



Research Journal of
**Environmental
Sciences**

ISSN 1819-3412



Academic
Journals Inc.

www.academicjournals.com

Evaluation of Modified Rapid H₂S Test for Detection of Fecal Contamination in Drinking Water from Various Sources

¹D.H. Tambekar, ¹S.R. Gulhane and ²Y.S. Banginwar
¹Post Graduate Department of Microbiology,
S.G.B. Amravati University, Amravati 444 602, India
²Institute of Pharmacy, Kaulkhed, Akola, India

Abstract: A comprehensive bacteriological analysis of 525 drinking water samples collected from railway stations, houses, rivers and lakes (surface water), tube wells and open wells was carried out using Manja's H₂S field test, MTFT (MPN) test, TTC (Eijkman test) and MFT test. Detection efficiency of faecal coliform contamination for H₂S field test (96-97%) was comparable to MPN test and more detection level over that of MFT and TTC. Efficiency of H₂S test varies with the source and decreased with the depth of the source of water. However, the H₂S test, compared to other tests, was more suitable, reliable, inexpensive, easy to perform and useful to detect fecal contamination in drinking water within 24 h, for places where time, man and laboratory facilities are very poor.

Key words: Rapid H₂S test, MPN test, drinking water contamination, coliforms

INTRODUCTION

Standard methods, which are available for detection of fecal contamination in drinking water, require trained analyst, bacteriological media and other supporting materials and facilities of microbiology Laboratory (WHO, 2002). In 1982, KS Manja (DRDO, Gwalior, India) developed a H₂S rapid field test based on production of hydrogen sulphide by bacteria that are associated with fecal contamination. The H₂S-producing bacteria are invariably present in feces and have very strong correlation between presence of H₂S-producing bacteria and fecal pollution of water. Hence by detecting the presence of H₂S-producing bacteria in water samples, fecal pollution of such water can be deduced. This rapid fields test needs no technical staff and the cost is lower than conventional bacteriological test for detection of fecal contamination in drinking water (Genthe and Franck, 1999).

The H₂S test was evaluated and reported (Sivaborvom, 1988; Kaspar *et al.*, 1992; Venkobachar *et al.*, 1994; Pillai *et al.*, 1999) favorable for detection of fecal contamination in drinking water from various source, including ground and surface water. Various modifications of H₂S test for detection of fecal contamination at various temperatures and incubation periods indicated that the test could be used in the field without any infrastructure (Rijal *et al.*, 2000; Mark *et al.*, 2002; Pathak and Gopal, 2005; Hirulkar and Tambekar, 2006). Though various people tested the validity of the H₂S test with MTFT or MFT for detection of fecal contamination of drinking water, but further validation and standardization is required by WHO and APHA as an alternative method for use in developing countries (WHO, 2002). In present study, composition of original medium was modified by substituting teepol with labolene (neutral pH) and evaluated the efficiency of this test to detect fecal contamination of drinking water from various sources.

Corresponding Author: D.H. Tambekar, Post Graduate Department of Microbiology, S.G.B. Amravati University, Amravati 444 602, India

MATERIALS AND METHODS

The H₂S test was prepared by replacing teepol with bile salt in the medium (Manja *et al.*, 2001). The H₂S medium (1 mL) was added in screw cap 30 mL bottle and sterilized at 121°C for 15 min. To each bottle, drinking water (20 mL) was inoculated for testing its bacteriological quality in duplicate. The bottles were then incubated at room temperature and 37°C for 24 to 48 h. Multiple Tube Fermentation Technique (MTFT) was performed by nine multiple tube dilution using double and single strength MacConkey medium (APHA, 1998). MFT test by using M-EC test agar (Hi-media Lab. Mumbai) and Eijkman test (detection of thermotolerant coliforms, TTC) by using Brilliant Green Bile Lactose Broth (BGLB) and indole test at 44.5°C were performed for each water sample as per standard protocol. The study was carried out over a period of four months from July to October 2006 and Water samples (525) collected from railway stations (173); all railways stations of Nagpur Bhusaval division of Central railway, India; houses from Amravati City (101); surface water (river, lake etc., 88), tube well (75) and open (dug) well (88) from salinity affected villages of Amravati district of Maharashtra State (India) were analysed by each of above tests. Blacking in H₂S medium was recorded as positive test after incubation period. Only MTFT positive (polluted) water samples were further subjected for Eijkman test in BGLB and Tryptone medium at 44.5°C for 24 h and positive results were recorded as gas in BGLB and indole positive. The sensitivity, specificity predictive values and efficiency of H₂S test were calculated as Table 1.

