sciendo

Nova Biotechnol Chim (2019) 18(2): 133-143
DOI: 10.2478/nbec-2019-0016

Nova Biotechnologica *et* Chimica

The efficiency of a low-cost hydrogen sulphide (H_2S) kit as an early warning test for assessing microbial rainwater quality and its correlation with standard indicators microorganisms

Mokaba Shirley Malema[1,2], Jean-Marc Mwenge Kahinda[1], Akebe Luther King Abia[3], Roman Tandlich[4,✉], Bongumusa M. Zuma[2] and Eunice Ubomba-Jaswa[5,6]

[1] *Council for Scientific and Industrial Research, Natural Resources and the Environment, PO Box 395, Pretoria 0001, South Africa*

[2] *Biotechnology Innovation Centre, Rhodes University, PO Box 94, Grahamstown, South Africa*

[3] *Antimicrobial Research Unit, College of Health Sciences, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa*

[4] *Environmental Health and Biotechnology Research Group, Division of Pharmaceutical Chemistry, Faculty of Pharmacy, Rhodes University, Artillery Road, PO Box 94, Grahamstown, South Africa*

[5] *Department of Biotechnology, University of Johannesburg, 37 Nind Street, Doornfontein, 2094, Gauteng, South Africa*

[6] *Water Research Commission, Lynnwood Bridge Office Park, Bloukrans Building, 4 Daventry Street, Lynnwood Manor, Pretoria, South Africa*

Article info

Article history:
Received: 31st October 2019
Accepted: 29th November 2019

Keywords:
Colilert®
Harvested rainwater
Hydrogen sulphide test
Membrane filtration
Water quality

Abstract

Testing microbial quality of the harvested rainwater remains a challenge in many countries. The H_2S test kit is a low-cost microbiological field-based test which can be used in areas where water testing facilities are limited. This study compares its efficiency with the standard indicators microorganisms in the detection of faecal contamination of rainwater in South Africa. A total of 88 rainwater samples were collected from various tanks in the Eastern Cape, South Africa over three months in 2016. The collected samples were analysed for faecal bacterial contamination using the H_2S test kit, Colilert-18/Quanti-tray®/2000 and the membrane filtration technique for faecal coliforms (MFT). The correspondence rate of the H_2S test kit with MFT was 88 %, while for the Colilert® it was 76 %. The H_2S test kit confirmed faecal contamination when concentrations of standards indicators microorganisms were 5 most-probable number of cells/100 cm^3 or higher. Overall, the best correspondence of the H_2S test kit with Colilert® was observed at *E. coli* concentrations above 50 most-probable number of cells/100 cm^3. Results of the H_2S test kit correlated better with MTF, while the medium used has strongly influenced the enumeration of faecal contamination. Results point to strong effect of media used and revealed the need to calibrate the correspondence between the standard indicator microorganisms and the H_2S test kit under local conditions for specific settings.

Introduction

In the year 2017, it was reported that diarrhoeal diseases were the second leading cause of death among children aged below 5 years (WHO 2017). Although the incidences of diarrhoeal diseases

✉ *Corresponding author:* roman.tandlich@gmail.com

mostly affect the low-income populations with poor access to safe water, sanitation, and urgent medical care, acute infectious diarrhoea is also a common cause of outpatient visits and hospital admissions in high-income regions (Wazny *et al.* 2013). Assessment of microbial quality of potable water is one of the major tools to prevent or decrease the likelihood of diarrhoeal disease outbreaks. Several methods are used to detect and quantify indicators microorganisms which are indicative of the faecal contamination of potable water, i.e. *Escherichia coli* and other faecal/thermotolerant coliforms (WHO 2008). If finite concentrations of these indicators microorganisms are detected in a sample of potable water, then faecal contamination is present in it and domestic use of such (untreated) potable water can result in diarrhoeal disease outbreaks.

However, the enumeration of indicators microorganisms in potable water can be a complex task and requires laboratory facilities (WHO 2011). These laboratory facilities might not be available where the microbial water quality must be tested, as reported for the case of South Africa (Luyt *et al.* 2012). Although *E. coli* is recommended as a standard indicator organism for this purpose, the hydrogen sulphide (H_2S)-producing bacteria are an alternative faecal indicator that has been shown to correlate with *E. coli* levels and their detection has been developed into field test products (Luyt *et al.* 2012; Tandlich *et al.* 2014; Murcott *et al.* 2015). The H_2S test kit was developed to equip public health workers with a simple field test kit that can detect faecal contamination in drinking water (Manja *et al.* 1982). A version of this method is the focus of the current study.

