Sensitivity analysis of field test kits for rapid assessment of bacteriological quality of water

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ABSTRACT

Bacterial testing kits are designed to provide rapid and cost-effective solutions for testing the bacterial contamination in potable water with reasonable accuracy. In this study, the culture method of bacteriological examination was used to critically evaluate the sensitivity of three different bacteriological test methods, namely, the Colilert-18h method, H_2S Test Medium (Powder) K019 and Bacteriological H_2S Strip Test, to provide an appropriate end-point use. Bacteriological sensitivity results of Colilert-18h were compared with those of the culture method. Once the Colilert-18h was found to be very sensitive to total coliform counts (TCC), the sensitivity analysis of the other two field kits was carried out. Results obtained from Colilert-18h showed that it is a highly sensitive and reproducible method as corroborated by the results of the spread plating method. Subsequently, the Colilert-18h method was used as a standard for critically evaluating the sensitivity of two available field kits, namely, H_2S Test Medium (Powder) K019 (sensitive for $\geq$4 TCC) and Bacteriological H_2S Strip Test (sensitive for $\geq$30 TCC), within 24–48 h of incubation, as suggested in their procedures. Thus, H_2S Test Medium (Powder) K019 is recommended for routine field testing for coliforms, as it is able to detect *Salmonella*, *Citrobacter* and other bacteria simultaneously.

Key words | coliforms, Colilert-18h, H_2S Strip Test, spread plating method

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INTRODUCTION

Contaminated drinking water along with inadequate supplies of water for personal hygiene and poor sanitation are the main contributors to an estimated 4 billion cases of diarrhoea each year causing 2.2 million deaths, mostly among children under the age of five (WHO 2000). All water is prone to bacterial contamination either at source or as a result of microbial re-growth in the pipe system (WHO 2004). Bacterial re-growth within the water pipeline distribution system is encouraged by higher initial temperatures, higher temperatures of water in the distribution system due to hot climates, lack of a residual disinfectant and possibly greater availability of nutrients due to the aggressive nature of the water to materials in contact with it (WHO 2004). The ever-increasing population has resulted in various types of contamination in most water bodies (WHO 2006). Fecal contamination in drinking water sources is a major cause of various waterborne infectious diseases and has assumed global dimensions. Although coliform bacteria have been internationally used as prime indicator of fecal contamination of water since the beginning of the 20th century (Singh & McFeters 1992), these may not be adequate as the sole indicator of recent fecal contamination in tropical water (Gawthorne *et al.* 1996). They appear to be a less sufficient indicator of waterborne pathogens, particularly in tropical and subtropical areas (Santiago-Mereado & Hazen 1987).

To mitigate the problem of unreliability of results of fecal contamination in tropical and subtropical areas using total coliform as indicator organisms, various methods have been developed which are based on the principle of colorimetry; these methods include the detection of hydrogen sulfide (H_2S)-producing bacteria and the use of defined substrate technologies. These tests are intended to meet the need for a reliable field test for use by village

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public health workers to detect fecal contamination in drinking water. Manja *et al.* (1982) reported the development of a simple method for detecting evidence of fecal contamination in drinking water based on detecting hydrogen sulfide-producing bacteria other than lactose-fermenting enteric bacteria, particularly the coliforms. The test is based on the readily observable formation of an iron sulfide precipitate on a paper strip (or in the water sample liquid) in a bottle or test tube, as a result of the reaction of H_2S with iron. The types of organism that have been found to produce H_2S are various and include *Citrobacter freundii*, *Salmonella typhimurium*, *Proteus mirabilis*, *Proteus vulgaris*, *Clostridium perfringens*, and some species of *Arizona*, *Klebsiella* and *Edwardsiella* (Manja *et al*. 1982; Kromoredjo & Fujioka 1991; Grant & Ziel 1996; Pillai *et al*. 1997). During the detection of hydrogen sulfide-producing bacteria, the blackening of sample water in a bottle is observed as a result of the reaction of H_2S with iron on a paper strip (or in the water sample liquid) in a bottle or test tube. Many researchers reported a strong correlation between the presence of fecal coliforms and of H_2S-producing bacteria in different water samples (Allen & Geldreich 1975; Manja *et al*. 1982; Kromoredjo & Fujioka 1991; Kaspar *et al.* 1992; Grant & Ziel 1996). Therefore, the 'H_2S test' may be used as reliable method for routine examination of bacteriological contamination of water in tropical, subtropical and temperate areas.

