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Full Length Research Paper

Evaluation of hydrogen sulphide test for detection of fecal coliform contamination in drinking water from various sources

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The assessment of H_2S field test for detection of potability of drinking water was evaluated by analysing 1050 water samples from various sources at room temperature and at 37ºC after 18, 24, and 48 h of incubation. The H_2S test showed 100, 84 and 89% correlation with Eijkman test, Membrane Filter Technique (MFT) and Most Probable Number (MPN) test for coliform, respectively. In comparisons with MPN the H_2S test showed 84% correlation with open well water, 80% with tube well water and 94% with hotels and restaurants water at room temperature, indicating decrease in efficacy of this test with depth of source of water. The test can be used in the field or in village level without any skilled personnel. Hence the test can be recommended for detection of fecal contamination in drinking water in the field where laboratory facilities are limited.

Key words: Rapid field test, MFT, MPN, Eijkman test, coliforms.

INTRODUCTION

The water quality and surveillance in most developing countries is inadequate to test all drinking water resource regularly, this is largely due to poor laboratory facilities, widely spread water sources and resource crunch. The 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. In such a scenario, a reliable and easy to use field test can help in effective monitoring of drinking water and water sources by users themselves. In 1982 K.S. Manja (DRDO, Gwalior, India) developed, a H_2S rapid field test, based on production of hydrogen sulphide by bacteria that are associated with fecal contamination. 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.

Sivaborvorn (1988) tested a variety of water (shallow and deep wells, rainwater, pond water) in Thailand by the H_2S test and by MPN test and found that these two tests agreed 85% and 88%. Kaspar et al. (1992) concluded that the H_2S test was not suitable for detection of coliforms in surface water and dug well water. Venkobachar et al. (1994) developed a modified H_2S test, which reduced the test time. Genthe and Franck (1999) evaluated the H_2S test and reported favorable to water samples from various source, including ground and surface water.

Pillai et al. (1999) evaluated various modification of H_2S test for detection of fecal contamination using feces diluted in distilled water. Rijal et al. (2000) compared two versions of the H_2S test, MPN and a membrane filter enumeration and recorded that the MPN, MF, modified version of the H_2S test achieved similar detection of bacterial contamination. Ratto et al. (1997) evaluated the original H_2S test at incubation temperatures of 22 and 35℃ and compared it to MPN and P-A total coliform (TC) and fecal coliform (FC) tests. The frequency of positive (unsuitable) samples was similar but not identical for all tests. Castillo et al. (1994) reported that the H_2S test produced about 10% more H_2S test positive samples than the coliform test. Marks et al. (2002) found 100% agreement between total coliform and H_2S results for raw waters and 81% agreement for treated waters. The H_2S test is particularly suitable in developing countries with

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Table 1. Composition of H_2S medium.

Chemicals	Quantity
Bacteriological grade peptone	20.0 g
Di-potassium hydrogen phosphate	1.5 g
Ferric ammonium citrate	0.75 g
Sodium thio-sulphate, A.R.	1.0 g
L-Cysteine HCl	0.125 g
Teepol or labolene (neutral pH)	1.0 ml
Total volume of the medium w/water = 50 mL	

ambient temperature between 25^0- 44^0C (Pathak and Gopal, 2005). The H_2S test was evaluated at various temperature and incubation period and reported that the test may be alternative to standard test and can be used in the field without any infrastructure (Tambekar et al., 2006; Hirulkar and Tambekar, 2006).

Though various people test the validity of the H_2S test with MPN or MFT for detection of fecal contamination of drinking water, further validation is required to opt the test as standard test for detection of quality of drinking water. Moreover W.H.O. and A.P.H.A. needs standardization of this method for use in developing countries (WHO, 2002). Hence attempt was made to evaluate the H_2S test for various incubation temperatures, incubation periods, and various sources and compared the results with known standard test for detection of fecal contamination in drinking water.

MATERIALS AND METHODS

A total of 1050 water samples collected from tube well (355 water samples), open well (355 water samples) and hotels and restaurants (340 water samples) were analysed by each of the test mentioned below.

The modified H_2S test medium of Manja's et al. (2001) was used in the study (Table 1). One ml of modified H_2S medium was added in each 30 ml screw cap battle and sterilized at 121°C at 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 (RT) and 37°C for 18, 24 and 48 h of incubation. The positive H_2S test or fecal contamination or pollution in drinking water indicated by change in color of the medium to black.

