reason would be the number of E. coli bacteria is very low compared to other interfering bacteria, metals and ions. Hence, the color produced by E. coli bacteria is not detected by the naked eye.

Effect of ferric chloride. Water samples # 19 and 20 contain E. coli along with ferric chloride. Ferric chloride can be used as an oxidizing agent to intensify the color produced after reacting with the GAL enzyme. However, it is observed that ferric chloride containing water samples did not increase the color intensity compared to other samples. Ferric chloride is not a stable compound, it reacts with moisture in ambient air and becomes very acidic. Enzymatic substrates do not work properly in acidic solutions.

Effect of negative controls. Water samples # 9 and 21 contain DI water without bacteria, metals and ions. These samples also act as negative controls. It is found that the enzymatic substrates did not produce any color for these samples.

3.1.2. Using fluorogenic substrates. Enzymatic substrates # C, E and H are fluorogenic substrates. Fig. 3 depicts the fluorescence development in several combinations of water samples containing E. coli and different interference bacteria, metals and/or ions after reacting with enzymatic substrates. Here, we have compared the fluorescence at the start of the testing i.e., at t = 0 min and after t = 60 min. The fluorescence emitted by the water samples was visualized under a simple hand-held ultraviolet (UV) lamp (Cole-Parmer 8 watt UV Lamp with Dual 365 nm Wavelength Light Tubes, Cole-Parmer, Montreal, QC, Canada). It is observed that the enzymatic substrates with a glucuronic acid molecule i.e., C and H did not produce any fluorescence after reacting with water samples with and without E. coli bacteria. It indicates that the GUD enzyme is not present in E. coli. The row with enzymatic substrate # E produced blue fluorescence after reacting with water samples containing E. coli (i.e., water samples # 1–3) whereas the substrate # E didn’t produce any fluorescence for water samples that do not contain E. coli even if they have other bacteria (i.e., water samples # 5–8). Water samples # 17 and 18 did not show any fluorescence though they contain E. coli. This may be because of the mixture of metallic and ionic compounds in these water samples. Water samples # 9 and 21 are negative controls containing DI water without any bacteria, metals and ions. Enzymatic substrates # C, E and H did not produce any color in DI water samples, which shows that the enzymatic substrates are working fine.

3.1.3. Selection of optimized chemical composition. It is clearly indicated that E. coli K-12 and ATCC 11229 used in this work contain a GAL enzyme only. Enzymatic substrates # A, B, E, F and G are good for detecting E. coli bacteria. Enzymatic substrate # E is a fluorogenic compound and it requires a UV lamp to identify the blue fluorescence produced by the GAL enzyme whereas the other substrates do not require a UV lamp. Enzymatic substrate # F is not able to produce color in the presence of cadmium and lead. Enzymatic substrate # B produces a yellowish color in the presence of E. coli which may pose a significant challenge for detection when the water samples contain some organic matter such as algae. Enzymatic substrates # A and G have good specificity in E. coli detection, however, the enzymatic substrate # G is expensive compared to enzymatic substrate # A. From this study, it is clear that enzymatic substrate # A (Red-Gal) is a promising substrate compared to other substrates as it has good specificity for detecting E. coli also it is relatively inexpensive. Hence, the optimized chemical composition for detecting E. coli is Red-Gal, B-PER and LTB.

3.2. Plunger-tube assembly method

In the 96-well plate method, we detected the bacteria in pre-concentrated samples containing a high concentration of E. coli bacteria (∼4 × 10^5 CFU mL^{-1}). The 96-well plate method is not suitable for detecting the E. coli in water samples containing a low concentration of E. coli. In addition, this 96-well plate method is not suitable for field use since it requires a pre-concentrator. In order to simplify the test method for field use, in the present work, we have developed a novel hydrogel based E. coli detection system using a plunger-tube assembly,
which can simultaneously pre-concentrate the bacteria from water samples and also provide qualitative colorimetric results in a simple six step procedure, as illustrated in Fig. 1(b). This test method is also suitable for water samples containing low concentrations of *E. coli* bacteria. The present plunger-tube assembly uses a hydrogel impregnated with an optimized chemical composition (Red-Gal, B-PER and LTB), which allows the user to identify *E. coli* by visualizing the change in color (pinkish red color appearance) within minutes of testing contaminated water. Fig. 4 shows the color change due to the presence of a known concentration of *E. coli* in contaminated water. It is observed that there is a pinkish red color appearing at the bottom of the tube to indicate the presence of *E. coli* whereas there is no color change in the DI water (control sample).

