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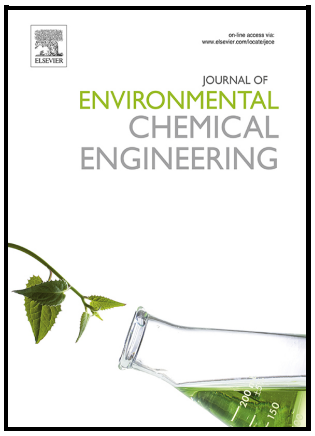

Field-testing solutions for drinking water quality monitoring in low- and middle-income regions and case studies from Latin American, African and Asian countries

N. Pichel, F. Hymnô de Souza, L.P. Sabogal-Paz, P.K. Shah, N. Adhikari, S. Pandey, B.M. Shrestha, S. Gaihre, D.A. Pineda-Marulanda, M. Hincapie, K. Luwe, S. Kumwenda, J.C. Aguilar-Conde, M.A.L.R.M. Cortes, J.W.J Hamilton, J.A. Byrne, P. Fernandez-Ibañez

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Field-testing solutions for drinking water quality monitoring in low- and middle-income regions and case studies from Latin American, African and Asian countries

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Abstract

This work highlights the need for a global approach to drinking water monitoring that involves facing several critical issues. Field tests that perform to very high standards of indicator microorganisms' detection and confidence and, at the same time, being available in rural and isolated locations of low-income settings are urgently needed. Commercially available field-testing solutions for *Escherichia coli* determination based on hydrogen sulfide and defined substrate methods were critically reviewed, considering their capabilities and limitations, compliance against the UNICEF Target Product Profile (TPP), technology performance, availability, and cost. None of the available tests meets the standards set by the UNICEF TPP, the biggest limitation being the requirement of a

power source. They need at least 18 to 24 h of incubation, hence they have not significantly decreased the amount of the time needed to complete an assay; and their applicability is generally limited by the sample volume. Additionally, there is still need for more accurate and standardised validation studies that open new opportunities for low-cost testing solutions in the field. On the other hand, traditional methods are the only ones legally authorised by national regulations in the case study locations, with a range of resources and technologies limitations. Despite the use of field kits is beginning to gain acceptance, its implementation in the field strongly relies on their availability and cost locally. Most field kits price exceed the maximum of 6 USD set by UNICEF, and they even cost significantly more when acquire from local distributors in developing countries.

Keywords: coliforms, safe water, waterborne diseases, drinking water testing.

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5 Conclusions

1. Introduction

Drinking water quality monitoring for faecal contamination plays a fundamental role for public health protection. Currently, at least 2 billion people use a drinking water source contaminated with faeces, including 144 million who rely on surface water (WHO, 2019). Bacteria such as *Escherichia coli* have been used as an indicator for contamination of water and related health risks, which led to more than 2.2 million deaths per year, predominantly among children under five in low- and middle-income countries (LMICs) (WHO, 2009; Postel, 2000; Asbolt, 2004; Efstratiou *et al.*, 2017). Therefore, to address this global health issue, the United Nations' Sustainable Development Goal SDG-6, focuses on ensuring safe drinking water and sanitation for all (United Nations, 2015). This uses faecal organisms monitoring as the indicator (indicator 6.1.1) to measure the proportion of population that can access drinking water in compliance with the standards (United Nations, 2015). In addition, water quality monitoring is crucial for determining where to focus efforts on improving water quality, ensuring the correct operation of water supplies, and validating control and preventative measures.

However, in LMICs, drinking water monitoring is barely covered by national drinking water programs. In fact, existing data from regulatory authorities is limited, especially for rural areas and populations using non-piped supplies (Rahman *et al.*, 2011; Rivett *et al.*, 2012; Westcoat *et al.*, 2016). These represents settings where monitoring is especially crucial since small water supply systems have been found to be at higher risk of contamination (Figueras and Borrego, 2010). According to the WHO and UNICEF Joint Monitoring Programme (JMP) in 2020, very high proportions (between 19.7% and 97%) of population rely on faecally contaminated water sources. In addition, it showed a higher percentage (13.5%) of contaminated water at point-of-use (POU) vs. point of collection (POC) because of inadequate storage (WHO/UNICEF, 2020).

The WHO indicates *Escherichia coli* as the most feasible and cost-effective indicator to monitor water quality for faecal pollution (WHO, 2017). Examination of drinking water for the presence of *E. coli*, which normally inhabits the bowel of human and other warm-blooded animal, provides conclusive evidence of recent faecal pollution (WHO, 2017). It is not found in great numbers in the environment, and it is less risky and relatively easier and cheaper to culture when compared with other enteric pathogens such as viruses and protozoa. Due to these features, *E. coli* is used as an indicator organism by international drinking water guidelines and national regulations. In general, drinking water must contain no faecal organisms; so, in all cases 'no detection' of *E. coli* in 100 mL sample is required to identify a water source as drinkable (WHO, 2017). In this regard, it is worth mentioning that basing water quality solely on indicator microorganisms presents limitations, as pathogens can be detected even in the absence of indicator microorganisms

(Richiardi *et al.*, 2023). However, the detection of all pathogens present in a water source is unfeasible, being *Escherichia coli* one of the most widely used indicator microorganism.

Detecting and enumerating *E. coli* has traditionally been based on the multiple-tube fermentation (MTF) method. This can be reformulated in alternative vessels to also report the most probable number (MPN) estimation of the bacterial counts. Alternatively, the membrane filtration (MF) method allows for direct colony observation and enumeration (CFU/volume of sample filtered in mL). The MTF and MF methods, commonly referred to as "conventional" or "traditional" methods, are complex to perform and time consuming. Some of these methodologies requires further confirmation tests to corroborate the results are truly from *E. coli*. Thus, to achieve consistent results, the use of these methods requires a wide range of laboratory equipment and skilled personnel, making them expensive and laborious. In addition, samples must be analysed within a maximum of 24 h after collection and they must be kept refrigerated (5 ± 3°C) (ISO 5667-3: 2018) prior to analysis. This is complex, impractical, and large expensive for rural communities or developing countries, for which the nearest laboratory might be at a significant distance from the drinking water collection sites. In places where laboratories are accessible, they are often overloaded and thus, only able to conduct infrequent testing of a limited number of supplies. In addition, sample transportation constitutes the major cost for water monitoring in rural areas (Crocker and Bartram *et al.*, 2014). As consequence, drinking water testing and monitoring in locations with limited resources is likely to be limited or inexistent, exacerbating the risk of waterborne diseases.

However, there is potential for optimization of monitoring programs by considering *on-site* water quality testing. On this regard, the UNICEF Target Product Profile (TPP) for rapid *E. coli* detection tests has sets the minimum requirements that a product intended for *E. coli* determination in water must meet for field-testing (UNICEF, 2023). These requirements include: (1) portable testing equipment; (2) *on-site* operation; (3) portable power source or no power requirement; (4) minimum number of consumables; (5) minimum life span of 2 years for hardware and 1 for consumables; (6) applicable in a broad range of water matrices (0 - 10 NTU, 5.5 – 8.5 pH and 600 - 1200 mg/L of salinity); (7) good performance (false positive rate <15%, false negative rate <15%, 80% detection and 90% confidence vs. reference methods) for qualitative (P/A in 100 mL of sample) or quantitative results (lower level of detection equivalent to 1 - 10 CFU/100 mL); (8) easily operated by non-technical users (minimum number of process steps, no reagent mixing, rapid incubation -preferred at room temperature 25 ºC- or no incubation required and easy interpretation of results); (9) reduced analysis time and final result time to ≤ 6 h; and (10) low cost (≤ 6 USD).

