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Full Length Research Paper

Suitability of the H_2S test for detection of fecal contamination in drinking water

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The suitability of H_2S field test to detect fecal contamination in drinking water was assessed by analysing 635 water samples from various sources at room temperature and at 37ºC after 18, 24, and 48 h of incubation. The H_2S test showed 216, 85, 96 and 85% correlation with Eijkman test, membrane filter technique, most probable number (MPN) test for coliform and membrane filter technique (MFT), respectively. Hence the test can be used for detection of fecal contamination in drinking water in the field where laboratory facilities are limited.

Key words: Rapid field test, MPN, fecal contamination, MFT, thermotolerant coliform (TTC).

INTRODUCTION

The water quality surveillance in most developing countries is inadequate to test all drinking water resource regularly. This is largely due to inadequate laboratory facilities and wide spread water sources. The standard methods, which are available for detection of fecal contamination in drinking water, required trained analyst, supporting materials and facilities in a microbiology laboratory. In such a scenario, a reliable and easy to use field test can help in effective monitoring of drinking water and water source by user themselves. In 1982, Manja et al. developed H_2S test, based on production of hydrogen sulphide by bacteria that are always associated with fecal contaminant. This rapid field test needs no technical staff and the cost of the H_2S test is very nominal than conventional bacteriological test for detection of fecal contamination in water (Manja, 2001).

Sivaborvorn (1988) tested 705 samples from a variety of water in Thailand (shallow and deep wells, rainwater, pond water) by the H_2S test and by coliform MPN. Based on agreement between a positive H_2S test and more than 10 MPN/100 ml as a coliform or fecal coliform positive, the two tests agreed 85 and 88% of the time, respectively. Pillai et al. (1999) evaluated various modifications in H_2S test for detection of fecal contamination using cysteine in the medium and incubation temperatures at 28-44ºC and reported that lower levels of fecal contamination were detected faster. Rijal et al. (2000) compared two versions of the H_2S test, MPN and a membrane filter enumeration on agar medium, while Ratto et al. (1989) evaluated the H_2S test at incubation temperatures of 22 and 35ºC and compared it to MPN and P-A total coliform (TC) and fecal coliform (FC) tests and stated that the H_2S test was an equally or more sensitive test than TC and FC tests. Castillo et al. (1994) also tested 622 water samples by the H_2S and coliform tests and reported that the H_2S test produced 10% more positive samples than the coliform test at both 32 and 35ºC. Genthe and Franck (1999) evaluated H_2S test for 413 water samples from various sources and showed 82% and 86% agreement with fecal coliform results with test incubation temperatures of 35 and 22ºC, respectively. Manja et al. (2001) compared the H_2S test (with cysteine in the medium, different sample volumes, different incubation times and different incubation temperatures) to MPN tests for coliforms for detecting fecal contamination in 686 water samples in India. Agreement of H_2S-positive and coliform- positive samples increased from 91% at 48 h to 95% at 72 h incubation. Pathak and Gopal (2005) evaluated the efficacy of H_2S test with 90 water samples at different incubation period and temperature and found that H_2S positive result was exhibited by 78% of samples.

According to WHO and APHA, these studies were found to be insufficient to declare this test as standard or alternative to conventional methods, therefore, this

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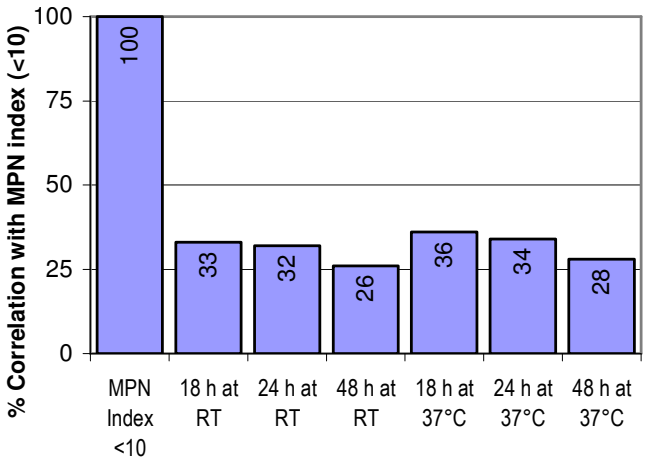

Figure 1. Correlation of MPN test (Index <10) with H_2S test.

comprehensive study was carried out to detect water quality by using Manja's (2001) H_2S field test, conventional most probable number (MPN) test, Eijkman test (thermotolerant coliform detection test), membrane filter technique (MFT), and employing microbiological analysis of water for assessment of this test at various temperature and incubation period.

MATERIALS AND METHODS

In the present study a total 635 water samples from tube well, open well and hotels and restaurants were analysed by H_2S test, standard MPN test, MFT and Eijkman test for coliform (APHA, 1998). The composition of H_2S test for 50 ml of medium was prepared accordingly Manja et al. (2001). One mL of Manja's H_2S medium was added in each 30 mL screw cap bottle and sterilized at 121°C for 15 min. To each 30 mL bottle 20 mL drinking water was inoculated for testing its bacteriological quality in duplicate. The bottles were then incubated at room temperature and 37°C for 18, 24 and 48 h. The positive H_2S test or contamination or fecal pollution in drinking water indicated by change in colour of the medium to black. Blacking in H_2S medium was recorded after 24 and 48 h of incubation at RT and at 37°C, respectively.