Fig. 5 illustrates the appearance of red color in the plunger-tube assembly for different known concentrations of *E. coli*, which were incubated at 37 °C for 1 h after performing the test. Note that the intensity of color change depends on the incubation time as well as the concentration of *E. coli*. The detection of *E. coli* was achieved within few minutes of incubation at 37 °C for higher concentrations of *E. coli* in the range of $4 \times 10^6$ CFU mL$^{-1}$ to $4 \times 10^5$ CFU mL$^{-1}$, whereas for concentrations in the range of 400 CFU mL$^{-1}$ to $4 \times 10^4$ CFU mL$^{-1}$, the detection time was around 60 min. The intensity of the color produced by *E. coli* after reacting with the chemical composition depends on the number of *E. coli* bacteria. The color produced in the water samples containing *E. coli* ($4 \times 10^6$ CFU mL$^{-1}$ to $4 \times 10^5$ CFU mL$^{-1}$) is able to be visualized in 5 min,
whereas the color produced in water samples containing *E. coli* (4 × 10^4 CFU mL\(^{-1}\) to 400 CFU mL\(^{-1}\)) is not able to be visualized in 5 min. It requires at least 60 min to visualize the color by eye. This is because the number of *E. coli* bacteria will grow in LTB (provided in optimized chemical composition) and produce more color over time. The hydrogel matrix is used to store the chemical composition only. The hydrogel does not play any role in *E. coli* detection. The plunger-tube assembly plays an important role in detection as it is able to screen out the target bacteria (*E. coli* in this case) from a given volume of water and concentrate it within the head-space between the plunger and the tube.

The effect of different kinds of bacteria, metals and ions on *E. coli* detection was also studied with the plunger-tube assembly. The results obtained with the plunger-tube assembly show the same behavior as we observed in the case of the 96-well plate method. Hence, for the sake of brevity, those results are not shown here.

The main advantages of the plunger-tube assembly over the 96-well plate method are the plunger-tube assembly can simultaneously pre-concentrate the bacteria from water samples and also provide qualitative colorimetric results in a simple 6 step procedure, which does not require a separate pre-concentration step, or the sequential addition of chemical reagents as used in the 96-well plate method. In summary, the plunger-tube assembly provides faster and simpler in-line colorimetric detection and accessibility of data through cellular devices.

4. Challenges and opportunities

Here, we outline some of the challenges of the hydrogel based plunger-tube assembly tool presented here. Any US EPA approved testing method would require the use of 100 mL of water samples. Currently, we are using off-the-shelf plunger-tube assemblies, which are procured from the standard vendor VWR, Canada and therefore, it comes only in sizes of 5 mL. Hence, as a scale-up operation, we would require making a specific mold for a 100 mL replica of the current plunger-tube assembly, which will make it compliant with the US EPA regulations. We believe that such a challenge actually creates better opportunity for augmenting the sensitivity and detection time for the proposed technology. With the larger volume of water available (100 mL compared to 5 mL), there will be higher probability of *E. coli* bacteria getting trapped in the hydrogel matrix, which will enhance the enzymatic based substrate reaction and thereby further reduce the detection time, particularly for low bacteria count. If we include the cost of manufacturing a mold for 100 mL, we anticipate the cost per test for 10 000 samples is around CAD 2–3. Such a low cost is primarily driven by the expectation that one would conduct a large number of such water quality tests for limited resource communities. This shows that this proposed technology has tremendous potential for penetrating water markets that are price sensitive and can emerge as a low-cost high-volume innovative solution for limited resource communities across the globe.

5. Conclusions

We formulated a new chemical composition comprising of enzymatic substrate, B-PER and LTB for the rapid and specific detection of *E. coli*. The effect of several kinds of bacteria, metals and ions on *E. coli* detection performance using the new chemical composition with different enzymatic substrates is studied. A chemical composition containing Red-Gal, B-PER and LTB showed optimal performance compared to other enzymatic substrates and it is selected as the optimized composition for rapid and specific *E. coli* detection. This newly formulated chemical composition is impregnated into a porous matrix, i.e., agarose hydrogel. The hydrogel matrix was dried and placed inside a plunger-tube assembly containing a 0.45 μm filter. This plunger-tube assembly would allow for simultaneous pre-concentration and detection of *E. coli* in contaminated water samples. We have demonstrated that we can detect very high levels of *E. coli* contamination (4 × 10^6 CFU mL\(^{-1}\) to 4 × 10^5 CFU mL\(^{-1}\)) in potable water within 5 minutes. The lowest levels of contamination (4 × 10^4 CFU mL\(^{-1}\) to 400 CFU mL\(^{-1}\)) can be detected under an hour using this method, which is much faster than any commercial method. The plunger-tube assembly method described here allows the user to detect *E. coli* by visualizing the appearance of pinkish red color within minutes of testing contaminated water. Additionally, this method does not require any microbiology instruments and trained personnel. The ease of the testing method makes it a potential candidate for field deployment in limited resource communities.

![Development of color in samples with decreasing concentrations of *E. coli* (CFU mL\(^{-1}\)) with the plunger-tube assembly, after 1 h incubation at 37 °C.](image-url)
Acknowledgements

The authors thank Dr Dasantila Golemi-Kotra from the Department of Chemistry, York University and her students for their help in providing bacterial strains (E. faecalis and S. enterica) and providing access to the biosafety cabinet (level 2) used in this study. Funds from the Lassonde School of Engineering as start-up grant and the Kanef Professorship to SKM are highly acknowledged.

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