The main objective of this work is to provide a critical analysis of several available field-testing solutions for drinking water monitoring. This is based on their capabilities, limitations, and their compliance against the UNICEF TPP (UNICEF, 2023), including technology performance, availability in rural and isolated locations and in low-income settings, and costs (per sample). Moreover, to discuss the current state of drinking water

monitoring in LMICs and to determine where efforts should be focused, recent case studies in Brazil, Colombia, Mexico, Malawi, and Nepal were performed. Case studies were jointly conducted by Ulster University (UK), University of San Paulo (Brazil), University of Medellin (Colombia), Centro de Tecnologia de Antioquia (Colombia), Cantaro Azul (Mexico), Med-Micro Research Laboratory (Nepal), and Kantipur Dental College Teaching Hospital & Research Centre (Nepal) following national level drinking water monitoring regulations and existing network monitoring infrastructure and coverage.

2. Existing field-testing solutions

Due to the above-mentioned limitations in different LMIC settings, there are limited capabilities to monitor all water supplies. Therefore, many well-known technologies are undergoing transition from lab-based to field-based tests. Commercial products for field testing of *E. coli* (identification and/or enumeration) in water, include the hydrogen sulphide (H_2S) method and the defined substrate method, as described below.

2.1 Hydrogen sulfide (H_2S) method

The H_2S method, or 'paper strip', was developed in the early 1980s by Manja *et al.* (1982). The medium composition is generally formulated by combining peptone, dipotassium hydrogen phosphate, ferric ammonium citrate, sodium thiosulfate and an inhibitor of the growth of non-enteric sulphur-reducing bacteria (e.g., bile salts, sodium deoxycholate or taurocholate) (WHO/SDE/WSH, 2002). However, its composition, along with supporting materials, test format, sample volume and incubation temperature, has been modified to improve its performance (WHO/SDE/WSH, 2002).

It detects all the H_2S-producing organisms in the sample, but it does not detect the presence of either a specific bacterium or group of bacteria (e.g., *E. coli* and total coliforms). *Enterobacter, Clostridia, Klebsiella, Escherichia, Salmonella, Acinetobacter, Aeromonas, Morganella, Citrobacter* and *Proteus* all give a H_2S positive reaction (Ratto *et al.*, 1989; Castillo *et al.*, 1994) by reducing sulfur to hydrogen sulfide forming a black iron sulfide precipitate in the presence of ferrous iron. The detection of black colour indicates the presence of these organisms and hence a positive result. The H_2S method has never been standardized. However, its performance has been extensively tested and compared against standard methods (Ratto *et al.*, 1989; Kaspar *et al.*, 1992; Venkobachar *et al.*, 1994; Grant and Ziel, 1996; Sivaborvorn, 1998; Castillo *et al.*, 1994; Martins *et al.*, 1997; Nagaraju and Sastri, 1999; Genthe and Frank, 1999; Pillai *et al.*, 1999; Nair *et al.*, 2001; Manja *et al.*, 2001; Muller and O'Reilly, 2002; Gupta *et al.*, 2008; Tambekar *et al.*, 2008; Chuang *et al.*, 2011; Kumar *et al.*, 2012; McNahan *et al.*, 2012; Yang *et al.*, 2013). The results of most of these studies indicated that the H_2S method detects the presence of faecal contamination with about the same frequency and with similar sensitivity to the reference methods. In addition, it detects other coliform bacteria such as *Clostridium perfringens* that is a faecal indicator more resistant to disinfection than *E. coli*. However, since the H_2S method is not specific for faecal organisms, it presents a high occurrence

of false results: 9-42.8% of false positives and 2.3-12% of false negatives (Nair *et al.*, 2001; Chuang *et al.*, 2011). As consequence, it has been clearly disclaimed for drinking water testing by several authors as requires confirmation test (Kaspar *et al.*, 1992; Nair *et al.*, 2001; Tambekar *et al.*, 2008; Chuang *et al.*, 2011; Islam *et al.*, 2017).

Nevertheless, the H_2S method has been widely used for drinking water testing in LMICs for more than two decades because it is an inexpensive, easy-to-use, and portable alternative for field-testing. In addition, reagents can be stored at room temperature (≤ 25 ºC) and have 12-24 months of shelf life. Due to its simplicity, it is even manufactured by local non-governmental organizations (NGOs) and community-based organisations for its use in rural and remote settings (Gupta *et al.*, 2008). Despite WHO and UNICEF having supported its use in developing countries for primary drinking water testing, they do not recommend its use for routine monitoring due to its low specificity and the lack of systematic studies on its standardization (WHO/SDE/WSH, 2002; Mosley and Sharp, 2005; UNICEF, 2007).

At the moment, there are at least six commercial brands for this test currently available on the market: PathoScreen™ Medium P/A and MPN (Hach®, USA), Ltek H_2S Water Test kit (Ltek Systems, India), Coliform P/A (H_2S) test vial (developed by ENPHO in 2001, Nepal), Bacteriological H_2S field test kit, bottle (Water Health Laboratories under contract with UNICEF, India) (Fig. 1), H_2S Test Medium (powder) K019 (HIMEDIA Laboratory Pvt. Ltd., India), and *Jal TARA-Aqua Check H_2S Vials/strip* (Taralife Sustainability Solutions Pvt. Ltd., India). Only two of them have had their performance compared against standard methods. PathoScreen™ was compared against Colilert-18/Quanti-Tray® (ISO 9308-2: 2012) showing a 79% of true positives, 9% of false positives and 12% of false negatives (Chuang *et al.*, 2011). Therefore, the authors recommended that it should not be used alone for drinking water testing, while better results were found when combining two methods (e.g., 100 mL H_2S test + 3M™ Petrifilm™ EC) than when used as a single test. Kumar *et al.* (2012) compared H_2S Test K019 performance against Colilert-18® showing it was able to detect coliform bacteria at concentrations > 6 colonies per 100 ml after 48 h of incubation. On the other hand, Phuyal *et al.* (2019) reported the ENPHO's H_2S test use for drinking water quality screening in Nepal. The H_2S tests price is <1USD for P/A and <2.5 USD for MPN (data from manufacturers and IndiaMART).

2.2 Defined substrate methods

Media incorporating fluorogenic and/or chromogenic enzyme substrates, the so-called 'defined substrate methods', were first introduced by Edberg *et al.* in 1991. They led to the development of a new generation of media for the specific detection of *E. coli* and total coliforms in water. These media are based on the ability to detect the presence and activity of specific and exclusive enzymes of *Escherichia coli* (β-D-glucuronidase) and total coliforms (β-galactosidase) using them as indicators of their respective organisms. The β-D-glucuronidase and β-galactosidase activity is measured by using chromogenic

and/or fluorogenic substrates. The chromogenic enzyme substrates act as the substrate for a specific enzyme and result in change colour, commonly from light-yellow to blue-green, due to the action of the enzyme. The fluorogen consists of a sugar or amino acid functionalised fluorophore, that converts UV to visible light when the sample is irradiated at 365 nm.