MPN test was performed by nine multiple tube dilution technique using double and single strength MacConkey purple medium for all water samples. MFT test by using M-EC, (M-1095, Hi-media pvt. Ltd, Mumbai, India) and Eijkman test (detection of thermotolerant coliforms) by using Brilliant Green Lactose Bile (BGLB) and indole test at 44.5°C were performed for each water sample as per standard procedure (APHA, 1998).

Blacking in H_2S medium was recorded after 24 and 48 h of incubation at RT and at 37°C. Only MPN positive water samples were further inoculated for Eijkman test in BGLB medium at 44.5°C for 24 h and positive results were recorded as gas in BGLB and indole positive at 44.5°C. Statistical analysis was made by computer based SPSS software.

RESULTS AND DISCUSSION

The H_2S method has been extensively studied by a number of investigators in different parts of the world. Such studies include evaluations of the original method, studies on modifications of the method and field-testing, usually with side-by-side comparison to other water quality tests. In some of these comparison studies the data are limited or have not been subjected to rigorous statistical analysis. However, the results of most studies suggest that the H_2S method detects fecal contaminated water with about the same frequency and magnitude as the traditional methods to which it was compared. In general, the sensitivity of the H_2S test appears about the same as other tests for fecal contamination of water, although, this aspect of the test has not been rigorously tested in some of the reported studies. Testing conditions and format, sample size, incubation temperature and incubation time influences test sensitivity and source of water. Because these conditions have differed among the different studies reported in the literature, it is difficult to make consistent comparisons and draw overall conclusions. However when comparisons with other methods of detecting fecal contamination were done, the H_2S method appeared to have sensitivity similar to the other methods, based on finding contaminated samples.

In present study a total of 1050 water samples were tested by standard MPN technique, MFT, H_2S method and Eijkman test. The results obtained after 18, 24 and 48 h of incubation of H_2S test, presence of coliforms by MPN method and confirmation of thermo tolerant coliform (TTC) fecal contamination by Eijkman test were recorded. Out of 1050 water samples analysed, 724 (69%) were positive by MPN test (>10 coliforms/100 ml), 767 (73%) by MFT test and 181 (17%) by Eijkman test. The results showed that H_2S test was 89% agreeable with MPN, 84% with MFT and 100% with TTC. Out of all 1050 water sample 326 water samples were negative by MPN test. Out of these 326 MPN negative water samples, 121 water samples were positive by MFT, indicating higher degree of detection of fecal contamination by MFT (Table 2).

On the basis of statistical analysis, it was shown that out of 724 (100%) MPN positive water samples, 161 (22%) in 18 h, 363 (50%) in 24 h and 621 (86%) in 48 h water samples were positive at RT, while at 37ºC, it was 308 (42%) in 18 h, 446 (62%) in 24 h and 643 (89%) in 48 h by H_2S test. It was shown that out of 767 (100%) MFT positive water samples, 21% in 18 h, 47% in 24 h, and 81% in 48 h water samples were positive at RT, while at 37ºC, 40% in 18 h, 58% in 24 h and 84 % in 48 h were positive by H_2S test. When compared with Eijkman (TTC) test, the H2S test was more than 100% agreeable (Table 2). It indicated that the efficacy of H_2S test was depend on incubation temperature and period and it was maximum up to 86 - 89% when compared to standard test. The incubation period had prominent effect on the

Table 2. Comparisons of H_2S test at various temperature and incubation period with MPN, MFT and TTC tests.

Result	MPN	Test	MFT	TTC	H_2S Test					
					RT/18H	RT/24H	RT/48H	37/18H	37/24H	37/48H
All test +ve	724 (69%)	+ve	767 (73%)	181 (17%)	161 (15%)	363 (35%)	621 (59%)	308 (29%)	448 (43%)	643 (61%)
Compare with MPN (100%)			100%	25%	22%	50%	86%	42%	62%	89%
Compare with MFT (100%)			100%	24%	21%	47%	81%	40%	58%	84%
All test -ve	326	-ve	283	869	889	687	429	742	602	407
Total	1050		1050	1050	1050	1050	1050	1050	1050	1050
MPN Index 0 - 9	326	+ve	121 (37%)	3 (1%)	12 (4%)	16 (5%)	55 (17%)	10 (3%)	19 (6%)	61 (19%)
		-ve	205	323	314	310	271	316	307	265
MPN Index 11-100	304	+ve	255	32	31	104	217	69	136	226
		-ve	49	272	273	200	87	235	168	78
MPN Index 101- 300	96	+ve	89	27	26	57	76	50	65	81
		-ve	7	69	70	39	20	46	31	15
MPN Index 460- 2400	324	+ve	302	119	92	186	273	179	228	275
		-ve	22	205	232	138	51	145	96	49
Total	**1050**		**1050**	**1050**	**1050**	**1050**	**1050**	**1050**	**1050**	**1050**

Table 3. Comparison of Efficacy of H_2S test at RT with MPN and MFT.

