original article

Efficiency evaluation of hydrogen sulfide-producing bacteria as an indicator in the assessment of microbial quality of water sources

Ali Shahryari, Mahnaz Nikaeen, Mehdi Hajiannejad, Maryam Hatamzadeh, Zahra Moosavian Kachuei[1], Hussein Saffari[1], Akbar Hassanzadeh[2], Mohammad Jalali[3]

Environment Research Center, Isfahan University of Medical Sciences (IUMS), Isfahan, Iran and Department of Environmental Health Engineering, School of Health, IUMS, Isfahan, Iran, [1]Vice-chancelleries for Health, Isfahan University of Medical Sciences, Isfahan, Iran, [2]Department of Epidemiology and Biostatistics, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran, [3]Food Security Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Address for correspondence:
Dr. Mahnaz Nikaeen, Environment Research Center, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: Nikaeen@hlth.mui.ac.ir

ABSTRACT

Aims: The object of this study was to assess the usefulness of the H_2S test for detection of fecal pollution of water in comparison to fecal indicator bacteria (FIB).
Materials and Methods: A total of 70 raw water samples were collected from drinking water sources in Isfahan province of Iran, aseptically in sterile containers during May-October 2012.The modified H_2S test medium of Manja *et al*. was used except that L-cysteine was added as an additional medium component. Total coliforms (TCs), fecal coliforms (FCs), and fecal streptococci (FS) were also estimated by multiple-tube fermentation method. Statistical analyses were carried out using SPSS 20 at the 95% confidence level (α = 0.05).
Results: It was found that out of 70 water samples assessed, 48.3%, 30.0%, 34.6%, and 32.9% of the samples were positive for TCs, FCs, FS, and H_2S, respectively. Analysis of data showed that 95.6%, 69.5%, and 76.9% of water samples, which were positive for H_2S test were also positive for TCs, FCs, and FS, respectively. The H_2S test was found to have the highest accuracy for the detection of FS, but it was not a suitable indicator for the prediction of FCs.
Conclusions: Our results showed that H_2S test is not a suitable alternative approach for routine water quality monitoring. However, the H_2S test could be used as an easy and economic test to assess the quality of drinking water in communities where manpower and sophisticated equipment are inadequate. More laboratory and field studies are required to assess the reliability of the method as an alternative method of traditional indicators.

Key words: Fecal indicator bacteria, H_2S test, microbial quality, water sources

Access this article online

Quick Response Code:

Website: www.ijehe.org

DOI: 10.4103/2277-9183.139740

INTRODUCTION

Fecal indicator bacteria (FIB) including total coliforms (TCs) and fecal coliforms (FCs) as well as fecal streptococci (FS) are used as traditional bacterial indicators for judging and verifying the possible presence or absence of pathogenic micro-organisms in drinking water.[1-3] However, the test of FIB requires trained technicians and developed laboratory

This article may be cited as:
Shahryari A, Nikaeen M, Hajiannejad M, Hatamzadeh M, Kachuei ZM, Saffari H, *et al*. Efficiency evaluation of hydrogen sulfide-producing bacteria as an indicator in the assessment of microbial quality of water sources. Int J Env Health Eng 2014;3:25.

equipment. In developing countries, particularly in thinly populated remote areas, lack of access to manpower and laboratory facilities is an obstacle to control and monitoring of microbiological quality of drinking water.[4,5] Under this situation, therefore, development of simple, rapid, and reliable methods to determine the fecal pollution of water samples are required.[4]

Hydrogen sulfide (H_2S) test as a reliable and inexpensive method for the detection of fecal pollution in drinking water could overcome this problem.[6] Many H_2S-producing bacteria are of fecal origin such as *Citrobacter freundii*, *Salmonella typhimurium*, *Proteus mirabilis*, *Proteus vulgaris*, *Clostridium perfringens*, and some species of *Arizona*, *Klebsiella*, and *Escherichia coli*. This test typically is performed with 10-100 mL of water samples inoculated into H_2S bottles and incubated at room temperature. Blackening of content in bottles due to reducing sulfate and other oxidized forms of sulfur indicates the fecal pollution of the water sample analyzed.[4,6-10]