β-D-glucuronidase activity although produced by 94-96% of *E. coli* strains (Manafi, 2000), it is also produced by other *Enterobacteriaceae*, and some *E. coli* strains are β-D-glucuronidase negative. These may lead to false positives and negatives respectively, yet the occurrence of these organisms and hence associated errors have been reported to be negligible (Köster *et al.*, 2003; Tavakoli *et al.*, 2008).

Although the target enzymes of these methods remain the same, a great number of reformulations have been introduced in the market for differing applications. These vary the chromogenic or fluorogenic substrates and the inhibitors used to be selective. They are offered as premeasured powders in single-use packs ready for the addition of liquids, as ready-to-use media plates for direct sample addition, or for use with portable filtration kits in MF. These liquid media test kits can provide qualitative data (P/A); or quantitative results via probable number (MPN) procedures usually requiring special MPN vessels; or even colony enumeration (CFU/mL) with a varied detection limit range in solid media formats. The currently available field-testing solutions based on the defined substrate methods are reviewed below. Supplementary Material S.1.1 shows the chromogenic and fluorogenic substrates and *E. coli*/TC results presentation of each solution. In addition, table 1 shows their compliance vs. the desirable TPP requirements (UNICEF, 2023); and table 2 a price comparison for each case study location (low- and middle-income) vs. a high-income country (UK).

2.2.1 Aquagenx®

The Aquagenx® CBT EC+TC (Fig. 1), developed at the University of North Carolina (Chapel Hill, USA), displays results as P/A (CBT EC+TC P/A) and also enables quantification using the Aquagenx Compartment Bags (CBT EC+TC MPN). The performance of the Aquagenx® Compartment Bag Test (CBT) for *E. coli* quantification has been compared against established methods such as membrane filtration (Stauber *et al.*, 2014, Wang *et al.*, 2017) and IDEXX Colilert Quanti-Tray®/2000 (Brooks *et al.*, 2017). These studies showed that it produces consistent results within a 95% confidence interval (Stauber *et al.*, 2014) in comparison with those produced by the reference methods. In addition, Aquagenx® have been widely used for water quality monitoring in developing countries (Heitzinger *et al.*, 2015: Brooks *et al.*, 2017; Symonds *et al.*, 2017; Goel *et al.*, 2019; Guo and Bartram, 2019; Ferrer *et al.*, 2020; Heitzinger *et al.*, 2020; Byrne *et al.*, 2021; Morgan *et al.*, 2021).

Figure 1. (Left) Hydrogen sulfide (H_2S) method. Field-testing vials manufactured and marketed by Water Health Laboratories under contract with UNICEF (IndiaMART Web). Yellow vessel indicates a negative result, while black vessel indicates a positive result. (Right) Aquagenx® Compartment Bag Test (CBT) for MPN. Pale yellow compartment indicates negative result, blue-green compartment indicates positive result for *E. coli*, and compartments that fluoresce blue under 365 nm UV light indicates positive for total coliforms.

2.2.2 Colilert®, Colilert-18® and Colisure®

In Colilert®, Colilert-18®, and Colisure® (IDEXX, USA) results can be either shown as P/A or quantified by using the MPN multi well cards Quanti-Tray® and Quanti-Tray® /2000.

Colilert® has been approved by the USEPA (USEPA, 1989a; USEPA, 1992), Association of Official Analytical Chemists Official Methods of Analisys℠ Program (AOAC® OMA℠) (AOAC® OMA℠ 991.15, 1994) and Standard Methods for the Examination of Water and Wastewater (APA, 6th ed., 1995). When compared against MF (in m-Endo, m-Endo agar LES and m-FC broth) and MTF (in Lauryl Sulphate Broth), Colilert® has shown no significant differences for *E. coli* and total coliforms detection and enumeration (Covert *et al,* 1989; Eckner, 1998; Buckalew *et al.*, 2006). In addition, Olstadt *et al.* (2007) reported a 0 % failure rate to detect P/A of total coliforms and *E. coli*.

Colilert-18® is an improved formulation of Colilert® and it is also approved by USEPA (USEPA, 1992) and AOAC® OMA℠ (AOAC® OMA℠ 991.15, 1994). Colilert-18 with Quanti-Tray® and Quanti-Tray®/2000 is a globally approved method for the detection and enumeration of total coliforms and *E. coli* in water (ISO 9308-2:2012). This medium has been validated against Lactose TTC with Tergitol-7 medium to prove that Colilert-18®/Quanti-Tray® was a more suitable alternative for *E. coli* enumeration presenting higher recoveries (Niemela and Fricker, 2003). Subsequently, Colilert-18® has been validated and/or compared to MF using several media (Tryptone Bile X-Glucuronide Agar, Chromocult Coliform Agar, m-Endo, m-TEC, Membrane Lauryl Sulphate Broth) showing equivalent results (Fricker *et al.*, 1997; Schets *et al.*, 2002; Chao *et al.*, 2004; Hörman and Hänninen, 2006; Pitkänen *et al.*, 2007; Kämpfer *et al.*, 2008; Boubetra *et al.*, 2011; Lusic *et al.*, 2016; Tiwari *et al.*, 2016; Vergine *et al.*, 2017). Olstadt *et al.* (2007) by contrast showed a 3.3% failure rate to detect total coliforms and *E. coli* in P/A tests.

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Colisure® is an EPA approved method for the detection of *E. coli* and total coliforms (USEPA, 1994). Colisure® has been compared to MF with mTEC and m-Endo agar showing greater results with a sensitivity of 96% and specificity of 100% (McFeters *et al.*, 1995). It presented a 20% failure rate to detect P/A of total coliforms and *E. coli* after 24 h incubation, which dropped to 0% after 48 h (Olstadt *et al.*, 2007).

2.2.3 ColiKat Rapid®

ColiKat Rapid® (Xebios Diagnostics GmbH, Germany) performance has been validated against Colilert-18®/Quanti-Tray® (ISO 9308-2: 2012) according to the ISO 17994 (ISO 17994: 2014) by an independent study which indicated that both methods are equivalent for enumeration of coliform bacteria, with ColiKat Rapid® showing a significantly higher recovery for *E. coli* (IWW, 2021).

2.2.4 Readycult® Coliforms 100

Readycult® Coliforms 100 is a medium manufactured by Merck KGaA (Germany). It has been approved as an USEPA alternative method (USEPA, 2002). Although there are no validation studies reported, its performance has been compared to other methods (Maheux *et al.*, 2008; Maheux *et al.*, 2011; Sandle, 2018), including the former ISO standard MF with Tergitol-7 media (Hörman and Hänninen, 2006) and the conventional MTF method (Wajid *et al.*, 2010). Readycult® Coliforms 100 showed a sensitivity of 94.4-97% and a specificity of 66-95.2%. In addition, it was shown to detect 442 of 468 *E. coli* strains, detecting none of the non-*E. coli* isolates (Maheux *et al.*, 2011).

