African Journal of Environmental Science and Technology Vol. 4 (2), pp. 084-091, February, 2010
Available online at http://www.academicjournals.org/AJEST
DOI: 10.5897/AJEST09.187
ISSN 1991-637X © 2010 Academic Journals

Full Length Research Paper

The effects of incubation period and temperature on the Hydrogen sulphide (H_2S) technique for detection of faecal contamination in water

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Accepted 8 December, 2009

A total of 171 water samples from 3 sources were analyzed for the presence of faecal contamination by standard MTF, P/A, EC-M and H_2S techniques at different temperatures and incubation times. Analysis of water samples by H_2S technique showed that the incubation period of H_2S bottles is highly dependent on temperature and concentration of faecal coliform bacteria. Incubation temperature was changed from 22 to 45°C. At higher temperatures (45°C) the bottles turned to black after a 6 h incubation period. Correlation of H_2S technique with P/A and MTF techniques were 75.4 and 71%, respectively. Furthermore, the P/A technique showed a correlation of 60.9% with standard MTF technique. In relation to the faecal coliform and by using EC-M technique, we obtained a correlation percentage of 65.1, 56 and 62.3% for standard MTF, H_2S and P/A techniques, respectively. This study indicated that incubation period and temperature had significant effects (P = 0.05) on the efficiency of H_2S technique. The times when H_2S bottles take to turn black is dependent on the number of faecal bacteria, an indicator of the risk that pathogenic organisms are present. Based on the results obtained in this study, we concluded that H_2S technique is a reliable method that can be used as an alternative for indication of faecal contamination and drinking water quality surveillance. By using this technique at high temperatures, rapid screening of large number of water samples in a short period can be profitable especially when the number of drinking water sources is high.

Key words: H_2S technique, MTF, P/A, EC-M, faecal contamination, drinking water.

INTRODUCTION

In developing countries the contamination of drinking water sources has been commonly reported (Sumantewari and Ramteke, 2003). The health hazards from polluted water are evident from the fact that about 80% of infectious diseases are water related. Since most of these diseases are transmitted through human faeces, the condition is more serious in densely populated areas with inadequate sanitation and sewerage facilities (Pillai et al., 1999). Micro-biological and chemical testing of drinking water quality should be performed to indicate whether water is safe to drink. Unfortunately, in many Pacific Islands the infrastructure needed to adequately monitor water quality is either non-existent or inadequate (Mosely and Sharp, 2005).

Sophisticated and costly equipment is required to test for indicator and enteric organisms; that is an incubator, filtration apparatus and chemical reagents, which must be stored under refrigeration (Mosely and Sharp, 2005).

The standard test for the coliform group may be carried out by the multiple-tube fermentation technique (MTF), presence-absence procedure, membrane filter (MF) technique or by enzymatic substrate coli form test. Each technique is applicable within the limitations specified and with due consideration of the purpose of the examination (Nicholas et al., 2001). Many limitations and complications have been associated with the faecal coliform assay, thereby raising questions about

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its continued appropriateness and usefulness in water testing (Doyle and Erickson, 2006).

In all cases, the time elapsed between collection and examination should not exceed 24h (Martins and Pellizari, 1990). Although there are several commercially available portable kits that make it possible to carry out on-site water quality testing, these are usually costly and require technical expertise to operate. An alternative low-cost test for faecal contamination in drinking water which is simple to use and easy to interpret is the hydrogen sulphide (H_2S) paper-strip test (Mosely and Sharp, 2005).

Various investigators have tested this method and various modifications of it in different tropic and temperate regions, including Indonesia, Peru, Paraguay and Chile, Nepal and South Africa and compared it to traditional bacterial indicators of faecal contamination of drinking water (Sivaborvorn, 1998). The results of their studies generally indicate that the method gives results comparable to the test for traditional bac-terial indicators of faecal contamination and is sometimes superior to these tests in detecting faecal contamination, based on other criteria for evidence of faecal contamina-tion. Furthermore, some studies indicate that the method worked well as a presumptive test for the detection of *Salmonella* (Sobsey and Pfaender, 2002).