Several researchers have attempted to determine the reliability of the H_2S test in the detection of fecal pollution in drinking water. Their investigation revealed that there is a good correlation between the H_2S test and traditional bacterial indicators.[5,6,10-18] Kromoredjo and Fujioka (1991) reported that the H_2S test is more efficient than the coliform test,[8] and Pathak and Gopal (2005) also demonstrated that the H_2S test is a sensitive and reliable test for the screening of bacteriological quality of water, particularly in emergency conditions such as outbreaks of water-borne infectious diseases.[7] The H_2S test is also recommended as a sufficiently reliable test in the field or at the village level without any skilled personnel[19] and could be used in many developing countries in emergencies for the detection of fecal pollution of drinking water.[20] Nair *et al*. concluded that in developing countries where the acceptable level of TCs is <10 MPN (most probable number), the H_2S method would be a good test to identify microbial pollution as a screening test for drinking water quality.[14]

However, the H_2S method has not been validated by the scientific communities such as the World Health organization (WHO).[21] Sobsey and Pfaender (2003) described that false-positive results in the H_2S test are a particular concern and recommended its use with caution, only where other alternative tests are infeasible.[4] In addition, Pathak and Gopal (2005) indicated that to improve the acceptability and authenticity of the H_2S test, more laboratory and field studies are required.[7] Wright *et al*. described that the accuracy and particularly specificity of the H_2S test is variable, and as optimal conditions for conducting the test remain unclear, they recommend that its performance be evaluated relative to standard methods, prior to its application in a new setting.[22] Based on these results and the WHO recommendation for further data about the usefulness of the H_2S test as an indicator,[4] this study was designed to assess the possibility of using the H_2S test as an indicator in the determination of fecal pollution of drinking water sources in comparison with FIB.

MATERIAL AND METHODS

This study was carried out over a seven-month period from May to November 2012. A total of 70 samples were taken from drinking water sources including well, spring, aqueduct, and river in Isfahan province, central part of Iran, with a semidry climate. In sterilized glass bottles, 500 mL of water samples were taken and transferred to the laboratory in an insulated box with cooling packs and were analyzed immediately on arrival at the laboratory. The microbiological parameters included TCs, FCs, and FS as FIB and H_2S test as an alternative fecal pollution indicator of water. FIB were assessed by multiple-tube fermentation technique (MFT) using double-strength medium in 10 series of tubes; results are reported as the MPN per 100 mL according to the *Standard Methods for the Examination of Water and Wastewater*.[23] The media and material used included lactose broth, brilliant green lactose bile broth, and EC broth for TCs and FCs; azide dextrose broth and PSE agar for FS.[23] Simultaneously, the H_2S test was performed similar to that of Manja *et al*. except that L-cysteine was added as an additional medium component. The concentrated medium used contained 40.0g bacteriological grade peptone, 3.0 g dipotassium hydrogen phosphate, 1.5 g ferric ammonium citrate, 2.0 g sodium thiosulphate, 0.25 g L-cysteine hydrochloride, 2 mL liquid detergent (Teepol), and 100 mL of water. Then, two mL of H_2S medium was added into each 30 mL screw cap bottle and sterilized at 121°C for 15 minutes. Then, 10 mL of water sample was inoculated to each H_2S medium which was formatted in a 10-tube MPN arrangement and incubated at 37°C for 24 to 48 hours. Any degree of black color in the medium within 24 to 48 hours due to iron sulfide precipitation was considered as a positive indication of fecal pollution[6,24] and results were reported similar to MPN-FIB. Statistical analyses were performed with SPSS 20. The relationship between the parameters was tested by chi-square and person correlation. A P value of <0.05 was consider significant.

RESULTS

In the present study, microbial quality of 70 drinking water sources was analyzed by the H_2S test in comparison to standard microbiological methods. The percentage of various samples of water sources that were considered microbiologically unacceptable because of the presence of analyzed indicators is presented in Table 1. It was found that out of 70 water samples assessed 48.3%, 30.0%, 34.6%, and 32.9% of samples were positive for TCs, FCs, FS, and H_2S, respectively. The results showed that 50% of the samples were contaminated by at least one microbial indicator. To identify the possible association between the H_2S test and

FIB, a correlation analysis was performed and the results are presented in Table 2. Table 3, 4, and 5 show the H_2S test characteristics in comparison to TCs, FCs, and FS tests as standard indicator bacteria, respectively.