RESULTS AND DISCUSSION

Out of 525 water samples tested, 413 were polluted by MTFT test (>10 coliforms 100 mL⁻¹), 184 by H₂S test in 24 h of incubation, 328 by H₂S test in 48 h of incubation and 151 polluted by MFT and 139 by TTC. However 106 (in 24 h) and 100 (in 48 h) water samples were safe by both H₂S and MPN test (<10 coliforms 100 mL⁻¹) indicating 54-79% efficiency agreement between these tests (Table 1).

On comparing H₂S test with standard tests to identify fecal coliform (FC), agreement rates (Hirulkar and Tambekar, 2006; Tambekar *et al.*, 2006; Grant and Ziel, 1996) was 90 to 140% indicated that the H₂S test is a more sensitive test than other FC tests. The H₂S test is more likely to overestimate the presence of FC than Total Coliforms (TC). This is also partly due to the greater specificity of the FC group (Table 2).

Out of 525 water samples tested, 413 samples showed MPN index more than 10. The predictive value for positive was 97% (in 24 h incubation) and 96% (in 48 h incubation) in H₂S test, when compared with MTFT whereas, the predictive value for negative was 31 and 51%, respectively for detection of fecal contamination in drinking water. The predictive values for positive in case of MFT and TTC was low, 32 and 37%, as these tests detect only *E. coli* and thermotolerant *E. coli*, respectively and H₂S test detect the associated microorganisms with faecal contaminations. The percent efficiency or accuracy of MTFT/H₂S.24, MTFT/H₂S.48, MFT/H₂S.48 and TTC/H₂S.48 was 54, 79, 48 and 58%, respectively, indicating good agreement between H₂S.48 and MTFT test. The specificity (95-89%) was also very high indicating good agreement or correlation between these two tests (Fig. 1).

Table 1: Calculation of sensitivity, specificity predictive value and efficiency of H₂S test with standard test

H ₂ S test	MTFT/MFT/TTC	
	Polluted	Safe
Polluted	True positive (a)	False positive (b)
Safe	False negative (c)	True negative (d)

Sensitivity = $a \times 100/a+c$, Specificity = $d \times 100/b+d$, Predictive value for +ve = $a \times 100/a+b$, Predictive value for -ve = $d \times 100/c+d$, Efficiency = $a+d \times 100/a+b+c+d$ (Accuracy)

Table 2: Comparison of rapid H₂S test with standard tests

a: Comparison of H ₂ S/24 test with MTFT				
H ₂ S/24	Source	MTFT		Total
		Polluted	Safe	
Polluted	Railway station water	36	4	40
	House hold water	0	0	0
	Surface water	62	0	62
	Tube well water	29	0	29
	Open well water	51	2	53
	Total	178	6	184
Safe	Railway station water	92	41	133
	House hold water	40	61	101
	Surface water	26	0	26
	Tube well water	44	2	46
	Open well water	33	2	35
	Total	235	106	341

b: Comparison H ₂ S/48 test with MTFT				
H ₂ S/48	Source	MTFT		Total
		Polluted	Safe	
Polluted	Railway station water	90	9	99
	House hold water	8	1	9
	Surface water	87	0	87
	Tube well water	56	0	56
	Open well water	75	2	77
	Total	316	12	328
Safe	Railway station water	38	36	74
	House hold water	32	60	92
	Surface water	1	0	1
	Tube well water	17	2	19
	Open well water	9	2	11
	Total	97	100	197

c: Comparison H ₂ S/48 test with MFT				
H ₂ S/48	Source	MFT		Total
		Polluted	Safe	
Polluted	Railway station water	25	74	99
	House hold water	1	8	9
	Surface water	29	58	87
	Tube well water	21	35	56
	Open well water	28	49	77
	Total	104	224	328
Safe	Railway station water	14	60	74
	House hold water	26	66	92
	Surface water		1	1
	Tube well water	3	16	19
	Open well water	4	7	11
	Total	47	150	197

d: Comparison H ₂ S/48 test with TTC				
H ₂ S/48	Source	TTC		Total
		Polluted	Safe	
Polluted	Railway station water	35	64	99
	House hold water	6	3	9
	Surface water	35	52	87
	Tube well water	21	35	56
	Open well water	25	52	77
	Total	122	206	328
Safe	Railway station water	8	66	74
	House hold water	2	90	92
	Surface water	0	1	1
	Tube well water	3	16	19
	Open well water	4	7	11
	Total	17	180	197