2.2.5 Colitag™

Colitag™ (CPI International, USA) (Fig. 2) is an USEPA approved method (USEPA, 2004). Some studies have tested its performance showing 0% failure to detect P/A of *E. coli* and total coliforms (Olstadt *et al.*, 2007). It has also been reported as an effective medium for faecal coliforms in a study developed in groundwater samples in Kentucky (Coyne and Shuler, 1994). Its use in developing regions for *E. coli* monitoring in drinking water has been reported for Mozambique (Arnal *et al.*, 2010) and sub-Saharan Africa (MacLeod *et al.*, 2019).

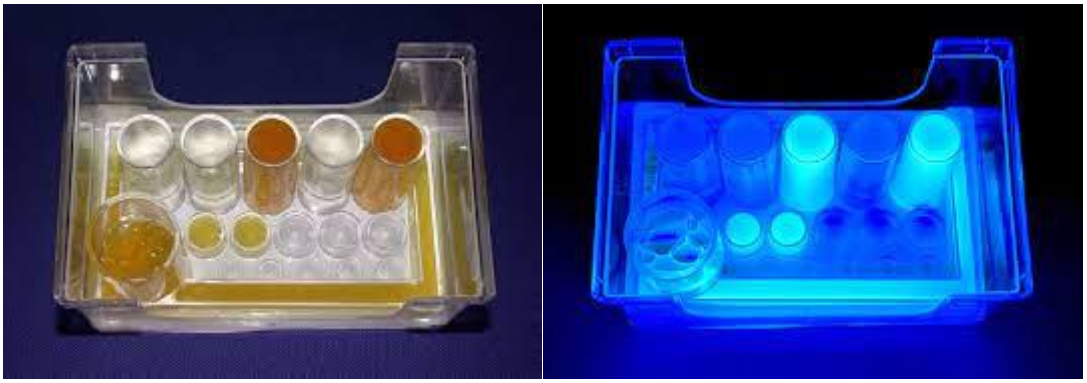

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Figure 2. Colitag MPN plate™ (left) yellow wells positive for coliforms; and (right) blue fluorescence wells under 365 nm UV light positive for *E. coli* (Colitag™ web).

*2.2.6 E*Colite*

The E*Colite test (Charm Sciences Inc., US) has been approved for use by the USEPA (USEPA, 1999). An independent validation of the E*Colite by Olstadt *et al.* (2007) showed a 20% failure rate to detect the P/A of total coliforms and *E. coli* after 24 h that subsequently reduced to zero if read after 48 h. Organisms such as *Aeromonas spp.*, which capable of galactosidase activity and hence false positives, were not completely suppressed by this formulation (Olstadt *et al.*, 2007).

2.2.7 Charm® PathoGel Test

PathoGel (Charm Sciences Inc., US) combines both defined substrate and hydrogen sulfide methods for coliforms, *E. coli* and hydrogen sulfide producing *Enterobacteriaceae* detection, also allowing *E. coli* colonies quantification. No comparisons vs. standard methods or field-based applications for drinking water testing have been reported so far.

2.2.8 Coliscan® EasyGel®

The Coliscan® EasyGel® (Micrology Laboratories, US) allows *E. coli* and total coliforms quantification. Coliscan® MF is used with membrane filters for larger volume samples, as required for drinking water sample monitoring. This method has been approved by the USEPA for the monitoring of surface water (USEPA, 2005).

Some research papers confirm that it has a sensitivity and specificity of 84% and 73% for total coliforms and 40% and 95% for *E. coli*, in comparison with Quanti-Tray® 2000 (Murcott *et al.*, 2015). In addition, Olstadt *et al.* (2007) reported a 0% failure rate to detect P/A of total coliforms and *E. coli*, with recovery not dependent on bacterial concentration or water matrix. However, it was unable to supress *Aeromonas spp.* (Olstadt *et al.*, 2007). It was also used for testing in the field as reported by Tune and Elmore (2009) and Wampler and Sisson (2011).

2.2.9 SimPlate®

The SimPlate® system (Biocontrol Systems Inc., US) consists of an MPN plate for the detection and enumeration of *E. coli* and total coliforms (Fig. 3). This was approved by the AOAC® OMA^{SM} (AOAC® OMA^{SM} 2005.03, 2005) and MicroVal in accordance with ISO 16140-6:2019 for foods. Its performance has been mainly evaluated in food samples (Townsend *et al.*, 1998, Russell, 2000; Feldsine *et al.*, 2005; Hauge *et al.*, 2017). No publications on its validation against drinking water reference methods nor field-based testing of water quality are currently available.

Figure 3. SimPlate® system: (left) pink wells indicate a positive result for total coliforms (Merck KGaA Web); (right) fluorescence in wells indicate a positive result for *E. coli* (Agrinea Web).

2.2.10 3M™ Petrifilm™ E. coli/Coliform count Plates

3M™ Petrifilm™ EC (hereafter Petrifilm), developed by 3M® Corporation (Minneapolis, US), is a compact ready-to-use plating medium intended for quantification in food and beverage products, environmental surfaces (AOAC® OMA℠ 2018.13; AOAC® OMA℠ 991.14; AOAC® OMA℠ 998.08) and environmental samples (MicroVal LR76, 2017).

Its performance in water matrices has been evaluated against MF methods reporting slightly lower counts for *E. coli* (0.04 log) and total coliforms (0.2 log) vs. mFC Agar (statistically not different), with corresponding correlation coefficients of 0.879 and 0.949 (Schraft and Watterworth, 2005). Baumgartner *et al.* (1993) and Hörman and Hänninen (2006) have also reported lower counts in comparison with MF in ECD Agar and Lactose Tergitol-7 (LTTC). In addition, Petrifilm showed weak sensitivity (true positive rate of 39.5-40%) but high specificity (79.2-95 %) vs. LTTC (Hörman and Hänninen, 2006), mFC (Schraft and Watterworth, 2005) and IDEXX Quanti-Tray® 2000 (Murcott *et al.*, 2015). Vail *et al.* (2003) found *E. coli* counts significantly correlated with those obtained by MF in mTEC, m-ColiBlue24® and Colilert-18/Quanti-Tray® 2000.

Petrifilm has been used for field studies in Africa and South America (Pearson *et al.*, 2008; Levy *et al.*, 2012; Seib *et al.*, 2012; Vivar *et al.*, 2016; Woodman *et al.*, 2019), one of them reporting incubation at ambient temperature (30 ± 2 ˚C) (Levy *et al.*, 2012). Additionally, it has been reported to be more suitable when used by untrained personnel, being chosen by two-thirds of local community groups over similar alternatives such as Coliscan® EasyGel® (Stepenuck *et al.*, 2011).

2.2.11 Compact Dry EC

Compact Dry EC (NISSUI Pharmaceutical Co., Ltd., Japan) consists of ready-to-use plates for enumeration in food and water samples. It is certified by the AOAC® OMA℠ for *E. coli* and coliforms enumeration in raw meat, fish, vegetables, and milk products (AOAC® OMA℠ 110402, 2020).

Its performance against conventional methods has been mainly evaluated in food samples, showing correlations >0.93 for food standard methods (Kodaka *et al.*, 2006; Hosokawa

and Kodaka, 2010). In water samples, a sensitivity and specificity of >99 and 97 % was found for incubation at 35 ± 2 ˚C for 24 h and at ambient temperature for 48 h, when comparing with MF in MI Agar (Brown *et al.*, 2020). It has been used in developing countries for water monitoring purposes (Agboli *et al.*, 2017; Navab-Daneshmand *et al.*, 2018; Johnson *et al.*, 2020).