Many (developing) countries and many areas around the world lack water testing facilities, due to financial constraints and a shortage of trained personnel which further hampers their ability to ensure accurate assessments (Luyt *et al.* 2012; Wright *et al.* 2012). A study on the overall cost of monitoring microbial drinking water quality in Sub-Saharan Africa indicated that financial constraints are assumed to be the main barrier to conducting water quality tests (Delaire *et al.* 2017). Conducting a microbial water quality test involves four types of expenses, namely consumables, laboratory equipment, labour (for collecting and analysing samples) and logistics such as transport and communication (Hunter *et al.* 2009; Crocker and Bartram 2014; Delaire *et al.* 2017). Delaire *et al.* (2017) also revealed that the estimated cost of monitoring piped water supplies in sub-Saharan African countries at the time of their study, based on the WHO recommendations, varied between 1403 USD (Liberia) and 1655 672 USD (South Africa) and amounted to 10931 000 USD per year for all the sub-Saharan African countries. Challenges relevant to this point have been reviewed for South Africa (e.g. Luyt *et al.* 2012). The H_2S test kit might provide a low-cost solution in this context, which can be field-based and could be used in the household settings (Delaire *et al.* 2017). In this way, the H_2S test kit could provide an early warning about faecal contamination at the point of consumption of the tested potable water.

Before the current study was conducted, the same version of the H_2S test kit used by the authors here had been tested on harvested rainwater in a pilot study of a community-based rainwater monitoring and treatment programme in the Grahamstown/Makhanda in the Eastern Cape Province of South Africa between March and June 2013 (Tandlich *et al.* 2014). Tandlich *et al.* (2014) collected a total of 55 samples from eight rainwater tanks during the period of the study. Results indicate that the modified H_2S test kit, which is a low-cost microbiological field-based test, was successfully used by non-governmental organisation volunteers to detect faecal contamination in harvested rainwater. Results further revealed a 71 % rate of correspondence between the membrane-thermotolerant *E. coli*, which had been grown on the m-TEC agar, and the H_2S test kit, which was also used, in the current study. In rural and certain peri-urban areas, the use of standard indicator microorganisms for faecal contamination of water samples, i.e. Colilert® for *E. coli* and the membrane filtration technique enumerating faecal coliforms on the m-FC agar (MFT), may not be a feasible choice due to the lack of investment capacity in building laboratories (US EPA 2016). Further reasons could include the long distances between the sampling sites and the laboratories, where analyses are carried out (Luyt *et al.* 2012).

Table 1. Sampling site characteristics and the description of the rainwater tanks from which the harvested rainwater was extracted.

Site	Location	Overhanging Tree branches and birds	Treatment	Rainwater usage	Tank age [years]	Roof type
S1	Rhodes University	Yes	None	Emergency Flushing of toilets	3	Tile
S2	Rhodes University	Yes	None	Emergency Drinking water source	3	Galvanized
S3	Rhodes University	Yes	None	Drinking	3	Galvanized
S4	Rhodes University	No	None	Emergency Flushing of toilets	6	Asbestos
S5	Rhodes University	Yes	Chlorinator	Drinking	-	Tile
S6	Rhodes University	No	None	Emergency Flushing of toilets	3	Tile
S7	Kenton (coastal)	No	None	Dishwashing	8	Tile
S8	Kenton (coastal)	No	None	Dishwashing	8	Tile
S9	Grahamstown west	No	None	Drinking	4	Galvanized
S10	Grahamstown west	No	None	Watering the garden	6	Galvanized
S11	Grahamstown west	No	None	-	6	Galvanized

.

Therefore, to strengthen testing programs, capacity building must not only include laboratory development and staff training (US EPA 2016). Use of simple and robust methods for testing microbial water quality, which do not require laboratory facilities or the trained microbiological personnel, and yet could provide early warning with respect to microbial rainwater quality at the point of use of the rainwater, in question, i.e. household level, are needed. The H_2S test kit fulfils these criteria (Tandlich *et al.* 2014).

The H_2S test kit is based on detecting the presence of faecal bacteria that produce H_2S (Sobsey and Pfaender 2002). In this test, the H_2S-producing bacteria of faecal origin reduce the thiosulphate in the kit medium to H_2S and hydrogen sulphide then reacts with the ferric salt (ferric ammonium citrate) to form an insoluble black ferrous sulphide precipitate (Mosley and Sharp 2005). Members of the *Enterobacteriaceae* group such as *Salmonella, Citrobacter, Klebsiella,* and *Proteus* can produce H_2S in such a medium (Sobsey and Pfaender 2002). Other non-enteric bacteria, typically absent in drinking water, can reduce thiosulphate into H_2S under anaerobic conditions, but their growth is inhibited by the addition of 0.5 % deoxycholate to the H_2S test kit medium (Sobsey and Pfaender 2002; Tandlich *et al.* 2014), as well as by conducting the H_2S test kit incubations under aerobic conditions.

This study aims to report on the comparison of the improved and modified H_2S test kit, which based on the medium of Venkobachar *et al.* (1994) and on further improvements by Tandlich *et al.* (2014), in the detection of faecal contamination of rainwater. Furthermore, the detection of faecal contamination in rainwater using the H_2S test kit and based on the two standard microorganisms that require enumeration, namely Colilert® and MFT, is reported. The study was also conducted in Grahamstown/Makhanda in the Eastern Cape, South Africa over three months in 2016.