The test is based on measuring bacteria that produce hydrogen sulfide under the test conditions employed. However, some coliform bacteria (e.g., *Citrobacter* spp.), some other enteric bacteria (e.g., *Clostridium perfringens*) as well as many other types of bacteria produce H_2S. The test measures the production (actually, the presence) of H_2S by its reaction with iron to form an insoluble, black precipitate of iron sulfide sulfide (Muller and Catherine, 2002). Given the low solubility of iron sulfide, the test can detect even small amounts of sulfide forma-tion or presence. Any source of H_2S in the sample can lead to a positive result. Sulfides can also be formed by abiotic chemical reactions. Many different bacteria, from a variety of habitats, including many of enteric origin, can release sulfide from proteins, amino acids and other reduced sulfur compounds by reduction reactions. There-fore, there are many possible sources of a positive result in the H_2S test (Sobsey and Pfaender, 2002).

In evaluations of the H_2S test, several investigators have attempted to identify by speciation, the bacteria present in positive H_2S tests. Castillo et al. (1994) found a large variety of bacteria, primarily various Enterobac-teriaceae and *C. perfringens*, in samples giving positive reactions in the H_2S test: *Enterobacter, clostridia, Klebsiella, Escherichia, Salmonella, Acinetobacter, Aeromonas* and *Morganella*. Ratto and coworkers (1989) found Citrobacter to be a common organism in positive H_2S tests. This suggests that while the test organisms may not be all coliforms they are organisms typically associated with the intestinal tracts of warm-blooded animals. Because some of these microbes may arise from faecal contamination of non-human origin, the test is not specific for human faecal contamination.

H_2S is a direct intermediate in three of these reactions: mineralization, sulfur oxidation and sulfate reduction, all of which can be mediated by various microbes (Sobsey and Pfaender, 2002).

Sulfides are produced by assimilatory and dissimilatory sulfate reduction. H_2S may result from the anaerobic decomposition by proteolytic bacteria (e.g., *Clostridia Vellionella*) of organic matter containing S amino acids (Assimilatory Sulfate Reduction) such as methionine, cysteine and cystine (Gabriel, 2005). Sulfate reduction is the most important source of H_2S in wastewater. It is the reduction of sulfate by strict anaerobes, the sulfate reducing bacteria (Dissimilatory Sulfate Reduction). In the absence of oxygen and nitrate, these strict anaerobic bacteria use sulfate as the terminal electron acceptor. They use low-molecular weight carbon sources (e.g., electron donors) produced via the fermentation of carbohydrates, proteins and other compounds. H_2 is also used as electron donor. These bacteria have very low cell yields (Gabriel, 2005).

In this study we evaluated the sensitivity, specificity, Predictive values for positive(+v) and negative(-v), accuracy and optimal condition of H_2S techniques in comparison with standard microbiological examination methods such as standard multiple tube fermentation and Presence /absence test for detecting faecal contamination of drinking water resources in developing countries. In this study the following targets have been defined: (1) Evalua-tion of the H_2S technique can be done by estima-tion of H_2S bacteria concentration which is obtained by using multiple dilutions and sample volumes in the MTF method; (2) Comparison of MTF (Most Probable Number), P/A (Presence/Absence) and H_2S techniques efficiencies has been done in relation to faecal conta-mination detection in drinking water; (3) Determination of optimum temperature and incubation period; (4) Determination of the accuracy and sensitivity of the H_2S technique.

MATERIALS AND METHODS

This research was conducted in Esfahan, Iran. And in this relation a total of 171 water samples from 3 sources were analyzed for the presence of faecal contamination by standard MTF, P/A, EC-M (*Escherichia coli* Medium) and H_2S techniques at different tempera-tures and incubation times. Most of the samples were collected and carried to the laboratory in sterile glass bottles from drinking water sources (ground waters, drinking water holding tankers and home piping). But some samples were prepared by adding various quantities of distilled water containing *E. coli* colonies (Pink to dark red with a green metallic surface sheen colonies developing on LES Endo agar) and were used because naturally contaminated (that is coliform and *E. coli* positive) drinking water samples were generally not available.