Rapid and online monitoring of potable water are important challenges for the water industry and health authorities as the detection and quantification of bacteriological contamination in drinking water seems to be a time-consuming task. The culture methods present serious limitations with regard to quantitative and qualitative assessment or the ability to intercept sporadic contamination events. The lack of availability of standard bacteriological tests for drinking water quality highlights the great need for developing rapid, simple and inexpensive tests for the bacteriological quality of drinking water with reasonable accuracy. This need is especially important for small community and household water supplies that lack the access to safe water and cannot afford conventional bacteriological testing of drinking water. On-site testing using portable equipment such as Colilert-18h and use of simplified tests, such as the H_2S tests, may help to overcome these constraints. Many alternative indicators and tests have been developed recently to detect fecal contamination of drinking water for rapid evaluation of biological quality. Field test kits are relatively simple, cost effective and do not demand expertise in evaluating water quality. Various H_2S test media have been studied, tested and used in many parts of the world as indicators of fecal contamination of drinking water (WHO 2000).

At present, many versions of the H_2S test methods and other portable bacteriological field test kits are available worldwide that differ in medium composition, preparation of the medium and supporting materials, test format and sample volumes, incubation time, incubation temperature and scoring of results. The evaluation of these methods is not being standardized worldwide and only some versions of these have been subjected to mutual evaluation or assessment with other bacteriological test methods for fecal contamination (WHO 2000). The objective of this study was to critically weigh the Colilert-18h based on IDEXX's patented Defined Substrate Technology and H_2S-based methods, specifically Bacteriological H_2S Strip Test and H_2S Test Medium (Powder) K019, for field applications.

MATERIALS AND METHODS

Preparation of samples

Controlled serial dilutions of water contaminated with fecal bacteria were prepared from secondary treated sewage from STP Jaipur (North) in which the total coliform count ranged from 10^5 to 10^6 per 100 mL, by adding 0.01% Tween-20. Extremely close serial dilutions were prepared in decreasing order of TCC ranging from 5,000 to 1 per 100 mL of water. These serial dilutions were filled aseptically in sterile vials provided along with the test methods. All experiments were run in triplicates to minimize the handling as well as statistical errors. These vials were kept in the incubator at a prescribed temperature for a prescribed period of time and results were monitored at regular intervals. Care was taken in the preparation and transfer of samples so that they did not deteriorate or become contaminated by other means (*Standard Methods* APHA 1999).

Methods used in bacteriological sensitivity analysis

Spread plate method

In this method, original samples were diluted serially to reduce the microbial population sufficiently to obtain separate colonies when plating, in the range of 30 to 300 cells per plate (Breed & Dotterrer 1916). One mL of diluted sample was transferred to the centre of an agar plate and spread evenly over the surface with a sterile bent glass-rod. After colonies were grown, they were counted with the help of a colony counter and the number of bacteria in the original samples was calculated. For this study, XLD (xylose lysine deoxycholate) agar medium and EMB (eosin methylene blue) agar medium were used in enumeration of TCC by the SPM method. XLD agar was used for the identification and enumeration of salmonellae, shigellae, *Pseudomonas* and *Citrobacter* (Taylor & Harris 1967). EMB agar was used for the identification and enumeration of Gram-negative coliforms such as *Escherichia coli, Serratia, Enterobacter* and *Klebsiella*. Diluted samples of testing water were spread on the XLD agar and EMB agar in triplicates to confirm the reproducibility as well as to avoid handling errors. Petri plates were incubated at 35 °C and observed at 24–48 h as described by Buck & Cleverdon (1960). Evans *et al.* (1981) observed that *Escherichia*, *Klebsiella*, *Citrobacter*, *Enterobacter*, *Pseudomonas* and *Hafnia* species comprise 95 to 98% of the lactose-fermenting, gas-producing, Gram-negative bacteria recovered from drinking water and hence the two media were expected to cover the majority of bacterial contamination.