The current paper is part of an ongoing project to establish the correspondence between the H_2S test kit and the standard indicator microorganisms used in South Africa and surrounding countries. Examples of previous studies included that of Tandlich *et al.* (2014), who compared the efficiency of the modified H_2S test kit to that of the m-TEC enumeration method. Angala *et al.* (2019) conducted a similar study in rural and urban parts of Namibia. The H_2S test kit was also used to study the microbial water quality in Harare, Zimbabwe in 2015 (Chirenda *et al.* 2015). However, the current study is the first one to evaluate the microbial quality of harvested rainwater and to compare the modified H_2S test kit and the Colilert® method. MFT is used for comparison purposes as this method was applied in previous studies (Tandlich *et al.* 2012; Nhokodi *et al.* 2016).

Experimental

Sample collection

Sampling was conducted weekly over a three-month period (June-September 2016) in the Eastern Cape from 11 tanks situated at Rhodes University, Kenton-on-sea (coastal) and in homes in the Grahamstown/Makhanda area. The 5 L bottles used for sampling were sterilised for sampling by first washing with antibacterial soap, rinsed with tap water followed by soaking in 30 % HCl for 5 minutes and finally rinsed with sterile distilled water. Sterile 5 L bottles were then used to collect rainwater samples by first rinsing the tap connected to the tanks with 70 % Ethanol and letting the tap run for 30 seconds before collection. Details about the sampling sites are provided in Table 1.Samples (total number/grand total = 88) were then transported to Rhodes University's Microbiology laboratory on ice for microbial analysis within 6 h after collection. GPS coordinates of the sampling sites are available from the authors upon request. The rainwater harvesting tanks were located either in public areas, where no one could have an expectation of privacy, or at private houses. Sampling at private residences took place in a participatory manner, i.e. only access to the sampling sites was negotiated with the household owners and no interviews or personal information was collected at any private house sampling sites. The results of the analyses from each private house were provided to the household owners/occupants expeditiously.

Microbial analysis

Preparation of the hydrogen sulphide (H_2S) test kits

The H_2S test kits were prepared as previously described by Tandlich *et al.* (2012), Luyt *et al.* (2011), Tandlich *et al.* (2014) and Angala *et al.* (2019). All the chemicals were purchased from MERCK, Johannesburg, South Africa). The H_2S test kits sampling was performed following the instructions previously described by Tandlich *et al.* (2014) with slight modifications of the incubation temperature. Briefly, five sterile H_2S test kits per sample were filled with 20 cm^3 of the collected rainwater (total volume of urine jars was 40 cm^3). The contents of the H_2S test kits were hand-shaken for about 10 seconds and incubated at 37 °C for 72 h instead of keeping the H_2S test kits in a warm place (room temperature) as described by Tandlich *et al.* (2014). The temperature of 37 °C is inside the working range of the H_2S test kit based on the medium by Venkobachar *et al.* (1994), as shown previously for water samples in South Africa by Genthe and Franck (1999). This temperature is feasible to be achieved in the household, e.g. incubating the H_2S test kits with sampled rainwater in the kitchen oven which is set to 37 °C, based on the use of low-cost thermometer to measure the temperature.

Table 2. Correspondence between the H_2S test kit and MFT and Colilert®.

		Colilert®		MFT	
		Presence[b]	**Absence[e]**	**Presence[c]**	**Absence[f]**
H_2S test	**Presence[a]**	52	0	46	6
	Absence[d]	21	15	5	31
MFT	**Presence**	51	0		
	Absence	22	15		

[a] Presence of faecal contamination indicates that all five H_2S kits in a particular sample from a given sampling site on a specific sampling occasion yielded a positive signal (see Fig. 1 for details);
[b] Presence of faecal contamination indicates that concentration of *E. coli* in the Colilert® analysis of the particular sample from a given sampling site on a specific sampling occasion yielded a concentration of 1 MPN/100 cm^3 or higher;
[c] Presence of faecal contamination indicates that concentration of faecal coliforms in the MFT analysis of the particular sample from a given sampling site on a specific sampling occasion yielded a concentration of 1 CFUs/100 cm^3 or higher;
[d] Absence of faecal contamination indicates that all five H_2S kits in a particular sample from a given sampling site on a specific sampling occasion yielded a negative signal (see Fig. 1 for details);
[e] Absence of faecal contamination indicates that concentration of *E. coli* in the Colilert® analysis of the particular sample from a given sampling site on a specific sampling occasion yielded a concentration below 0 MPN/100 cm^3;
[f] Absence of faecal contamination indicates that concentration of faecal coliforms in the MFT analysis of the particular sample from a given sampling site on a specific sampling occasion yielded a concentration below 0 CFUs/100 cm^3.

The incubated H_2S test kits were checked every 12 h for any colour change. A black colour indicated the presence of H_2S-producing organisms of faecal origin (positive), while no colour change indicated the absence of H_2S-producing organisms of faecal origin. Both positive (*E. coli* ATCC® 25922) and negative (sterile distilled water) controls were included in each test. Incubations were done using the same equipment (Tandlich *et al.* 2014).