H_2SRT.48	H_2SRT.24	H_2SRT.18	MPN						
			MPN Index		0-9	11 -100	101- 300	460-2400	Total
-ve	-ve	-ve	MFT	-ve	186	19	1	1	207
				+ve	85	68	19	49	221
			Total		271	87	20	50	428
+ve 55	-ve	-ve	MFT	-ve	15	12	3	5	35
				+ve	24	101	16	82	223
			Total		39	113	19	87	258
	+ve 16	-ve	MFT	-ve	2	14	2	6	24
				+ve	2	59	29	89	179
			Total		4	73	31	95	203
		+ve 12	MFT	-ve	2	4	1	10	17
				+ve	10	27	25	82	144
			Total		12	31	26	92	161

efficacy of H_2S test and it is from 22 to 86% at RT, while at 37ºC, it is from 42 to 89%. There was higher degree of percentage correlation between H_2S test and Eijkman test when the MPN index was 460 to 2400 (Table 2). This evident that H_2S producing organisms are having coexistence with coliforms especially of fecal origin.

Out of 326 MPN negative, 121 (37%) water samples were MFT positive, while at RT out of 55 MPN negative, 10 (18%), 16 (29%) and 55 (100%) water samples were H_2S test positive in 18, 24 and 48 h, respectively (Table 3). Out of 61 MPN negative water samples at 37ºC, 10 (16%), 19 (31%) and 61 (100%) were H_2S test positive in 18, 24, and 48 h, respectively (Table 4)

A total of 1050 water samples, 355 from open well, 355 from tube well and 340 from hotels and restaurants were analysed. Out of 355 open well water samples, 256 were positive by MPN, 254 by MFT, 69 by TTC and 225 by H_2S test. When the efficacy of H_2S test was compared with MPN test for open well water, it showed 21, 53 and 84% at RT and 44, 65 and 89%, while with MFT it was 44, 65 and 86% in 18, 24 and 48 h of incubation respectively and with TTC it was 326% (Figure 1b). Out of 355

Table 4. Comparision of efficacy of H_2S test at 37^0C with MPN and MFT.

H_2S37.48	H_2S37.24	H_2S37.18	MPN		MPN Index				Total
					0-9	11-100	101-300	460-2400	
-ve	-ve	-ve	MFT	-ve	180	15	1	1	197
				+ve	85	63	14	47	209
			Total		265	78	15	48	406
+ve 61	-ve	-ve	MFT	-ve	19	16	1	4	40
				+ve	23	74	15	44	156
			Total		42	90	16	48	196
	+ve 19	-ve	MFT	-ve	2	9	2	4	17
				+ve	7	58	13	45	123
			Total		9	67	15	49	140
		+ve 10	MFT	-ve	4	9	3	13	29
				+ve	6	60	47	166	279
			Total		10	69	50	179	308

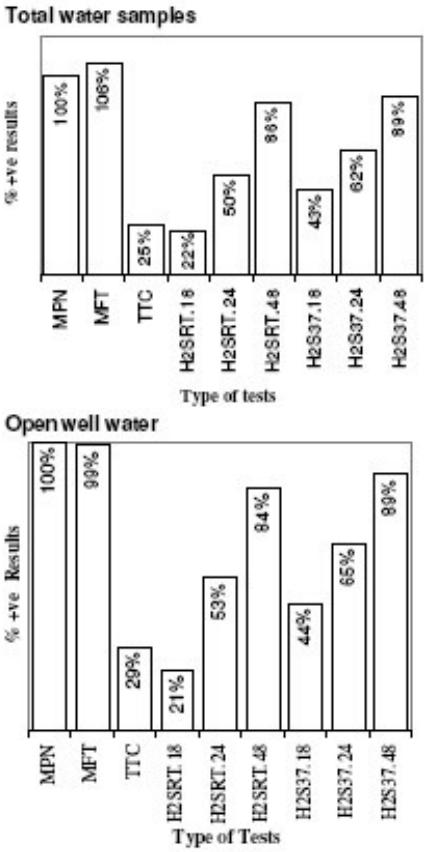

Figure 1a and 1b. Efficacy of H2S test based on source of water, incubation temperature and period. (**1a:** Total water samples, **1b:** Open well water).