On the other hand, some samples were prepared by adding same quantities of distilled water containing faecal coliforms (Blue colonies developing on M-FC medium -Faecal membrane filtration procedure). Four different volumes of each simulated contaminated drinking water sample were filtered through 0.45 µm pore size cellulose ester MFs in triplicate (APHA et al., 2005).

Table 1. Comparison of two techniques for detection of efficiency (accuracy), predictive value for +ve, predictive value for –ve, specificity and sensitivity.

MPN(Most probable number) or P/A(Presence/Absence) test			
Safe	**Polluted**		
(b) False Positive	(a)True Positive	Polluted	H_2S Test
(d)True Negative	(c) False Negative	Safe	

Sensitivity = $\frac{a}{a+c}\times 100$, Specificity = $\frac{d}{b+d}\times 100$, Predictive value for +ve = $\frac{a}{a+b}\times 100$, Predictive value for - ve = $\frac{d}{c+d}\times 100$, Accuracy = $\frac{a+d}{a+b+c+d}\times 100$.

The standard tests for coliforms were done by the multiple-tube-fermentation technique and presence-absence procedure (through the presumptive and confirmed phases). The culture medium which was used for presumptive and confirmed phases was lauryl tryptose broth (double concentration) and brilliant green lactose bile broth (BGBB). Gas production in BGBB tubes was an indicator for detecting faecal cloiforms after a 48 h incubation period at 44.5°C. The presence of *E. coli* in confirmed positive samples was determined by faecal coliform test (EC medium). Presence-Absence (PA) test was performed as described by standard methods for the examination of water and wastewater. Screw-cap 250 ml milk dilution Bottles (capacity 150 ml) were filled with 50 ml media (double strength) and autoclaved. A 100 ml sample was inoculated and the bottles were incubated at 35 ± 0.5°C for 48 h (APHA et al., 2005).

H_2S test was performed as described by Kaspar et al. (1992). The H_2S medium consists of 20 g peptone, 1.5 g dipotassium hydrogen phosphate, 0.75 g ferric ammonium citrate, 1g sodium thiosulfate, 1 ml Teepol and 50 ml water (Sobsey and Pfaender, 2002). Briefly, 2 ml of concentrated medium that was prepared in our laboratory was introduced into small screw-cap 30 ml glass vials. The cap of the bottle was tightly screwed and the sample mixed completely. The vials were autoclaved and then stored in refrigerator at 4 - 5°C. A 20 ml water sample was inoculated and vials were incubated at 37°C for 48 h. Every 6 - 12 h the samples were examined for changes in colour. The date and time of each observation were recorded on the report forms and the observations were recorded as follows: (–) = no change; (+) = slight change, the water was turned gray; (++) = the water was partially black; (+++) = the water samples itself were noticeably black.

The influence of some parameters including incubation period, incubation temperature and concentration of faecal coliform were tested for H_2S method. After 6, 12, 24 and 48 h, the samples were analyzed for a colour change to black. The samples were stored at ambient temperature (20 - 23°C during the study period), 37 ± 0.5 and 44 ± 0.5°C. The efficiency (accuracy), predictive value for +ve, predictive value for -ve, specificity and sensitivity of H_2S method in comparison to a references point (such as MTF and P/A methods) were examined on the basis of Table 1 and following equations (Manafi and Kremsmaier, 2001; Chandrasekhar, 2001):

RESULTS AND DISCUSSION

A total of 171 water samples from 35 sources were analyzed for the presence of faecal contamination by standard MTF, P/A, EC and H_2S test (Table 2). More than 90% (91.66%) of positive samples in standard MPN technique were positive for H_2S method with black precipitation. In addition, 73.3 and 60% of total samples were positive for H_2S and P/A test; respectively. Stronger colour changes were noted at higher MPNs (Table 2).