Colilert®

Bacteria *E. coli* in rainwater samples was enumerated in triplicates using the Colilert-18®/Quanti-tray®/2000 (IDEXX Laboratories, Inc., Johannesburg South Africa). Briefly, 100 cm^3 of water sample was mixed with the Colilert-18® reagent, allowed to dissolve and transferred to a Quanti-tray®/2000. The trays were then sealed with a Quanti-tray® sealer and incubated at 37 °C for 18 – 24 h. Positive *E. coli* (ATCC® 25922) culture (inoculated in sterile water) and negative (sterile water) controls were included. Following incubation, the Quanti-tray®2000 were examined under UV for fluorescent wells. The MPN/100 cm^3 values were recorded according to a tabulation of 95 % confidence intervals provided by the manufacturer, i.e. the MPN concentrations are derived from a statistical distribution, which reports an MPN value of the concentration for the respective indicator microorganism. The statistical distribution provides an estimate of the likely variability of the MPN concentration through the 95 % confidence interval. In further text of this article, the most probable number per 100 cm^3 of the sampled rainwater is used to report bacterial concentrations measured by Colilert® and designed as MPN/100 cm^3.

Membrane filtration technique (MFT)

The MFT was performed in triplicates by filtering 100 cm^3 of the harvested rainwater sample through a 0.45 µm sterile membrane filter (Millipore, Tokyo, Japan). The membrane filters were placed on m-FC agar (Merck, South Africa) plates and incubated at 44.5±0.2 °C for 24 h. After incubation, (presumptive) faecal coliforms were counted as blue colonies. Concentrations of faecal coliforms are reported as the number of colony-forming units per 100 cm^3 and designated as CFUs/100 cm^3 in further text.

Statistical analysis

Data analysis was done using the Statistical Package for the Social Sciences (SPSS) ver. 16.0. The non-parametric Spearman's rank correlation was used to determine if there was any correlation between the three methods for the detection of faecal contamination (Colilert®, MFT and H_2S test). Furthermore, a 2 × 2 contingency table (95 % confidence level) method (Mack and Hewison 1988) was used to determine sensitivity, specificity, positive predictive value, negative predictive value, false positive, false negative and accuracy. The correspondence rates (*CR*) were calculated as shown in Eq. 1.

$$CR = \frac{100\times(a+d)}{grand\ total} \quad (1)$$

In Eq. 1, *a* is the total number of true positive results, *d* is the total number of true negative results and the term *grand total* represents the total number of samples tested, i.e. 88. A true positive result was recorded, if the H_2S test kit recorded a positive signal for faecal contamination in all five kits for particular sample from a given sampling site on individual sampling occasion and the indicator microorganism concentration of 1 MPN/100 cm^3 or 1 CFUs/100 cm^3 or higher was measured in the same sample. A true negative result was recorded, if the H_2S test kit recorded a negative faecal contamination signal in all the five kits for particular sample from a given sampling site on individual sampling occasion and the indicator microorganism concentration below 0 MPN/100 cm^3 or below 0 CFUs/100 cm^3. These results were evaluated in line with positive and negative controls.

Results

Efficiency of the H_2S test to detect faecal bacteria in harvested rainwater

A total of 88 samples from 11 harvested rainwater tanks were analysed to determine the performance

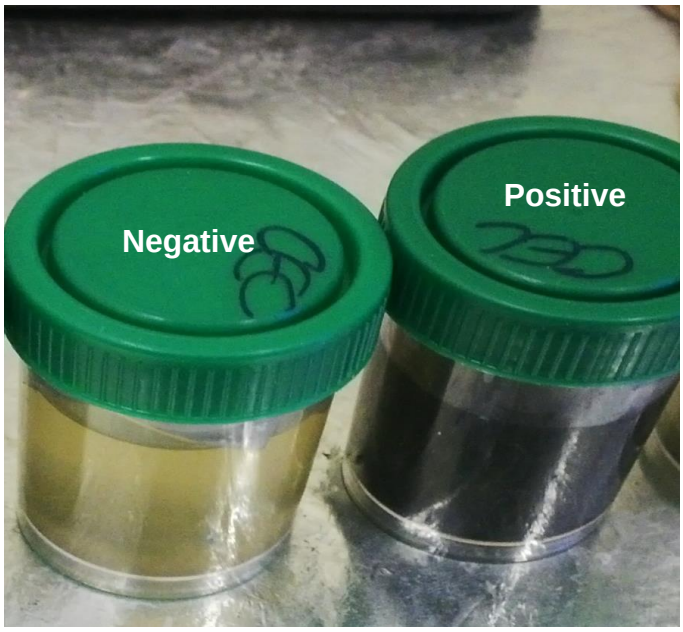

Fig. 1. Modified H_2S kit on model samples indicating negative (left) and positive (right) results. These were used as a reference to evaluation of the individual kits collected throughout the study at sampling sites S1-S11 (Table 1).

of the H_2S test kit in the detection of faecal contaminants compared to two standard methods (Colilert® and MFT). Correspondence between the standard indicator microorganisms are shown in terms of presence and absence in Table 2. Fig. 1 shows the difference between a negative and a positive H_2S test kit. The *CR* values and related parameters of comparison between the H_2S test kit and the standard indicators microorganisms are shown in Table 3.