DISCUSSION

The H_2S test is considered as a simple and low-cost alternative method to the traditional bacterial indicators in remote areas for which laboratory equipment for microbial quality monitoring of water is not readily available.[6] Analysis of data showed that a high positive correlation was found between H_2S and TCs, FCs, and FS, respectively [Table 2]. The Rijal and Fujioka (1995) study showed a significant correlation between the H_2S test and FIB. They found that the MPN-H_2S was always equal to or exceeded the MPN-TC and MPN-FC count in their test.[25] According to the chi-square analysis, 95.6%, 69.5%, and 76.9% of water samples which were positive for H_2S test were also positive for TCs, FCs, and FS, respectively. Castillo *et al*. (1994) and Ratto *et al*. (1997) reported that the agreement between the H_2S and TC is 75.0% and 87.5%, respectively.[11,26] Although the highest agreement was observed between positive samples of the H_2S test and TCs, the H_2S test was found to have the highest accuracy for the detection of FS. This observation is supported by Ramteke (1995) from three alternative tests for the detection of bacteriological water quality indicators.[27] Therefore, the H_2S test could be a useful indicator for the presence or absence of FS [Tables 3-5].

False-positive results were seen as H_2S-positive samples were found to not contain TCs, FCs, and FS in 1.4%, 10%, and 8.6% of the samples, respectively. Previous studies applying the H_2S test to groundwater samples have also demonstrated false-positive results, where H_2S-positive samples contained no FCs.[24,28] The positive H_2S test in samples that contained no detectable coliforms and FS could be a result of the presence of heterotrophic bacteria which have the ability to produce a positive H_2S test. False-negative results also were seen as H_2S-negative samples were found to contain TCs, FCs, and FS in 17.1%, 7.1%, and 5.7% of the samples, respectively [Tables 3-5]. Similar results were obtained by Nair *et al*. who reported false-negative results in some samples containing low level of coliform bacteria, <5 CFU/100 mL.[14] In the study of Genthe and Franck (1999) false-positive and false-negative results of the H_2S test were observed in 4.9% of the samples as compared with MPN test of FCs. However, a false positive is less likely to lead to a risk of disease and could be conservative in terms of human safety. In contrast, there is a great concern about the false-negative results of alternative indicators such as the H_2S test. In this case, the alternative indicator dose is not able to identify fecal polluted water and the water could be consumed.[13]

The observation of H_2S data revealed that this method has the highest sensitivity (83.2%) for the detection of FS. However, the highest specificity of the H_2S test was observed in the detection of TCs. A study conducted by Anwar et al. reported that H_2S strip test is 87.24% sensitive and 100% specific for the detection of bacterial contamination of water with a positive predictive value of 100% and a negative predictive

Table 1: Detection of indicator organisms in various samples

Source	No of samples	% of positive samples			
		TC	FC	FS	H_2S
Well	40	42.5	20.0	26.7	27.5
Spring	10	40.0	30.0	33.3	20.0
Aqueduct	15	60.0	46.7	54.5	53.3
River	5	80.0	60.0	40.4	40.0
Total	70	48.3	30.0	34.6	32.9

FC: Fecal coliform, FS: Fecal streptococci, H_2S: Hydrogen sulfide, TC: Total coliform

Table 2: Correlation matrix of analyzed microbial indicators

	H_2S	FS	FC
TCs	0.805**	0.767**	0.851**
FCs	0.797**	0.833**	
FS	0.792**		

**Starred correlations are significant at $P < 0.001$, FCs: Fecal coliforms, FS: Fecal streptococci, H_2S: Hydrogen sulfide, TC: Total coliform

Table 3: Comparison of sensitivity, specificity, positive predictive value, negative predictive value of H_2S test with total coliforms

		Multiple-tube test (total coliforms)		
		Positive	Negative	Total
H_2S test	Positive	22	1	23
	Negative	12	35	47
Total		34	36	70
Sensitivity	64.7%	Positive predictive value	95.65%	
Specificity	97.22%	Negative predictive value	74.46%	

H_2S: Hydrogen sulfide

Table 4: Comparison of sensitivity, specificity, positive predictive value, negative predictive value of H_2S test with fecal coliforms

		Multiple-tube test (FC)		
		Positive	Negative	Total
H_2S test	Positive	16	7	23
	Negative	5	42	47
Total		21	49	70
Sensitivity	76.19	Positive predictive value	69.56	
Specificity	85.71	Negative predictive value	89.36	

Table 5: Comparison of sensitivity, specificity, positive predictive value, negative predictive value of H_2S test with fecal streptococcocci