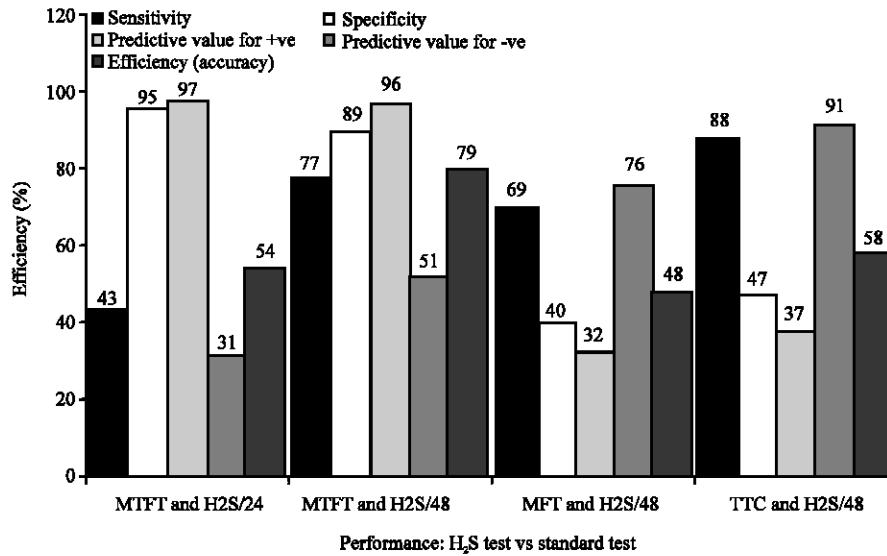


Fig. 1: Sensitivity, specificity, predictive value and efficiency of H₂S test

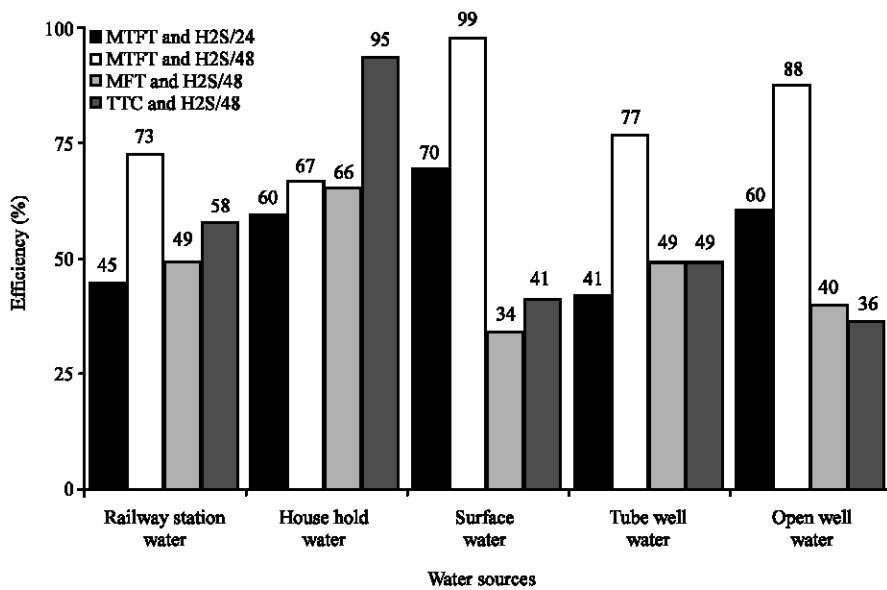


Fig. 2: Efficiency (accuracy) of H₂S test with water from various sources

The efficiency of H₂S test either in 24 h or 48 h incubation when compare with standard water quality tests was varies with source of drinking water. When compared with MTFT, it was 70-99% in surface water, 60-88% in open (dug) well water, 41-77% in tube well water, 45-73 in railway station water and 60-67% in house hold water indicating good agreements between these two test for surface, open well and household water (Fig. 2). This clearly indicated that more coliforms per 100 mL lead to more accurate H₂S test and good agreement.

H₂S test indicated good agreement with TTC (95%) and MFT (66%) in household water. Higher the MPN index, higher the agreement between modified H₂S test, MFT and TTC, indicating fecal

thermotolerant coliform always associated with H₂S producing microorganisms. Thus indicated that H₂S test can detect the fecal pollution efficiently and efficiency of this test was upto 99% and decreased with the depth of the water source. Therefore the test is more reliable for surface, open well and household water where there's direct faecal contamination due to human activities.