From data in Table 3, comparison of the H_2S test as the new method and MFT as the standard method showed a sensitivity of 93 % and the *CR* value of 88 % The H_2S test showed a false positive of 16 % and a false negative of 9.8 % compared to the MFT. Using Colilert® as the standard method, the H_2S test showed a sensitivity of 71 % and a specificity of 100 % to detect the presence or absence of faecal bacteria in harvested rainwater (Table 3) when compared to Colilert®, the H_2S test had a false positive result of 0 % and a false negative of 29 %. The *CR* between the H_2S test kit and Colilert® was equal to 76 % (see Table 3 for details).

Performance of the H_2S test kit in the detection of faecal bacteria from individual rainwater tanks

The H_2S test detected faecal bacteria in all the tanks except at sampling site 11 (Fig. 2). Sampling site 11 presented low bacterial counts using Colilert® compared to the other rainwater harvesting tanks throughout the sampling period. At sites S1 S2, S4 and S5, there was a similar pattern between the two methods (H_2S and MFT) in the detection of faecal bacteria. Furthermore, the H_2S test detection rate was higher in S6, S8, S9 and S10 when compared to MFT. Table 4 shows the measured concentrations of the standard indicator microorganisms and the H_2S test kit results.

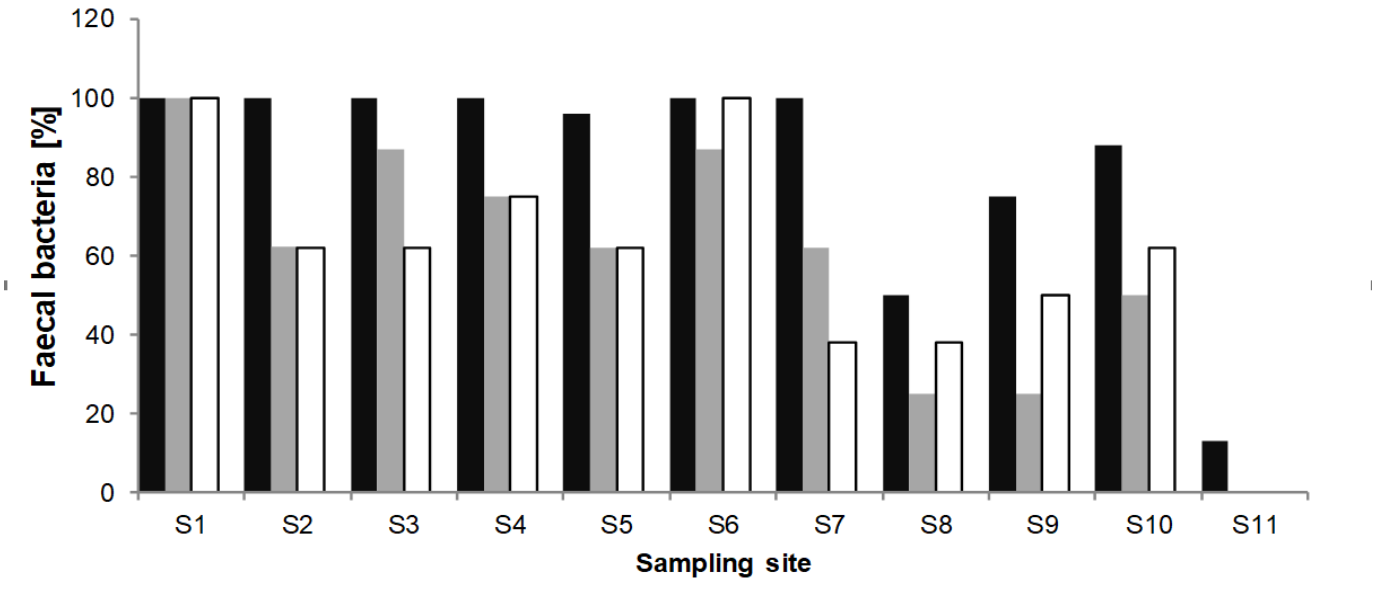

Fig. 2. Performance of the used test types on the detection of faecal bacteria. The portion of the total samples per respective sampling site detected by Colilert (black columns), Membrane filtration (grey columns) and H_2S test (white columns).

Table 3. Comparison of H_2S test with Colilert® and MFT for performance efficiency.

Parameter	H_2S *vs.* MFT [%]	H_2S *vs.* Colilert® [%]	MFT *vs.* Colilert®[%]
Sensitivity[1]	93	71	70
Specificity[2]	88	100	100
Positive predictive value[3]	92	100	100
Negative predictive value[4]	91	42	41
CR[5]	**88**	**76**	**75**

Sensitivity[1] = 100×true positive results/(true positive results + false negative results);

Specificity[2] = 100×true negative results/(true negative results + false positive results);

Positive predictive value[3] = 100×true positive results/(true positive results + false positive);

Negative predictive value[4] = 100×true negative results/(true negative results + false negative);

CR[5] as defined in Eq. 1.

Spearman's correlation coefficient calculations (Table 5) indicate a high degree of correlation between the results of the H_2S test kit and Colilert®/MFT, i.e. the *r* values are higher than 0.60 in both cases (see below).