		Multiple-tube test (FS)		
		Positive	Negative	Total
H_2S test	Positive	20	6	26
	Negative	4	40	44
Total		24	46	70
Sensitivity	83.23	Positive predictive value	76.92	
Specificity	88.23	Negative predictive value	90.91	

value of 76.25%. In addition, the samples which were negative for TCs by multiple-tube method were also negative by the H_2S strip method.[12] Sensitivity and specificity of the H_2S test was 76.19% and 85.71%, respectively, for the detection of FCs contamination of water with a positive predictive value of 69.56% and a negative predictive value of 89.36% [Table 4]. Mack and Hewison (1988) reported that for an H_2S test to be useful, the sensitivity and specificity should be 80% at least, and the positive predictive value and negative predictive value should be 100% to accurately screen the water samples. However, they received a sensitivity and specificity of 61.5% and 62.9%, respectively, with the H_2S method in the analysis of drinking water samples.[29] Although in our results, both sensitivity and specificity of the H_2S for FS detection was above the value recommended by Mack and Hewison, (1988) positive predictive value (PPV) and negative predictive value (NPV) were less than 100%. In general, the results show that the presence or absence of FS is most accurately indicated by the H_2S test. Several studies have proved that FS are better predictive indicators of drinking water quality.[27] However, H_2S producing bacteria are not suitable alternative approach for routine water quality monitoring.

For the H_2S bacteria test to be an acceptable tool to evaluate water quality for the presence or absence of fecal pollution, data are needed indicating the micro-organisms that produce positive results in the test and conditions under which test results indicate actual fecal contamination of water; ultimately, a quantitative version of the test is needed to estimate the magnitude of fecal contamination.[10] Analysis of the data showed that there is no statistical significant difference ($P>0.05$) between all four methods (TCs, FCs, FS, and H_2S tests) in detection rate of positive water samples. This result supports the finding of the study reported by Ramteke which showed that all three methods (TCs, FS, and H_2S tests) detected the presence of indicator bacteria in water samples obtained from the spring, stream, and groundwater.[27] The highest percent of positive H_2S test was found in aqueduct and river samples. The result is consistent with Pathak and Gopal who reported that maximum H_2S-positive (100%) results were found in open well and surface water samples. They reported that H_2S test-positive samples are more than those of coliform and FC tests for untreated drinking water and may provide a greater margin to consumers than the coliform test.[7]

CONCLUSION

In general, our results show that the H_2S test is not a suitable alternative approach for routine water quality monitoring. However, the H_2S test could be used as an easy and economical test to assess the quality of drinking water in communities where manpower and sophisticated equipment are inadequate. More laboratory and field studies are required to assess the reliability of the method as an alternative to traditional indicators.

ACKNOWLEDGMENT

This research was conducted with funding from the vice chancellery for research at the Isfahan University of Medical Sciences (Grant No: 390530) as a part of PhD dissertation.