2.2.12 m-ColiBlue24®

m -ColiBlue24® (Hach/Millipore Billerica, US) uses membrane filtration allowing *E. coli* quantification. This product was approved by the USEPA for *E. coli* and total coliforms determination in drinking water (USEPA, 1999). Its performance has been tested by Olstadt *et al.*, (2007) showing a failure rate to detect the presence or absence of total coliform bacteria and *E. coli* of 23%. In addition, it was incapable of detecting *E. coli* at concentrations $<10^3$ CFU/100 mL. It was unable to supress *Aeromonas spp.* at different concentration levels (10^3-10^9 CFU/100 mL) and showed dependence on water matrix due to a poor acid-neutralizing capacity. Comparison with conventional methods showed it provided highly correlated results to MF with m-FC, m-TEC and m-ENDO agar (Grant, 1997; Hamilton *et al.*, 2005), and even better results than MF with Lactose TTC agar (Bernasconi *et al.*, 2006) with a specificity ranging from 65% (Jensen *et al.*, 2001) to 97.7% and a sensitivity of 100% (Grant, 1997). m-ColiBlue24® has been used for field testing by Goodwin (2003) in Brazil, Vanderwaag *et al.*, (2009) in Nicaragua, Genter *et al.*, (2019) in Uganda and Yimer and Damer (2021) in Ethiopia.

Journal Pre-proof

Table 1. Compliance of marketed field-testing solution for *E. coli* determination in water against the desirable UNICEF Target Product Profile (UNICEF, 2023).

	Microbial performance					**User requirements**														**Others**
Product	*E. coli*	Total coliforms	Qualitative test: P/A in 100	Quantitative test: level of	100 mL assay volume	Portable[1]	*On-site* operation[2]	Easily operated by non-	Reduced number of	No reagents mixing[5]	Reduced analysis time[6]	Not power requirement or	Ambient temperature	Storage at ambient	≥12-month shelf-life for	≥2-year shelf-life for	Cost per test ≤6 USD[9]	Easy results interpretation[10]	Time to get result ≤6 h	Evaluated against RM or	Marketed as a kit including all consumables[11]
Aquagenx® CBT EC+TC P/A	■	■	■		■	■	■	■	■	■	■	■	■	■	■		■			■	■
Aquagenx® CBT EC+TC MPN	■	■		■	■	■	■	■	■	■	■	■	■	■	■			■		■	■
Colilert®	■	■	■		■	■		■	■	■	■			■	■			■		■	
Colisure®	■	■	■		■	■		■	■	■	■			■	■			■		■	
Colilert-18®	■	■	■		■	■		■	■	■	■			■	■			■		■	
Colilert-18®/Quanti-Tray®	■	■		■	■					■				■	■	■		■		■	
ColiKat Rapid®	■	■	■		■	■		■	■	■	■			■	■		■	■		■	
ColiKat Rapid®/Quantification Tray	■	■		■	■					■				■	■	■		■		■	
Readycult® Coliforms 100	■	■	■		■	■	■	■	■	■	■	■	■	■	■			■		■	
Colitag™	■	■	■		■	■		■	■	■	■				■			■		■	
Colitag™/Colitag MPN plate™	■	■		■	■	■				■					■			■		■	
E*Colite	■	■	■		■	■		■	■	■	■			■	■			■		■	■
Charm® PathoGel Test	■	■	■	■	■	■		■	■	■	■			■	■			■			■
Coliscan® EasyGel®	■	■				■		■	■	■	■				■		■	■		■	■
Coliscan® MF	■	■		■	■	■				■					■		■	■		■	■
SimPlate®	■	■				■				■				■	■			■			
3M™ Petrifilm™ plates	■	■				■		■	■	■	■				■		■	■		■	
Compact Dry EC	■	■				■		■	■	■	■			■	■		■	■		■	
Compact Dry EC + MF	■	■		■	■	■				■				■	■			■		■	
m-ColiBlue24®	■	■		■	■	■				■					■			■		■	

[1]Lightweight, no bigger than carry-on suitcase that can accommodate at least 10 tests in a working day; [2]No laboratory, typically in a clean space without water and electricity or a reliable electricity access; [3]Training

process (maximum half day) that can be understood by non-technical users; [4]The number of process steps is ≤ 2 (e.g., add the medium to the water sample and gently mix); [5]It does not require the mixture of 2 or more reagents; [6]Time necessary to conduct a single test (media preparation, analytical procedure, and disposal) excluding time required for transportation and incubation; [7]Incubation can be performed at variable temperatures between 20 - 25 °C; [8]The product does not require to be stored bellow room temperature (20 – 25 °C); [9]Cost includes all consumables (for the largest pack commercialized) and the specific equipment required pert test (not including incubator). Exchange rate applied: 1 GBP=1.36 USD. Quotations obtained from manufacturers or distributors in UK; [10]Present qualitative/quantitative results either through clear visual cues or text. Easy but a degree of subjectivity; [11]It includes all consumables required to perform a test; RM: reference methods.

3. Drinking water monitoring in developing communities – case studies

3.1 Brazil

Brazilian drinking water regulation requires that water for human consumption must be absent of total coliforms and *E. coli* in 100 mL sample (GM/MS nº 888, 2021), in compliance with WHO guidelines (WHO, 2017). Both *E. coli* and total coliforms analysis are mandatory, and the analytical method used must be recognized by national or international organizations such as WHO, USEPA or UNICEF (GM/MS nº 888, 2021). The monitoring program may differ depending on the features of the water source (source type, such as ground or surface water, and past detection of faecal contamination), as well as the type of the water supply system (size, in terms of population covered, and treatment technology).

The GM/MS nº 888 (2021) regulation defines three types of water supply systems: (1) public water supply system, (2) collective and (3) individual alternative water supply solutions. Public water supply system refers to the population with access to piped water. Alternative water supply solutions are those who rely on un-piped water, using water from wells, cisterns, water trucks, springs, or fountains. Alternative supplies can be collective, providing water for a group of households, or individual, providing water for no more than one household (GM/MS nº 888, 2021). The type of water supply affects the monitoring program, regarding the number of samples or sampling locations and those responsible of conducting water quality checks. For public water supply systems and collective solutions, water quality assessment from source to consumption is the responsibility of the public/private organization managing the supply system. However, for individual alternative solutions, local governments are responsible for the monitoring and the householder is responsible for maintenance, which has proven to be a challenge due to the lack of resources (Oliveira *et al.*, 2017), leading to a lack of information about safe drinking water coverage. Frequency and number of samples for drinking water monitoring in Brazil according to GM/MS nº888 (2021) is reported in Supplementary Material S.2.1.

In this context, only 28% of households in rural settings were covered by public water supply systems in 2010. The remaining using alternative solutions (FUNASA, 2019; IBGE, 2011). Thus, drinking water monitoring for 72% of rural households relies on their owners. Data from 2014-19 showed that total coliforms and *E. coli* were present in 60% and 30% of drinking water samples analysed in rural areas, respectively. Considering only individual alternative water supply solutions, these numbers reached 78% for coliforms and 45% for *E. coli* (SISAGUA, 2021).