Discussion

Efficiency of the H_2S test to detect faecal bacteria in harvested rainwater

The H_2S test method was assessed in this study for its efficiency in the detection of faecal bacteria in harvested rainwater. The H_2S test showed a higher *CR* value against MFT, i.e. 88 %, as compared to the Colilert® method where the *CR* value was equal to 76 %. In a previous study by Tandlich *et al.* (2014), the *CR* value of the H_2S test was test in comparison to the membrane filtration method was equal to 71 %. Based on the calibration in Grahamstown's surface water (Tandlich *et al.* 2012), the *CR* value was estimated to be equal to 99.4 % in the five-kit test variant of the H_2S test kit method used in the current study (Nhokodi *et al.* 2016). The media used for the enumeration of the particular standard indicator microorganism will have an influence on the recovery of cells, i.e. the measured bacterial concentration. Based on the *CR* values measured in this study, it can be seen that the H_2S test kit corresponds better with MFT than with the Colilert® method. Therefore, the *CR* values have to established/measured/calibrated under the local conditions and for the given source of water.

Results of the current study further indicate that the H_2S test kit performed better, i.e. achieved higher correspondence rates with the standard indicator microorganisms, at higher bacterial concentrations above 50 MPN/100 cm^3 in the rainwater samples. This observation could explain the difference in correspondence rate between Colilert® and the H_2S test kit. However, the H_2S test kit was positive for faecal contamination, when concentrations of standards indicators microorganisms were 5 most-probable number of cells/100 cm^3 or higher. Therefore, the H_2S test kit may be used as a compliance test to monitor the quality of harvested rainwater at the household level. It should also be emphasised that should the H_2S test show a positive result, the water should not be used for any potable purposes such as drinking without treatment. The contaminated harvested rainwater could be treated at household level using treatment options such as boiling and chlorination prior to use.

The modified H_2S test kit used in this study was first introduced and piloted by Luyt *et al.* (2011) and locally produced at a cost of 0.34 USD using basic laboratory chemicals and everyday household materials such as urine jars. At present, that price might have increased as the South African Rand depreciated against the USD. The H_2S test kit is a good early warning system that does not require laboratories or sophisticated equipment to establish whether or not water is contaminated by faecal bacteria (Luyt *et al.* 2011). A study by Wallis (1991) in Thailand reported that rainwater tanks had a 20 % false positive and 41 % false negative using the H_2S test compared to the MFT. These results indicate that the medium used in this study performed better than previous studies in the literature, as the rate of false positives was 16 % and the rate of false negatives were at 9.8 % when H_2S was compared with MFT.

Table 4. Comparison of the results of faecal contamination in the rainwater samples obtained with modified H_2S kit, measured by Colilert® and with measured faecal coliform counts (MFT)[a].

Sampling	S1			S2			S3			S4			S5			S6		
occasion	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S
1	>2419.6	>300	+	1161	>300	+	652	>300	+	>2419.6	>300	+	10	0	-	>2419.6	>300	+
2	1073	>300	+	99	0	-	437	>300	+	5	0	-	19	0	-	346	0	+
3	1769	>300	+	576	38	+	533	>300	+	153	0	+	< 1	0	-	1081	>300	+
4	408	>300	+	206	0	-	435	76	-	666	>300	+	325	54	+	986	>300	+
5	962	>300	+	511	0	-	819	0	-	293	>300	-	634	>300	+	613	>300	+
6	1859	81	+	673	53	+	1841	114	-	724	48	+	734	140	+	994	>300	+
7	1859	>300	+	678	28	+	>2419.6	>300	+	627	42	+	977	>300	+	979	>300	+
8	994	>300	+	569	42	+	>2419.6	>300	+	750	>300	+	977	>300	+	>2419.6	>300	+

[a]Microbial concentrations were expressed in MPN/100 mL for *E. coli* and CFUs/100 cm^3 for MFT.

Table 4. *Continued*

Sampling	S7			S8			S9			S10			S11		
occasion	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S	Colilert	MFT	H_2S
1	181	9	+	202	5	+	8	0	+	< 1	0	-	9	0	-
2	506	6	+	187	18	+	< 1	0	-	448	>300	+	< 1	0	-
3	237	7	+	86	0	+	76	0	-	338	50	+	< 1	0	-
4	185	4	-	< 1	0	-	651	>300	+	8	0	-	< 1	0	-
5	66	0	-	76	0	-	1120	56	+	267	44	+	< 1	0	-
6	847	0	-	< 1	0	-	36	0	-	198	0	-	< 1	0	-
7	583	5	-	< 1	0	-	59	0	+	218	6	+	< 1	0	-
8	445	0	-	< 1	0	-	< 1	0	-	312	0	+	< 1	0	-

[a]Microbial concentrations were expressed in MPN/100 mL for *E. coli* and CFUs/100 cm^3 for MFT.

Table 5. Spearman's rho correlation coefficients for the results of tests used to assess the faecal contamination of the sampled rainwater.

		Correlations	**Colilert®**	**MFT**	**H_2S**
Spearman's rho	**Colilert®**	r_s	1.000	0.869**	0.686*
		P	–	0.001	0.020
		N	11	11	11
	MFT	r_s	0.869**	1.000	0.865**
		P	0.001	–	0.001
		N	11	11	11
	H_2S	r_s	0.686*	0.865**	1.000
		P	0.020	0.001	–
		N	**11**	**11**	**11**

* The calculated correlation coefficient was significant at the 0.01 level (2-tailed).