Colilert-18h method with Quanti-Trays

The Colilert-18h method is used for the simultaneous detection and confirmation of total coliforms and *E. coli* in fresh and marine water. It is based on IDEXX's patented Defined Substrate Technology (DST) (www.idexx.com). When specific substrates attached to a chromogen are included in a growth medium, microorganisms possessing the target enzyme metabolize the substrate, resulting in release of the chromogen. The release of chromogen manifests itself in a color change of the substrate. Microorganisms other than the target cannot grow and metabolize and do not affect the test (Hanko 2000). In the method, targeted microbes metabolize the indicator nutrients in the Colilert-18h medium provided along with the equipment. Coliforms including *E. coli* metabolize ortho-nitrophenyl galactopyranoside using the enzyme b-galactosidase to produce ortho-nitrophenyl. This results in a yellow coloration of the testing vial. *E. coli* also metabolize 4-methyl-umbelliferyl glucuronide using the enzyme b-glucuronidase to produce 4-methyl-umbelliferone which fluoresces under long wave (365 nm) ultraviolet light.

The working principle of Colilert-18h with Quanti-Tray technology has some parallels with traditional MPN (most probable number) methodology. As in traditional MPN methods, Colilert-18h with Quanti-Tray may also be used for enumeration of indicator organisms in water samples where there is a selected number of indicator organisms sought in the presence of many other bacteria. As for traditional MPN methods, the confirmed result obtained is an estimate or 'most probable number'. Enumeration of numbers of total coliform and *E. coli* species by using Colilert-18h were achieved through the use of a 'Quanti-Tray'; 100 mL of water sample mixed with Colilert-18h medium provided along with the instrument was dispensed into a Quanti-Tray with 97 vials. After the appropriate incubation period (18–22 h) at temperature of 35 ± 0.5 °C, the number of vials positive for total coliforms and *E. coli* were counted. Results were then calculated from the relevant MPN table supplied along with the instrument.

H_2S Test Medium (Powder) K019

HIMEDIA Laboratory Pvt. Ltd, Mumbai, is the manufacturer of the H_2S Test Medium (Powder) K019. The test medium was recommended for detection of *Salmonella* and *Citrobacter* from water samples. This test kit detects *Salmonella* Typhimurium and *Citrobacter freundii* on the basis of production of hydrogen sulfide even in the absence of *E. coli* (WHO 2006). The medium was rich in growth factors and nitrogen sources that enhance the production of H_2S by targeted microbes in the sample medium. Standard H_2S test medium has been modified by adding L-cysteine which provides sulfur to reduce the variability of growth of *Salmonella* sp. (Hu *et al.* 1995; Pillai *et al.* 1997). The presence of cysteine in the growth factor makes it more sensitive as well as less time consuming for detection of *Salmonella* serotype

Typhimurium and *Citrobacter freundii* (Hu *et al.* 1995; Pillai *et al.* 1997). In addition, the medium is sufficiently buffered which makes it selective against the growth of Gram-positive microorganisms, and also contains ferric salt which is reduced by targeted species of microorganism to form a black precipitate of iron sulfide which makes them visible (Allen & Geldreich 1975). After 24–48 h of incubation, a change of color to black is the indication of *Salmonella* and *Citrobacter* contamination in water and if the water sample color in testing vials changes to yellowish brown with haze, this indicates bacteriological contamination other then *Salmonella* and *Citrobacter* and that the water is not fit to drink.