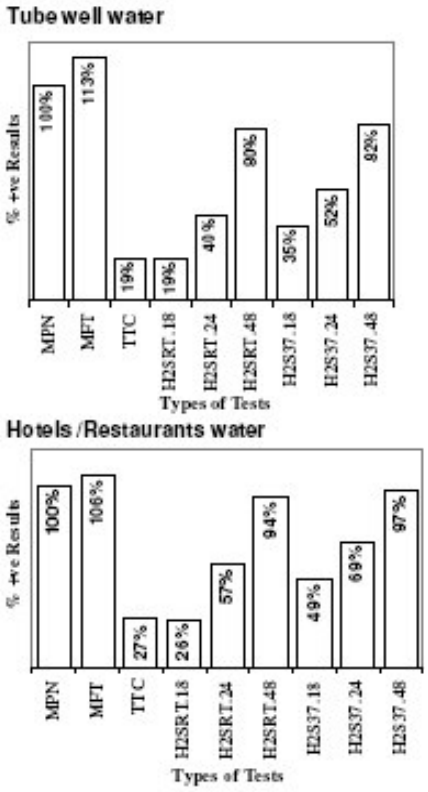

Figure 1c and 1d. Efficacy of H2S test based on source of water, incubation temperature and period. (**1c:** Tube well water, **1d:** Hotels / Restaurants water).

tube well water samples, 234 were positive by MPN, 265 by MFT, 37 by TTC and 177 by H_2S test. When the efficacy of H_2S test was compared with MPN test for tube well water, it showed 19, 40 and 80% at RT and 35, 52 and 82%, while with MFT it was 16, 33 and 71% in 18, 24 and 48 h of incubation, respectively (Figure 1c). Out of

340 hotel and restaurant's water samples, 235 were positive by MPN, 249 by MFT, 62 by TTC and 220 by H_2S test. When the efficacy of H_2S test was compared with MPN test for hotels and restaurant's water, it showed 27, 57 and 94% at RT and 49, 69 and 97%, while with MFT it was 46, 50 and 90% in 18, 24 and 48 h of incubation respectively (Figure 1d).

When the H_2S test is compared with standard tests to identify FC, the agreement rates ranged from 90 to 94.4% by Grant and Ziel (1996), 111.1% by Castillo et al. (1994), and 140% by Ratto et al. (1997). Grant and Ziel (1996) also found an 80% agreement with C*lostridium perfringens,* which were known to be of strong fecal origin. These numbers showed that the H_2S test is a very good surrogate (>90% correlation) for the standard test to identify FC. From the previous studies cited above, it appears that the H_2S test is a more sensitive test than other FC tests. The H_2S test is more likely to overestimate the presence of FC than TC. This is also partly due to the greater specificity of the FC group.

The study indicated that the efficacy of H_2S test also depend on source of water and it was 84 - 89% in open well, 80 - 82% in tube well water and 94 - 97% in hotels and restaurant's water. It also indicated that the efficacy of H_2S test decreased with depth of water source as tube wells and open wells water usually less fecal contaminated as compared with hotels and restaurant's water. The water samples, which were negative by MPN and MFT but positive by H_2S test (false positive), may be due to non-fecal originated or soil inhabitant microorganisms.

On these results it was clearly indicated that when MPN count was very low i.e. less than 10 coliform per 100 ml or negative MPN, the percentage correlation with H_2S test was almost 100%. When the MPN test was positive or higher MPN count the percentage correlation with H_2S also increased from 84 - 97%. This clearly indicated that more coliforms per 100 ml lead to more accurate H_2S test and good correlation.

Conclusion

The study concluded that the H_2S test is more accurate for detection of fecal contamination in drinking water, where water gets contaminated by unhygienic storage, handling or collection such as in hotels and restaurants. The study also concluded that the H_2S test is a simple and versatile test that can be carried out in the field for suitable indicator of potable water quality and for the routine monitoring of water for detection of fecal contamination in the field as well as epidemics of water born diseases and applicable to tropical and subtropical potable waters. It was also found that H_2S test was more suitable alternative to conventional MPN method and most useful to detect fecal pollution in drinking water especially at village level. It could be employed for routine testing where time, man power and laboratory facilities are too meager. Therefore, this test is recommended for the routine monitoring of water for recent fecal contamination in the field where technical expertise and incubation equipment are not readily available.

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