** The calculated correlation coefficient was significant at the 0.05 level (2-tailed).

False negatives occur when standard methods indicate the presence of contamination, a positive result but the new test indicates that the water is safe, a negative result. Alternatively, false positives occur when a new test indicates that a water source is contaminated, a positive result when in fact it is not. The improvement in detection ability of the H_2S kit in this current study can be attributed to the modification that the kit had undergone, i.e. addition of 0.5 % deoxycholate to the H_2S test kit medium. Furthermore, a false positive result is less likely to lead to a risk of disease because it would result in the contaminated water not being used, subjected to additional testing or treated. However, a false negative is of great concern as it means the contaminants are not detected by the new test (Sobsey and Pfaender 2002) and hence users might be exposed to microbial contaminants that could make them sick. In the current study, the H_2S test gave a false negative of 9.8 % with MFT and 29 % when compared to the Colilert® method. Further development and modification of the H_2S test kit might be required to align its monitoring role with the Colilert® system. One possible modification could be optimisation of the bile salt(s) added to the H_2S test kit medium.

Performance of H_2S test on the detection of faecal contaminants from individual rainwater tanks

Analysis from the current study showed that in most cases where rainwater samples had high faecal bacterial counts, the H_2S test showed a positive test as well (Table 4). The observation between the Colilert® and the H_2S test suggests that the H_2S test is more efficient when the *E. coli* count in the rainwater samples is ≥ 50 MPN/100 cm^3. In almost all the tested samples (S1-S10) the performance of the H_2S test was satisfactory and proved to be competent when compared to MFT and Colilert®. Water samples with low faecal bacterial counts resulted in negative H_2S test. This finding suggests that rainwater samples from the Eastern Cape Province of South Africa, in which low concentrations of indicator microorganisms are measured, the H_2S test kit results should be approached with caution. It is advisable to validate the H_2S test against standard methods, prior to distributing the test in a new setting. In addition, further part of the ongoing project, which the current study is part of, will have to focus on the environmental stress of indicator microorganisms in the harvested rainwater and the factors affecting the cultivability of the H_2S test kit bacteria. Knowledge of the quality of the water to be tested is very important in order to minimize false negatives, especially in cases where contamination is seldom encountered. With sufficient public education on the H_2S test, households would be in charge of their own water safety. If results indicate high risk, households would be instructed to treat their water to make it fit for human consumption (Matwewe *et al.* 2018).

Vasudevan and Tandon (2008) conducted research on the microbial quality of rainwater from roof surfaces and performed similar tests using MPN, MFT and H_2S and their results were in agreement with the results obtained in this study in terms of the positive correlations observed between the 3 methods during high bacterial counts. Vasudevan and Tandon (2008) further stated that in some cases, H_2S showed that the water was potable while the MPN method disagreed. This finding differs from the current study in that no false positives were identified between Colilert® and H_2S. Omar and Bhutada (2016) studied the bacteriological assessment of drinking water and reported that the H_2S test recorded, 20 positive samples out of the 38 samples tested, while 24 samples were positive for faecal contamination with MFT. The study from Omar and Bhutada (2016) further reported that 10.52 % of the total number of samples tested as false negatives when the H_2S test was compared to MFT. Based on the low difference between the H_2S and the MFT observed in their study, the authors concluded that the H_2S test was a reliable and alternative method for the detection of faecal contamination during drinking water quality surveillance and screening of large numbers of samples in a short duration in the field where laboratory facilities are limited.

Conclusions

With an average *E. coli* concentration of 362 MPN/100 cm^3, the H_2S test can overall be

considered an effective tool in the rainwater microbial quality monitoring. The H_2S test was the most efficient in detection of faecal contamination, if the Colilert® concentrations of *E. coli* were above 50 MPN/100 cm^3. With the *E. coli* concentrations below 50 MPN/100 cm^3, the H_2S test kit was not always sensitive enough, nor accurate enough in detection of faecal contamination in rainwater tested. The H_2S test kit results correlated well with MFT in detection of faecal contamination. Comparing results from this study, with those from previous studies in the Eastern Cape, indicates the *CR*, sensitivity and other parameters should be measured under local conditions, i.e. to establish the efficiency of the H_2S test kit in the detection of faecal contamination of the specific source(s) of the potable water.

Acknowledgement

This study was supported by funds from the Parliamentary Grant of the Council for Scientific and Industrial Research (CSIR Project ECHS043) and the Water Research Commission (WRC project K5/2593). Rhodes University provided access to monitoring sites and laboratory space for sample analysis.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Angala HAN, Tandlich R, Ngqwala NP, Zuma BM, Moyo S (2019) Citizen science, treatment and compliance monitoring of microbial water quality in Namibia. *In* Proceedings from the 11th International Conference: Air and Water Components of the Environment, Babes-Bolyai University, Cluj-Napoca, Romania 22-24th March 2019, p. 323-338.

Chirenda TG, Srinivas CS, Tandlich R (2015). Assessment of the City of Harare's capacity to treat municipal water and conduct the necessary analyses on microbial water quality, the use of alternative water sources and its impact on public health. Water SA. 41: 691-697.