Bacteriological H_2S Strip Test

TARA Aqua Check developed the H_2S vials/strip at its own facility, TARA Environment Monitoring facility, Delhi. The test is based on formation of an iron sulfide precipitate on a paper strip (or in the water sample liquid) in the test bottle as a result of the reaction of H_2S with iron librated from the targeted microorganism. The test was intended to detect the bacteria associated with fecal contamination by the activity of the microorganism in reducing organic sulfur to the sulfide oxidation state as H_2S gas which then reacts rapidly with iron to form black iron sulfide precipitate. *Citrobacter* was one of the common coliform genera responsible for a positive H_2S test (Allen & Geldreich 1975). The advantage of the method is its simplicity, low cost and effectiveness in the absence of a typical microbiological laboratory or field laboratory. In the procedure of the H_2S Strip Test, a sterilized paper strip treated with media is kept in a sterilized glass bottle. The bottle is filled with the water sample and kept for 24–48 h at room temperature (25 to 37 °C). If bacteria are present in the sample, they produce hydrogen sulfide, which turns the water sample black.

RESULTS AND DISCUSSION

Quantitative analysis of secondary treated effluent of STP north

This set of experiments was carried out to analyze the quantitative load of bacteria in controlled samples prepared from secondary treated effluent of STP Jaipur (North) before testing the sensitivity of three available test kits. Based on morphological identification, the total coliform species in samples were counted by using the spread plate method giving a result of 5,220 TCC/100 mL. Of the total coliform count of 5,220 per 100 mL, 26% were *Pseudomonas*, 22% *Klebsiella*, 18% *Serratia/Hafnia*, 10% *Enterobacter*, 13% *E. coli* and 11% *Citrobacter*.

Comparative analysis of TCC by spread plate method and Colilert-18h method

Serial dilutions prepared from secondary treated effluent of STP Jaipur (North) were used as the samples for comparative analysis of the spread plate method and Colilert-18h method. Results of TCC obtained from both methods (SPM and Colilert-18h) are shown in Table 1. The test results obtained from the Colilert-18h method and the standard plate method were analyzed by taking polled data of both methods for comparison of variance by the 2-tailed student t-test as shown in Table 2. Student t-test statistics which were applied at 80% confidence interval proved that results obtained by both methods (standard plate and Colilert-18h method) were significantly similar. The reason for the slightly higher count consistently reported with SPM compared with that of Colilert-18h could be dilution factor errors, as the dilution

Table 1 | Comparison of TCC enumerated by the spread plate and Colilert-18h method

TCC/100 mL by spread plate method			TCC/100 mL by Colilert-18h method		
Plate no.	Plate count	Average count	Quanti-Tray no.	TCC count	Average count
Plate 1	5,430	5,220	Tray 1	4,812	4,830
Plate 2	5,280		Tray 2	4,480	
Plate 3	4,950		Tray 3	5,200	
Plate 1	1,190	1,037	Tray 1	921	904
Plate 2	970		Tray 2	961	
Plate 3	950		Tray 3	830	
Plate 1	515	470	Tray 1	437	412
Plate 2	465		Tray 2	403	
Plate 3	430		Tray 3	397	
Plate 1	232	221	Tray 1	182	179
Plate 2	218		Tray 2	187	
Plate 3	212		Tray 3	167	

Table 2 | Statistical analysis of results obtained by the spread plate and Colilert-18h method

Variance (SPM)	Variance (Colilert-18h)	Polled variance	Polled standard deviation	Test statistics at 80%	Standard tα/2- value at 80%
60,300	129,861.333	95,080.667	308.351	1.549	1.573
17,733.333	4,507	11,120.1667	105.452	1.545	
1,825	465.333	1,145.167	33.840	2.099	
105.333	108.333	106.833	10.336	4.977	