MPN test was performed by nine multiple tube dilution technique using double and single strength MacConkey medium. MFT test by using M-EC test agar (Hi-media Lab. Mumbai) and Eijkman test (detection of thermotolerant conforms) by using BGLB and indole test at 44.5°C were performed for each water sample as per standard procedure. Only MPN positive water samples were further inoculated for Eijkman test at 44.5°C for 24 h and positive results were recorded as gas in BGLB and indole positive.

RESULTS AND DISCUSSION

Over the last two decades, several investigators have assessed the H_2S test with various modifications such as medium composition, incubation period and temperature in different tropic and temperate regions including Indonesia, Peru, Chile, Nepal and South Africa, and compared it to conventional water bacteriological methods. Ratto et al. (1989) evaluated the H_2S test at 22 and 35°C and compared it to MPN and fecal coliform tests for 20 potable water samples and concluded that H_2S test was a more sensitive test than total coliform (TC) and fecal coliform (FC) tests. Castillo et al. (1994) concluded that the simplicity and low cost of the H_2S test makes it very applicable to tropical and subtropical potable water test. Genthe and Frank (1999) evaluated the H_2S test for specificity by using seeded positive and negative samples and reported favorable results. Pillai et al. (1999) concluded that positive H_2S results were generally obtained in 18 to 48 h of incubation at 25-44°C.

A Comparative study has been earlier performed for assessment of H_2S test at various incubation temperature and period with standard tests (Manja et al., 2001). The present study was carried out with 635 drinking water samples collected from different sources such as tube wells, open wells and hotel and restaurants. Out of total 635 samples, 297 (47%) were found potable by MPN, 108 (17%) by MFT and 83 (13%) by Eijkman test. Out of the 297 potable water samples (from MPN test), H_2S test was negative in 99 (33%), 97 (32%) and 78 (26%) at room temperature and 106 (36%), 101 (34%) and 84 (28%) at 37°C after 18, 24 and 48 h of incubation period, respectively (Figure 1). Out of total 635 water samples, 338 (53%), 399 (63%) and 82 (13%) water samples were nonpotable by MPN, MFT and Eijkman test, respectively Whereas H_2S test positive in 73 (22%), 159 (47%) and 320 (95%) at room temperature and 158 (47%), 214 (63%) and 326 (96%) at 37°C after 18, 24 and 48 h of incubation period, respectively (Table 1).

The present study showed 22, 47 and 95% correlation at room temperature and 47, 63 and 96% correlation at 37°C of H_2S test with MPN test, which is in concordance with previous studies on the H_2S test (Figure 2). The H_2S test also showed 216% correlation with Eijkman test (thermotolerant coliform, TTC) (Figure 3) and 85% correlation with MFT at 37°C after 48 h of incubation (Figure 4). At room temperature and at 37°C, the efficacy of H_2S test as compared to MPN test was 95% and 96%, indicating practically no difference in the test results with incubation temperature within 48 h.

The incubation period had significant effect on the efficiency of H_2S test. As incubation period increased from 24 to 48 h, the efficiency also increased from 47% to 95 at room temperature and 63% to 96% at 37°C.
Moreover the efficiency of H_2S test also increased to 83% at room temperature and 85% at 37°C with the increased in incubation period from 24 to 48 h, when compared with MFT. In MFT, the medium used was M-EC test agar, which detects only both human and nonhuman originated *Escherichia coli* and not the total coliform. H_2S test detect fecal associated H_2S producing organisms. As compare to TTC, the efficacy of H_2S test was very high because TTC detected only human thermotolerant *E. coli*, which may be low in the tested water samples.

Thus study indicated that the H_2S test is a reliable and alternative indicator of fecal contamination in drinking

Table 1. Comparison of H_2S test with coliform density (MPN Index) MFT and Eijkman test (thermotolerant coliforms).

MPN index	No. of Samples	H_2S Test at RT			H_2S Test at 37°C			TTC	MFT
		18 h	24 h	48 h	18 h	24 h	48 h		
Coliform density (<10)	297	99	97	78	106	101	84	108	83
Coliform density (>10)	338	73	159	320	158	214	326	82	399
Total	635	172	256	398	264	315	410	190	482

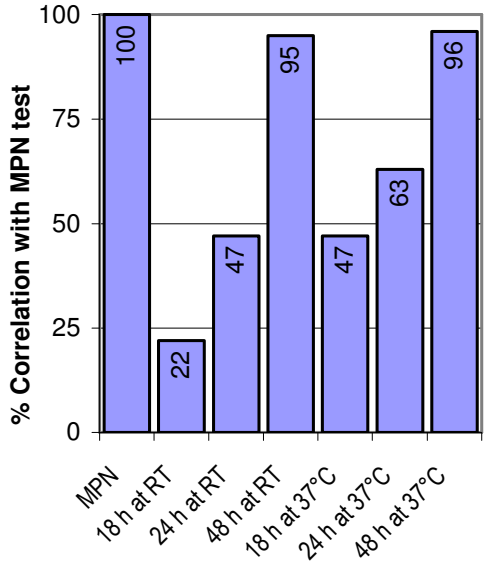

Figure 2. Correlation of MPN test (Index >10) with H_2S test.