Crocker J, Bartram J (2014) Comparison and cost analysis of drinking water quality monitoring requirements *versus* practice in seven developing countries. Int. J. Environ. Res. Public Health 11: 7333-7346.

Delaire C, Peletz R, Kumpel E, Kisiangani J, Bain R, Khush R (2017) How much will it cost to monitor drinking water quality in Sub-Saharan Africa? Environ. Sci. Technol. 51: 5869-5878.

Genthe B, Franck M (1999) A tool for assessing microbial quality in small community water supplies: An H_2S strip test. *In* WRC Report No. 961/1/99, 33 pages. to: Water Research Commission. by: Division of Water, Environment and Forestry Technology, CSIR, Stellenbosch, South Africa.

Hunter PR, Pond K, Jagals P, Cameron J (2009) An assessment of the costs and benefits of interventions aimed at improving rural community water supplies in developed countries. Sci. Total Environ. 407: 3681-3685.

Luyt CD, Tandlich R, Muller WJ, Wilhelmi BS (2012) Microbial monitoring of surface water in South Africa. Int. J. Environ. Res. Public Health 9: 2669-2693.

Luyt CD, Muller WJ, Tandlich R (2011) Low cost tools for microbial quality assessment of drinking water in South Africa. Healthmed. 5: 1868-1877.

Mack K, Hewison K (1988) Report No. 47: Evaluation of a hydrogen sulfide screening test. Thai-Australian Northeast village water resource project.

Manja KS, Maurya MS, Rao KM (1982) A simple field test for the detection of faecal pollution in drinking water. Bull. World Health Organ. 60: 797-801.

Matwewe F, Hyland K, Thomas J (2018) Locally produced hydrogen sulphide detecting water quality test kits increase household level monitoring in rural Tanzania. J. Water Health. 16: 359-368.

Murcott S, Keegan M, Hanson A, Jain A, Knutson J, Liu S, Tanphanich J, Wong TK (2015) Evaluation of microbial water quality tests for humanitarian emergency and development settings. Procedia Eng. 107: 237-246.

Mosley LM, Sharp DS (2005) The hydrogen sulphide (H_2S) paper-strip test: a simple test for monitoring drinking water quality in the Pacific Islands. *In* South Pacific Applied Geoscience Commission (SOPAC) and WHO Technical report 373, Suva, Fiji.

Nhokodi T, Nqowana T, Collings D, Tandlich R, Köhly N (2016) Civic engagement and environmental sustainability in teaching and learning at a higher education institution in South Africa. Acta Technol. Dubnicae 6: 66-82.

Omar SJ, Bhutada SA (2016) Bacteriological assessment of drinking water using H_2S test. Br. J. Med. Health Res. 3: 2394-2967.

Sobsey MD, Pfaender FK (2002) Evaluation of the H_2S method for detection of fecal contamination of drinking water, sanitation and health. Public Health, 44.

Tandlich R, Luyt CD, Ngqwala NP (2014) A community-based rainwater monitoring and treatment programme in Grahamstown, South Africa. J. Hydrocarb. Mines Environ. Res. 5: 46-51.

Tandlich R, Luyt CD, Muller WJ (2012) Faecal contamination source identification using a combination of chemical and microbial biomarkers. *In* WRC Report No. KV 295/12 (Water Research Commission: Pretoria, South Africa.

US EPA (2016) Water Quality Laboratory Capacity Building https://www.usaid.gov/west-africa-regional/fact-sheets/usepa-water-quality-laboratory-capacity-building (accessed November 4, 2016).

Vasudevan P, Tandon M (2008) Microbial quality of rainwater from roof surfaces. J. Sci. Ind. Res. 67: 432-435.

Venkobachar C, Kumar K, Talreja A, Iyengar I. (1994) Assessment of bacteriological quality using a modified H_2S strip test. J. Water Supply Res. T. 43: 311-314.

Wallis I (1991) Simple test to detect contamination of drinking water. *In* Project report Marine and Freshwater Research Center, No 11/91. Land and Water Resources Research and Development Corporation, Canberra, ACT, 12 p.

Wazny K, Zipursky A, Black R, Curtis V, Duggan C, Guerrant R, Levine M, Petri jr. WA, Santosham M, Scharf R, Sherman PM, Simpson E, Young M, Bhutta ZA (2013) Setting research priorities to reduce mortality and morbidity of childhood diarrhoeal disease in the next 15 years. PLoS Med. 10: e1001446.

Wright JA, Yang H, Walker K, Pedley S, Elliot J, Gundry SW (2012) The H_2S test versus standard indicator bacteria tests for faecal contamination of water: Systematic review and meta-analysis. Trop. Med. Int. Health. 17: 94-105.

World Health Organisation (WHO 2008) Guidelines for drinking-water quality, Third edition, World Health Organization, Geneva, Switzerland.

World Health Organisation (WHO 2011) Guidelines for drinking water quality, Fourth edition, World Health Organization, Geneva, Switzerland.

World Health Organisation (WHO 2017) An update on diarrhoeal disease, World Health Organization, Geneva, Switzerland.