requirement in the case of the spread plate method was much higher than that for Colilert-18h. The Colilert-18h method was found to be sensitive in a range of 1–2,500 total coliforms/100 mL and it could also detect the difference of a single coliform count in two consecutive samples. Very high sensitivity reported by this method might be because of the specificity of substrate to the target microorganism as well as the large number of wells used in the Quanti-Tray for statistical interpretation of results. This method does not require trained manpower for enumeration of total coliforms in water samples. The results were highly reproducible with respect to the standard spread plate technique used routinely worldwide. As this method utilizes two nutrient-indicators, ortho-nitrophenyl-β-galactoside and 4-methylumbelliferyl-β-D-glucuronide, which can be metabolized by the coliform enzyme β-galactosidase and the *E. coli* enzyme β-glucuronidase, respectively, this overcomes the drawback of false negative results attributed to the presence of *E. coli* that does not have β-glucuronidase. A major characteristic of this technology is that more than one enzyme can be assayed at the same time provided that each is attached to a chromogen of a different color which results in enumeration of both *E. coli* as well as total coliforms in a single experiment. The Colilert-18h method simultaneously detects and enumerates total coliforms and *E. coli* directly from water samples without the requirements of confirmation (Hanko 2000). As the results of Colilert-18h were cross-checked by standard spread plate method and were found comparable, this method was subsequently used as the standard in analyzing the sensitivity of two other field test kits.

Bacteriological sensitivity analysis of H_2S Test Medium (Powder) K019

Sensitivity analysis of this method was made by using the same sewage sample as used in the previous methods which contained coliforms and waterborne pathogenic bacterial species. The indication of color change in testing vials during the experiment for the presence of *Salmonella*, *Citrobacter* and other bacterial contamination was observed at regular intervals during the entire 48 h of incubation. Table 3 summarizes the results of bacteriological sensitivity analysis of H_2S Test Medium (Powder) K019 for which positive results were obtained corresponding to different incubation periods. The first clear indication of the presence of *Salmonella* and *Citrobacter* was found after 18 h of incubation when the TCC was ≥ 360 per 100 mL. The subsequent indications of color change to black due to *Salmonella* and *Citrobacter* contamination in testing vials were observed at 24, 36 and 48 h of incubation when TCC/100 mL was ≥ 120, ≥ 36 and ≥ 36, respectively. Although it has relatively less sensitivity, this kit is also sensitive for some other bacteria in addition to *Salmonella* and *Citrobacter*; therefore, it gives the additional benefits of detecting other bacterial contamination. If the color of the medium changes to yellowish brown with haze, it indicates an H_2S-negative (*Salmonella* and *Citrobacter* absent) reaction; however, it does show the presence of some other bacteria and hence can check whether or not water is fit for drinking. The rapid indications of color change in testing vials due to bacterial contamination other than *Salmonella* and *Citrobacter* were observed at 18 h of incubation when TCC/100 mL was ≥ 4. The subsequent incubation of 24, 36 and 48 h also gave the same sensitivity results for other bacterial contamination as given at 18 h of incubation.

Thus, according to the results, the H_2S test medium method was found to be sensitive for ≥ 4 coliform/100 mL after 48 h of incubation. The major advantage of using this medium is that it can detect *Salmonella*, *Citrobacter* and other bacteria, simultaneously. The warmer water temperatures may also contribute to the growth of coliforms, thermotolerant coliforms and *E. coli* and the greater survival

Table 3 | Bacteriological sensitivity analysis of H_2S Test Medium (Powder) K019 for *Salmonella*, *Citrobacter* and other bacterial contamination

	Sensitivity within 18 h		Sensitivity within 24 h		Sensitivity within 36 h		Sensitivity within 48 h	
TCC/100 mL Detected by Colilert-18	*Salmonella* and *Citrobacter*	Others	*Salmonella* and *Citrobacter*	Others	*Salmonella* and *Citrobacter*	Others	*Salmonella* and *Citrobacter*	Others
6,000	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4,800	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3,600	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2,400	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
1,200	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
600	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
480	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
360	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
240	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
120	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
60	No	Yes	No	Yes	Yes	Yes	Yes	Yes
48	No	Yes	No	Yes	Yes	Yes	Yes	Yes
36	No	Yes	No	Yes	Yes	Yes	Yes	Yes
24	No	Yes	No	Yes	No	Yes	No	Yes
12	No	Yes	No	Yes	No	Yes	No	Yes
6	No	Yes	No	Yes	No	Yes	No	Yes
4–5	No	Yes	No	Yes	No	Yes	No	Yes
3–4	No	Yes	No	Yes	No	Yes	No	Yes
2–3	No	No	No	No	No	No	No	No
1	No	No	No	No	No	No	No	No