CONCLUSIONS

The H₂S test (48 h incubation) was comparable to MPN method based on presence of thermotolerant coliforms. Thus the H₂S was found to be more suitable, reliable, inexpensive, easy to perform and most useful to detect faecal contamination in drinking water within 24 to 48 h. It also proved suitable to assess microbiological quality of drinking water and useful in routine screening for large number of samples for places where time, man and laboratory facilities are very poor. In principle, the test does not conform to the conventional standards of bacteriological testing of water samples and cannot replace the conventional MTFT, MFT or TTC test. However, the H₂S test is easy to perform, user-friendly, screening test, suitable for handling by untrained personnel for community participation in monitoring of rural drinking water sources and low cost rapid test, hence recommended for the routine monitoring of water for recent faecal contamination in the field or villages where technical expertise, infrastructure and incubation equipment are not readily available.

REFERENCES

- APHA., 1998. Standard Methods for the Examination of Water and Wastewater. Andrew, D.E., L.S. Classer and A.E. Greenberg (Eds.), 20th Edn., Am. Public Health Assoc., Washington DC., Part, 9000: 9-140.
- Genthe, B. and M. Franck, 1999. A tool for assessing microbial quality in small community water supplies: An H₂S strip test: Water research commission. Division of Water, Environment and Forestry Technology, CSIR, Stellenbosch, South Africa. WRC Report No. 961/1/99: 1-33.
- Grant, M.A. and C.A. Ziel, 1996. Evaluation of a simple screening test for faecal pollution in water. *J. Water SRT-Aqua*, 45: 13-18.
- Hirulkar, N.B. and D.H. Tambekar, 2006. Suitability of the H₂S test for detection of fecal contamination in drinking water at various temperatures and incubation period. *Afr. J. Biotechnol.*, 5: 1025-1028.
- Kaspar, P., I. Guillen, D. Revelli, T. Meza, G. Velasquez, H. Mino de Kaspar, L. Pozzoli, C. Nunez and G. Zouiek, 1992. Evaluation of simple screening test for the quality of drinking water systems. *Trop. Med. Parasitol.*, 43: 124-127.
- Manja, K.S., M.S. Maurya and K.M. Rao, 1982. A simple field-tests for the detection of faecal pollution in drinking water. *Bull. World Health Org.*, 60: 797-801.
- Manja, K.S., R. Sambasiva, K.V. Chandra Shekhara, K.J. Nath, S. Dutta, K. Gopal, L. Iyengar, S.S. Dhindsa and S.C. Parija, 2001. Report of study on H₂S test for drinking water, UNICEF, New Delhi.
- Mark, D.S. and F.K. Pfaender, 2002. Evaluation of H₂S method for detection of faecal contamination of drinking water. WHO/SDE/WSH/02.08, Geneva.
- Pathak, S.P. and K. Gopal, 2005. Efficiency of modified H₂S test for detection of fecal contamination in water. *Environ. Monit. Assess.*, 108: 59-65.
- Pillai, J., K. Mathew, R. Gibbs and G. Ho, 1999. H₂S Paper strip method: A bacteriological test for faecal coliforms in drinking water at various temperatures. *Water Sci. Technol.*, 40: 85-90.
- Rijal, G.K., R.S. Fujioka and C.A. Ziel, 2000. Evaluation of the hydrogen sulphide bacterial test. A simple test to determine the hygienic quality of drinking water. Abstracts of the General Meeting of the American Society for Microbiology Abstract. *Am. Soc. Microbiol.*, pp: 354.

- Sivaborvorn, 1988. Development of simple test for bacteriological quality of drinking water (Water quality control Southeast Asia). Department of Sanitary Engineering, Mahidol University. Thailand Center File 3-P-83-0317-03. International Development Research Center, Canada.
- Tambekar, D.H., N.B. Hirulkar, S.R. Gulhane, Y.S. Banginwar and N.S. Bhajipale, 2006. Efficacy of rapid H₂S test for detection of fecal contamination in drinking water. *J. Curr. Sci.*, 9: 139-144.
- Venkobachar, C., D. Kumar, A. Talreja and I. Lyengar, 1994. Assessment of bacteriological quality using a modified H₂S strip test. *Aqua (Oxford)*, 43: 311-314.
- WHO., 2002. Indicators (draft documents) OECD. World Health Organization, Geneva, pp: 1-62.