GM/MS nº 888 (2021) regulation indicates that *E. coli* and total coliforms determination in drinking water must be performed by using standardized MTF, MF and defined substrate methods. Thus, the microbiological analysis guidelines are focused on laboratory-based methodologies, and there is no recommendation for *on-site* water quality monitoring. Currently, the most common methods used are MF + Chromocult® medium, IDEXX Colilert® methods, ReadyCult® Coliforms 100, Compact Dry EC, Aquagenx®, Colitag™ and m-Coliblue24® (FUNASA, 2013).

Table 2. Availability and price comparison of field-testing solutions current available on the market between low-, middle- and high-income countries (UK, Brazil, Colombia, Malawi and Nepal).

	Price USD/test ([1])				
Product	UK ([4])	Brazil ([5])	Colombia ([6])	Malawi ([7])	Nepal ([8])
Hydrogen sulfide method (P/A)	1.6	NA	NA	4.8	0.62
Aquagenx CBT EC+TC P/A	4.8	11.97	24.36	18.5	16.57
Aquagenx CBT EC+TC MPN	6.8	16.37	34.51	22.3	18.59
Colilert® ([2])	6.3	2.15	NA	NA	1.46
Colisure® ([2])	6.3	5.11	NA	NA	2.03
Colilert-18® ([2])	6.3	9.41	NA	11.2	1.75
ColiKat Rapid® ([2])	4.2	-	-	NA	-
Readycult® Coliforms 100 ([2])	4.9	3.70	17.72	NA	20.22
Colitag™ ([2])	7	1.95	17.0	NA	NA
E*Colite ([2])	11.6	4.24	NA	NA	NA
Charm® PathoGel Test	3.5	-	-	NA	-
Coliscan® EasyGel®	1.8	NA	NA	NA	1.59
Coliscan® MF([2])	4.1 + 13.23([3])	NA	NA	NA	-
SimPlate®	8.8	11.94	NA	NA	3.39
3M™ Petrifilm™ plates	1.9	2.93	11.8	3.2	NA
Compact Dry EC		2.11	2.95	NA	NA
m-ColiBlue24® ([2])	9.2	3.98	3.79	NA	11.31

NA: not available locally; ([1]) Quotations obtained in January 2023; ([2]) Only medium included; ([3]) Portable filtration apparatus; ([4]) Exchange rate applied 1 GBP=1.36 USD; ([5]) Exchange rate applied 1 BRL=0.1946 USD; ([6]) Exchange rate applied 1 COP=0.00025 USD; ([7]) Exchange rate applied 1 MWK=0.049 USD; ([8]) Exchange rate applied 1 NPR=0.0084 USD

3.2 Colombia

In Colombia, the 'acueducto' companies are those authorized to manage the water bodies through a concession from the Autonomous Regional Corporations (CAR), being responsible for the treatment, supply, and monitoring of drinking water (Resolución 2115/2007). Additionally, they must report results in the format of a risk index integrating both microbiological and physicochemical parameters (Water Quality Risk Index, IRCA) (Resolución 2115/2017). The sampling frequency and the number of samples vary depending on the population covered by the drinking water provider (Supplementary Material S.2.2). In rural setting, frequencies are lower than in urban areas. However, in cases where a rural area is considered at a high-risk by the authorities, higher frequencies may apply (Resolución 622/2020). When the water providers are not able to meet 2115/2007 regulation, establishing drinking water monitoring requirements, CARs and

every Health Sectional Office are responsible for water monitoring. In addition, they also perform routine microbiological analyses every 2 months for small water providers (≤ 5,000 inhabitants) and monthly for populations up to 100,000 inhabitants (Resolución 2115/2017). Currently, 97% of the urban population has access to safe water while, in rural areas, despite 71% having access to water services, only 46.4% have potable water (JMP, 2021).

E. coli is the faecal indicator for microbiological water quality monitoring in Colombia. Water must not contain *E. coli* in 100 mL of sample to be considered as drinkable (Resolución 2115/2017). The National Institute of Health and the Colombian Institute of Technical Standards and Certification are the government entities that approve and support the methods proposed by any interested party. The methods used must have a sensitivity ≥ 95% and a detection limit of 1 CFU/100 mL. Currently, the approved methods are ISO 9308-1 (MF + Chromocult®) (ISO 9308-1: 2014) and ISO 9308-2 (Colilert-18/Quanti-Tray®) (ISO 9308-2: 2012). However, through resolution 1303/2008, Colitag™ was accepted as an alternative P/A method for *E. coli* and total coliforms detection in drinking water (Resolución 1303/2008). The cost of a Colitag™ test in a private Colombian laboratory varies between 17 and 22 USD. In addition, as per 622/2020 regulation, water providers in rural regions are permitted to utilise *on-site* techniques such portable labs or *E. coli* P/A field kits (Resolución 622/2020).

3.3 Mexico

Drinking water regulation in Mexico sets that water must not contain *E. coli* or thermotolerant coliforms in 100 mL of sample (NOM-127-SSA1-2021), being MTF the authorised method for their quantification (NOM-210-SSA1-2014). The National Water Commission (CONAGUA) and the Federal Commission for Protection against Sanitary Risks (COFEPRIS) are responsible for drinking water quality monitoring for supplying systems providing water to populations greater than 50,000 inhabitants and fewer than 50,000 inhabitants, respectively. However, any governmental agency covers monitoring in rural areas. This responsibility is placed on the municipalities, which is challenging because, along with technical and resources constrains, the use of field kits is not allowed requiring prior approval by the Mexican certifying authorities.

According to the Mexican National Institute of Statistic and Geography, 21% of the Mexicans reside in rural regions (INEGI, 2020), and according to WHO/UNICEF (2010), 3 million people nationwide lack access to safe drinking water (WHO/UNICEF, 2010). In this regard, NGOs, academic institutions, and research organizations make an effort to monitor the quality of drinking water in these vulnerable areas. They do so by importing field equipment that has been validated by international organizations.

3.4 Malawi

Drinking water management in Malawi is based on a decentralised policy, where districts hold the responsibility for management, maintenance, and oversight of the water service. In addition, there are multiple stakeholders involved including ministries, water boards,

NGOs, donors, research organizations and the private sector introducing complexity to the water sector. The Ministry of Agriculture, Irrigation and Water Development is the lead agency; however, it is not directly involved in the water management. Water boards are responsible for providing piped water in the urban and peri-urban areas, which is supplied mainly by surface water sources. There are a total of five water boards: Lilongwe, Blantyre, Northern Region, Central Region, and Southern Region. These facing several challenges as aging of existing networks which leads to waterborne outbreaks due to cross contamination from sewer lines (CREW, 2019). In rural areas, NGOs, church organizations and rural water supply departments hold the responsibility. The water supply in this instance is reliant on boreholes systems and unimproved water sources. Currently, Malawi reports that 80% of the population has access to safe water. However, access is uneven, being specially restricted in rural areas, where 42% of the population relies on faecal contaminated sources (NSO & ICF, 2011).