of some enteric bacteria, notably *Salmonella*, compared with coliforms (Hazen 1988; Jimenez *et al*. 1989; Iverson & Fleay 1991; Townsend 1992). Therefore, targeted bacteria, especially *Salmonella*, in test procedures gives additional bacteriological safety in analysis of water samples in a warmer environment.

Bacteriological sensitivity analysis of H_2S Strip Test

H_2S Strip Test kit is usually used for the detection of TCC in water samples and it is again a colorimetric method. The indications of color change in testing vials during the experiment for the presence of TCC were observed at regular intervals during the entire 48 h of incubation. Table 4 shows the total coliform counts at which positive results were obtained with the corresponding incubation periods. The first clear indication of change in color of the sample for the presence of TCC was observed after 18 h of incubation, when the TCC was ≥1,000 per 100 mL. Subsequent indications of color change due to bacterial contamination in testing vials were observed at 24, 36 and 48 h of incubation when TCC/100 mL was ≥400, ≥100 and ≥30, respectively. The sensitivity of the H_2S Strip Test was found to increase up to 10 TCC/100 mL when the incubation was extended to 72 h. This increased detection may be due to multiplication of microbes in water samples producing an observable change in color following prolonged incubation. Any further increase in incubation time did not increase the detection sensitivity; therefore, this test kit was found to be sensitive for ≥30 coliform/100 mL for 48 h of incubation.

The Bacteriological H_2S Strip Test, and H_2S test medium may prove useful alternative indicators of fecal contamination for water quality surveillance and screening of a large number of water samples in a short duration, particularly during any outbreak of waterborne disease among a rural population.

Table 4 | Bacteriological sensitivity analysis of H_2S Strip Test for total coliform count

TCC/100 mL	Sensitivity within 18 h	Sensitivity within 24 h	Sensitivity within 36 h	Sensitivity within 48 h	Sensitivity within 72 h
5,000	Yes	Yes	Yes	Yes	Yes
4,000	Yes	Yes	Yes	Yes	Yes
3,000	Yes	Yes	Yes	Yes	Yes
2,000	Yes	Yes	Yes	Yes	Yes
1,000	Yes	Yes	Yes	Yes	Yes
500	No	Yes	Yes	Yes	Yes
400	No	Yes	Yes	Yes	Yes
300	No	No	Yes	Yes	Yes
200	No	No	Yes	Yes	Yes
100	No	No	Yes	Yes	Yes
50	No	No	No	Yes	Yes
40	No	No	No	Yes	Yes
30	No	No	No	Yes	Yes
20	No	No	No	No	Yes
10	No	No	No	No	Yes
5	No	No	No	No	No
4	No	No	No	No	No
3	No	No	No	No	No
2	No	No	No	No	No
1	No	No	No	No	No

These two kits are simple, one step, single tube, presence–absence (P–A) tests, suitable for field applications, with a long shelf-life and easy transportation and operation.

CONCLUSION

Online monitoring of microbial quality is a major challenge; therefore, field test kits give an alternate method of quick prognosis of any possible epidemiological events. A major advantage of using H_2S-based test kits is that the test medium used for identification of target microorganisms is stable and it is possible to pre-dispense the medium into sterile containers for storage and transport over long distances. The present study suggests a methodology to analyze the sensitivity of field kits against Colilert-18h results. Out of the two different field tests, the H_2S Strip Test and H_2S test medium, the sensitivity for total coliform detection was found to be $\geq$30 total coliform count/100 mL and $\geq$4 total coliform count/100 mL, respectively, for 48 h of incubation, as suggested in their procedures.

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