REFERENCES

1. WHO. Guidelines for drinking-water quality 3rd ed. Geneva, Switzerland: World Health Organization; 2008.
2. EPA. National primary drinking water regulations (a), United State Environmental Protection Agency, 2009.
3. Ashbolt NJ, Grabow W, Snozzi M, Snozzi M. Indicators of microbial water quality. In: Lorna Fewtrell L, Bartram J, editors. London, UK: IWA Publishing; 2001;289-316.
4. Sobsey M, Pfaender FK. Evaluation of the H_2S method for detection of fecal contamination of drinking water. Geneva, Switzerland: WHO; 2003.
5. Dufour AP, Snozzi M, Koster W, Bartram J, Ronchi E, Fewtrell L. Assessing microbial safety of drinking water: Improving approaches and methods: International Water Assn; 2003.
6. Manja KS, Maurya MS, Rao KM. A simple field test for the detection of faecal pollution in drinking water. Bull World Health Organ 1982;60:797-801.
7. Pathak SP, Gopal K. Efficiency of modified H_2S test for detection of faecal contamination in water. Environ Monit Assess 2005;108:59-65.
8. Kromoredjo P, Fujioka RS. Evaluating three simple methods to assess the microbial quality of drinking water in Indonesia. Environ Toxicol Water Qual 1991;6:259-70.
9. Pillai J, Mathew K, Gibbs R, Ho GE. H_2S paper strip method—A bacteriological test for faecal coliforms in drinking water at various temperatures. Water Sci Technol 1999;40:85-90.
10. McMahan L, Devine AA, Grunden AM, Sobsey MD. Validation of the H_2S method to detect bacteria of fecal origin by cultured and molecular methods. Appl Microbiol Biotechnol 2011;92:1287-95.
11. Castillo G, Duarte R, Ruiz Z, Marucic MT, Honorato B, Mercado R, *et al*. Evaluation of disinfected and untreated drinking water supplies in chile by the H_2S paper strip test. Water Res 1994;28:1765-70.
12. Anwar MS, Chaudhry NA, Tayyab M. Bacteriological quality of drinking water in Punjab: Evaluation of H_2S strip test. J Pak Med Assoc 1999;49:237-40.
13. Genthe B, Franck M. A tool for assessing microbial quality in small community water supplies: An H_2S strip Test. Water Research Commission: Division of Water, Environment and Forestry Technology, CSIR, Stellenbosch, South Africa, 1999.
14. Nair J, Gibbs R, Mathew K, Ho GE. Suitability of the H_2S method for testing untreated and chlorinated water supplies. Water Sci Technol 2001;44:119-26.
15. Hirulkar N, Tambekar DH. Suitability of the H_2S test for detection of fecal contamination in drinking water. Afr J Biotechnol 2006;5:1025-8
16. Gupta S, Sheikh MA, Islam MS, Rahman KS, Jahan N, Rahman MM, *et al*. Usefulness of the hydrogen sulfide test for assessment of water quality in Bangladesh. J Appl Microbiol 2008;104:388-95.
17. Young Hwang RE. Six-month field monitoring of point-of-use Ceramic water filter by using H2S paper strip Most probable number method in San Francisco Libre, Nicaragua. Massachusetts Johns Hopkins University, 2003.
18. Smith MK. Microbial contamination and removal from drinking water in the TERAI region of Nepal Massachusetts Johns Hopkins University, 2001.
19. Tambekar DH, Hirulkar NB, Gulhane SR, Rajankar PN, Deshmukh SS. Evaluation of hydrogen sulphide test for detection of fecal coliform contamination in drinking water from various sources. Afr J Biotechnol 2007;6:713-7.
20. Mosley LM, Sharp DS. The Hydrogen Sulphide (H2S) Paper-Strip Test: SOPAC technical report 373, Feb. 2005, Available from: http://www.sopac.org/data/virlib/TR/TR0373.pdf [Last accessed on 2005 Dec 04].

21. WHO. Evaluation of the H_2S Method for Detection of Fecal Contamination of Drinking Water. Water, Sanitation and Health Department of Protection and the Human Environment. Geneva: World Health Organization; 2002.
22. Wright JA, Yang H, Walker K, Pedley S, Elliott J, Gundry SW. The H2S test versus standard indicator bacteria tests for faecal contamination of water: Systematic review and meta-analysis. Trop Med Int Health 2012;17:94-105.
23. APHA. Standard methods for the examination of water and wastewater. 21th ed. Washington D. C.: American Public Health Association, American Water Works Association, and Water Environment Federation; 2005.
24. Pant H, Sarfaraz S, Iyengar L. Evaluation of L-Cystine-amended H_2S test for the detection of faecal pollution in potable water: Comparison with standard multiple tube fermentation method. World J Microbiol Biotechnol 2002;18:321-4.
25. Rijal G, Fujioka R. A Homeowners Test for Bacteria in Cistern Waters. Proceedings of the 1995 Regional Conference on International Rainwater Catchment Systems Association; 1995.
26. Ratto A, Dutka BJ, Vega C, Lopez C, El-Shaarawani AH. Potable water safety assessed by coliphage and bacterial tests. Water Res 1989;23:253-5.
27. Ramteke PW. Comparison of standard most probable number method with three alternate tests for detection of bacteriological water quality indicators. Environ Toxicol Water Qual 1995;10:173-8.
28. Kaspar P, Guillen I, Rivelli D, Meza T, Velazquez G, Mino de Kaspar H, *et al*. Evaluation of a simple screening test for the quality of drinking water systems. Trop Med Parasitol 1992;43:124-7.
29. Mack KF, Hewison K. The PATH/NEVWRP Tests. Thai-Australian Northeast village water resoure project, Report No 47: Evaluation of a Hydrogen Sulphide Screening Test, 1998.

Source of Support: Isfahan University of Medical Sciences, **Conflict of Interest:** None declared.