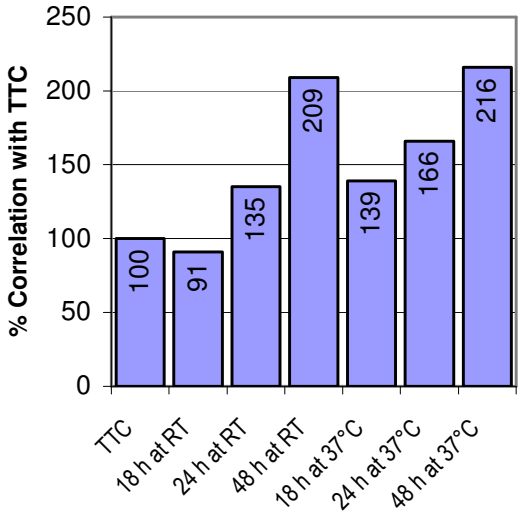

Figure 3. Correlation of TTC test with H_2S test.

water quality surveillance and screening of large number of water samples in short duration in the field where laboratory facilities are limited. Therefore, the Manja's

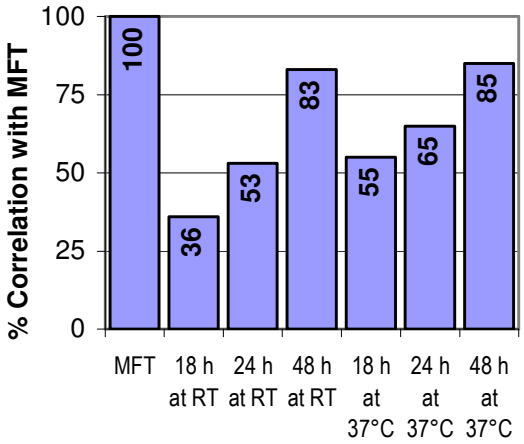

Figure 4. Correlation of MFT test with H_2S test.

H_2S test, a simple and versatile test, can be carried out in the field within a broad rang of incubation temperature and is recommended for the routine monitoring of water for detection of fecal contamination.

REFERENCES

APHA (1998). Standard Methods for the Examination of Water and Wastewater (20th ed.). Eds. Andrew D Eaton, Lenore S Classer and Arnold E Greenberg. American Public Health Association, Washington DC.

Castillo G, Duarte R, Ruiz Z, Marucic MT, Honorato B, Mercado R, Coloma V, Lorca V, Martins MT, Dutka BJ (1994). Evaluation of disinfected and untreated drinking water supplies in Chile by the H2S paper strip test. Water Res. 28:1765-1770.

Genthe B, Franck M (1999) A tool for Assessing Microbial Quality in Small Community Water Supplies: An H2S Strip Test. WRC Report No. 961/1/99, 33 pages. to: Water Research Commission. by: Division of Water, Environment and Forestry Technology, CSIR, Stellenbosch, South Africa.

Manja KS, Maurya MS, Rao KM (1982). "A Simple field tests for the detection at Fecal Pollution in drinking water. Bulletin of the World Health Organizations 60:797-801".

Manja KS, Sambasiva R, Chandra Shekhara KV, Nath KJ, Dutta S, Gopal K, Iyengar L, Dhindsa SS, Parija SC (2001) Report of study on H_2S test for drinking water, 96 pages, UNICEF, New Delhi.

Pathak SP, Gopal K (2005). Efficiency of modified H_2S test for detection of fecal contamination in water. Environment monitoring and assessment 108: 59-65.

Pillai JK, Mathew R Gibbs, Ho G (1999). H_2S Paper strip method-A bac-

teriological test for fecal coliforms in drinking water at various temperatures. Water Sci. Technol. 40:85-90.
Ratto A, Dutka BJ, Vega C, Lopez C, El-Shaarawi A (1989). Potable water safety assessed by coliphage and bacterial tests. Water Res. 23:253-255.
Rijal GK, Fujioka RS, Ziel CA (2000) "Evaluation of the hydrogen sulphide bacterial test. A simple test to determine the hygienic quality as drinking water. Abstracts of the General Meeting of the American Society for microbiology abstract Q354, page 622. Amer.Soc. Microbial, Washington, D.C."
Sivaborvorn (1988). Development of simple test for bacteriological quality of drinking water (Water quality control southeast Asia) Department of sanitary engineering, Mahidol University. International Development Research Center, Canada. Thailand center file 3-P-83-0317-03.
WHO (2002) Indicators (draft document). OECD, World Health Organization, Geneva.