According to Malawi regulations, water must not have *E. coli* in 100 mL of sample to be declared drinkable (Waterwork Act 72:01, 2014). Water quality monitoring in small water supply systems, community boreholes and protected wells, serving more than 60 % of the population, is not consistently performed. These supply systems are mostly found in rural areas and may remain for years without having any testing. NGOs occasionally perform water quality tests, but only if they support the provision of water in the area. Furthermore, an increase of extreme natural disasters such as floods has recently led to cholera outbreaks as consequence of the damage of drinking water and sanitation infrastructures (UNICEF Malawi, 2023), making the need for drinking water testing even more relevant in the context of global climate change.

3.5 Nepal

According to the 2011 Nepalese population census, most of the country's population relies on piped water, tube well or hand-pumped water, covered well "*Kuwa*" water, uncovered well water, spout water, and river or stream water (UN RC/HC Nepal, 2011). Recent data from the Department of Water Supply and Sewerage Management reported that 51.69% of the population have access to improved drinking water sources, while the remaining 48.31% rely on un-piped locally and privately managed systems like private tube wells (DWSSM, 2019). However, what qualifies as ‘improved’ drinking water sources do not necessarily correspond to potable water. Behind the global headlines, disparities still exist between rural and urban areas. Appropriate treatment practices prior to drinking are followed by 30% of households in urban areas, compared to 12% in rural areas (DHS, 2016). Thus, the population with a true access to safe drinking water is likely to be significantly lower than the report estimates. In fact, 82.2% of population use drinking water contaminated with *E. coli* (WHO/UNICEF, 2020). As result, the incidence of faecal pollution detection in drinking water and waterborne diseases outbreaks have increased (Burlakoti *et al.*, 2020). The functional status of water schemes and the quality of water remained low, with *E. coli* bacteria polluting 71% of all water sources, 91% of which are used by the poorest quintile (Warner *et al.*, 2008). Results from samples collected for the Nepal Multiple Indicator Cluster Survey 2019 revealed that water from 17 in 20

households, and three in four sources were contaminated with *E. coli* (UNICEF, 2021). Malla *et al.*, (2018) in a study conducted in the Kathmandu valley, reported *E. coli* presence in every water source tested. A survey carried out in Province 2 in Nepal (southern lowland), where more than two thirds of the people use shallow tube well water sources, showed that 89% of samples from POC and 96% from POU were contaminated with *E coli* (Progress Inc., 2018). Another study in western Nepal found that 32% of POC samples and 9% of POU samples were *E. coli*-free, while 58% of the latter presented intermediate or high-risk for *E. coli* levels (Daniel *et al.*, 2020).

In Nepal, there are a few governmental and private water testing laboratories. When present, they are located in the country's major cities. Microbial quality screening of drinking water is lacking in rural areas. These facilities often use WHO-recommended MF method and MPN for examination of water. Coliform (MPN/100 mL) (0 in 95% of samples) and *E coli* (MPN/100 mL) (0 in 100% of samples) are often used as indicators of faecal contamination in water following national regulation on drinking water monitoring (National Drinking Water Quality Standard 2062, 2005). Consumers who want to test their water sources for coliform bacteria can use the testing facilities for a small fee. Government-run water producers and distributors, such as Kathmandu Upatyaka Khanepani Limited (KUKL), test drinking water at the source and throughout the distribution system on a regular basis. Although there is a paucity of data on coliform testing of drinking water in Nepal's rural areas, as public awareness of water safety grows, several NGOs, international non-governmental organizations (INGOs), and private water companies are employing field kit approaches for *E. coli* testing. Coliform (P/A) test vial (ENPHO) is a regularly used kit for coliform testing in drinking water. Each vial costs approximately Rupees 75 (around 0.70 USD) (Eco Concern Pvt. Ltd.). The Department of Food Technology and Quality Control (DFTQC) has also given permission to use hydrogen sulfide (H_2S) kits in rural areas where MF procedures are not available. However, it is recommended to test the water at least once a year in one of the DFTQC laboratories present in every province of Nepal (Burlakoti *et al.*, 2020). Water Aid Nepal and their partners NGOs frequently prefer to use DelAgua and Wagtech device-based techniques (WaterAid, 2011). This device approach is more expensive (approximately 4,000 USD) than kit methods, and they also necessitate a compact laboratory set-up. Thus, they are not affordable to be used in rural areas.

4. Challenges and prospects

Unlike H_2S methods, defined substrate methods are specific for the detection of *E. coli* and total coliforms bacteria in water. The products commercialized based on these technologies provide improvements over traditional techniques by reducing time for preparation and use of microbiological equipment. This is especially relevant for medium preparation, since it is usually provided as a powder in premeasured single use packs to be directly added to the water sample, or ready-to-use media plates. For methods in which MF or the use of tray sealers is not required (Aquagenx, IDEXX P/A, ColiKat Rapid® P/A, Readycult Coliforms 100, Colitag™ P/A, E*Colite, Charm® PathoGel Test,

Coliscan® EasyGel®, Simplate®, 3M™ Petrifilm™ plates and Compact Dry EC), the analysis time and costs have also been reduced. Most of the methods are portable, with a reduced number of process steps being easily operated by non-technical users. They can provide qualitative data (Aquagenx® CBT EC+TC P/A, Colilert®, Colisure®, Colilert-18®, *ColiKat Rapid*®, Readycult® Coliforms 100, Colitag™ and E*Colite), or quantitative results via MPN (Aquagenx® CBT EC+TC MPN, Colilert-18®/Quanti- Tray®, *ColiKat Rapid*®/Quantification Tray, Colitag™/Colitag MPN plate™ and SimPlate®), or even colonies enumeration (Charm® PathoGel Test, Coliscan® EasyGel®, Coliscan® MF, 3M™ Petrifilm™ plates, Compact Dry EC and m-ColiBlue24®). In addition, international standards (ISO and USEPA) have approved the use of Colilert®, Colisure®, Colilert-18®, Colilert-18®/Quanti-Tray®, Readycult® Coliforms 100, Colitag™, E*Colite, Coliscan® EasyGel® and m-Coliblue24® for water testing.

However, it has been observed that currently available solutions still present limitations that complicate their application for field-testing. One of the biggest limitations is that they require a power source for incubation. Only Aquagenx® and Readycult® Coliforms 100 have no power requirements by allowing ambient temperature incubation (20-25 °C). When temperatures different from room temperature are required, portable incubators using batteries or body belt incubators using the body heat could be used as substitutes to electrical incubators in those areas lacking laboratory equipment, electricity or where continuous access to electricity is not present. These portable solutions have space limitations, restricting the number of samples than can be analysed per day. In this respect, it should be noted that cold storage as required by Colitag™, Coliscan®, 3M™ Petrifilm™ plates and m-ColiBlue24® is also problematic for their use in humanitarian emergency situations and in resource-poor communities. In addition, the field kits have not provided any substantial reduction of the time needed to complete each assay due to the requirement for incubation. None of the products discussed give a response in less than 6 h as set by the UNICEF TPP, requiring a minimum of 18-24 h of incubation increasing to 48 h when incubated at ambient temperature.

The utility of Coliscan® EasyGel®, SimPlate®, 3M™ Petrifilm™ plates, Compact Dry EC is limited by the assay volume (<100 mL in qualitative tests or a detection limit >10 CFU-MPN per 100 mL in quantitative tests), making them unsuitable for testing drinking water sources. Even though some kits are compatible with membrane filtration, thereby increasing the volume assay (Coliscan® MF and Compact Dry EC), MF makes the analytical procedure longer and more complex for its use by non-specialized users. Membrane filtration techniques also require extra equipment for vacuum and sterilization of membrane filtration apparatus. In the case of Colilert-18®/Quanti-Tray®, *ColiKat Rapid*®/Quantification Tray, Coliscan® MF, Compact Dry EC coupled with MF and m-ColiBlue24®, the need of power and laboratory equipment such as tray sealers or membrane filtration system can hinder their use in the field.

Chromogenic results presentation and interpretation, despite the ease of presentation, it has an inherent degree of subjective around identifying and interpreting a colour change. Additionally, UV torches with emission at 365 nm are necessary to identify *E. coli* presence by fluorescence emission in IDEXX methods, ColiKat Rapid®, Readycult® Coliform 100, Colitag™, E*Colite, Charm® PathoGel Test and SimPlate®. The blue colour indicating the presence of *E. coli* or total coliforms vastly improved interpretation

of results over the traditional yellow colour still used in Colilert®, Colisure®, ColiKat Rapid® and Colitag™, which can hinder results interpretation in turbid, rust or heterotropic-bacteria contaminated samples.

Case studies showed that despite these countries have the legal and institutional framework for drinking water monitoring, the multiple challenges presented by rural settings (remote locations, no access to equipped laboratory, technicians, or electricity) and the lack of resources leads to a limited or non-existent monitoring of water intended for human consumption. Currently, only traditional laboratory-based methods are legally authorised in the study locations. Although the use of field-testing solutions is beginning to gain acceptance, contributing to the optimization of monitoring programmes, its applicability will likely depend on their availability and cost locally. Most field kits cost more than the maximum of 6 USD set by UNICEF, and they even cost significantly more when acquire from local distributors in developing countries.

5. Conclusions

This paper presents a detailed review of marketed field-testing solutions for *E. coli* and total coliforms determination in drinking water, considering their capabilities and limitations, compliance against the UNICEF Target Product Profile, and including technology performance, availability, and cost. It also discusses the current situation of drinking water monitoring for microbiological parameters in Brazil, Colombia, Mexico, Malawi and Nepal, representing a range of low- and middle-income settings.

Data produced and critically analysed in this study shows that safe drinking water and proper water quality monitoring remain a distant reality for many around the world, especially in rural isolated communities in LMICs. While there is potential for optimization of monitoring programs by considering *on-site* testing, none of the field-testing solutions currently available on the market meet all the requirements set as desirable by the UNICEF Target Product Profile. Each method has its own set of drawbacks, particularly with respect to regulatory approval and the need of incubation and hence electricity limiting field applicability. Substantial work remains to be done to develop field tests that address the need for electricity, whilst reducing response time and costs. They also need to accomplish the requirements of technology performance, and availability in rural an isolated locations and in low-income settings, which remain a great barrier to achieve safe drinking water for all. Although there has been a great advancement on the testing of commercial testing products in the field in the last decades, there remains need for more accurate and standardised validation studies to open new opportunities for low-cost and available testing solutions in the field. Thus, there is still a need for the development of more adequate water quality testing solutions for *E. coli* determination in water that allows *on-site* operation in low- and middle-income settings.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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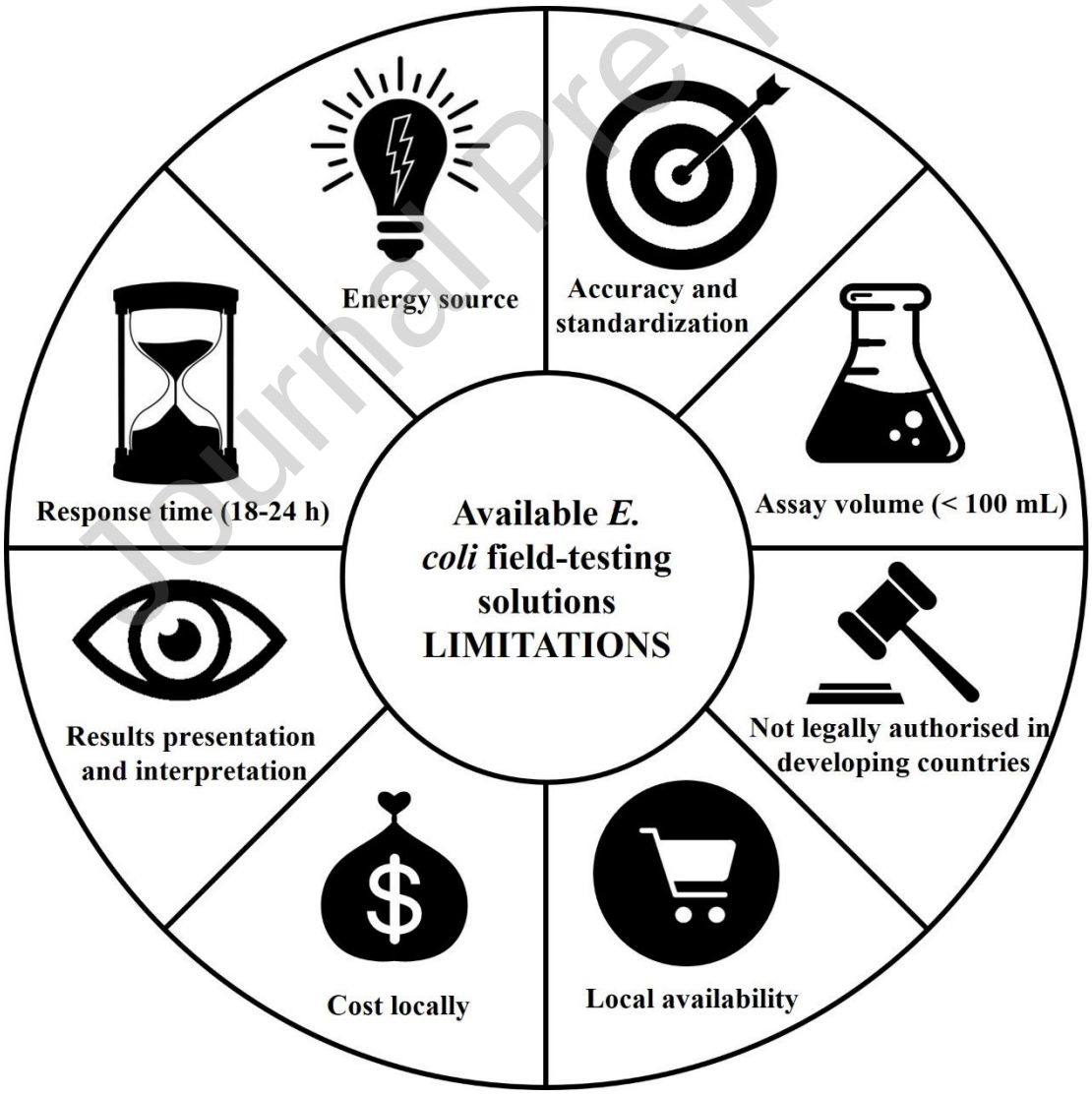

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