On the other hand, the H_2S and P/A technique showed negative result when the MPN of sample was less than approximately 3.6 index/100 ml. When a range of temperatures from 22 to 45°C were tested, the bottles at 22 and 35°C did not turn black after 12hr. The study highlights the poor performance of the H_2S technique at lower temperature (less than 35°C).

The results shown in Table 3 show that a gray colour was produced at 6 h incubation at 45°C and that black colour did not appear until 12 h. Gray colour appeared after 12 h at 35°C. At room temperature (22°C) the incubation period may be increased to more than 48 h, because at reduced temperature cellular metabolic activities and growth decreases, therefore there will be less expression of products of microbial metabolic activities such as hydrogen sulphide formation.

The incubation period required for the H_2S bottles is highly depended on the incubation temperature and the concentration of faecal coli-forms as shown in Figure 1. An increase in the incubation period was observed with lowering the concentration of faecal coliforms at all temperatures. The bottle containing microbial concentration of ≤ 2 cfu/100 ml did not turn black at first 24 h of incubation. Black colour precipitation was also observed at the bottom of all bottles. But the colour of precipitation was changed with microbial concentration variation in bottles. It was also noticed that the black colour developed at 45°C within 6 h when concentrations were ≥ 5 cfu/100 ml (due to the high concentration of H_2S producing bacteria). Black colour developed at 45°C within 6 h when concentrations were ≥ 5 cfu/100 ml.

As shown in Figure 1, when the bacterial concentration in sample is low (2 > CFU/100 ml), the incubation period is very important to obtain true results. But the cardinal point is the minimum concentration to give true results. The present study showed that the bacterial concentration of ≤2 CFU/100 ml is necessary to obtain true results.

The sensitivity of the H_2S technique is, however, still debatable. Some research showed that as low as 1 CFU/100 ml of the bacteria will give positive results while

Table 2. Comparison of MPN (Most probable number), P/A (Presence/Absence), EC-M (*E. coli* Medium) and H_2S techniques for detection of faecal contamination.

No. of Positive Test													
EC-Medium			H_2S				P–A		Standard MPN			Total no. of H_2S Sample	Source
MPN (Index/100 ml)	N0. +	Total	+++	++	+	-	P	Total	MPN (Index/100 ml)	N0. +	Total		
2.2	2	10	0	2	3	0	2	2	12	7	10	5	1
2.2	2	10	5	0	0	0	2	2	> 23	10	10	5	2
6.9	5	10	1	4	0	0	2	2	12	7	10	5	3
3.6	3	10	0	0	5	0	0	2	6.9	5	10	5	4
< 1.1	0	10	0	0	5	0	0	2	6.9	5	10	5	5
< 1.1	0	10	0	0	0	5	0	2	< 1.1	0	10	5	6
2.2	2	10	2	3	0	0	1	1	9.2	6	10	5	7
1.1	1	10	0	0	0	5	0	1	3.6	3	10	5	8
< 1.1	0	10	5	0	0	0	1	1	> 23	10	10	5	9
< 1.1	0	10	5	0	0	0	1	1	> 23	10	10	5	10
< 1.1	0	10	0	0	0	5	0	1	< 1.1	0	10	5	11
1.1	1	10	1	2	0	0	1	1	> 23	10	10	3	12
2.2	2	10	0	3	0	0	1	1	23	9	10	3	13
2.2	2	10	0	0	3	0	1	1	12	7	10	3	14
1.1	1	10	0	0	3	0	0	1	5.1	4	10	3	15
< 1.1	0	10	0	0	0	5	0	1	< 1.1	0	10	5	16
< 1.1	0	10	0	0	2	3	1	1	< 1.1	0	10	5	17
< 1.1	0	10	0	1	1	3	0	1	< 1.1	0	10	5	18
< 1.1	0	10	0	0	0	5	0	1	< 1.1	0	10	5	19
6.9	5	10	5	0	0	0	1	1	6.9	5	10	5	20
< 1.1	0	10	2	0	4	0	1	1	< 1.1	0	10	6	21
> 23	10	10	2	3	1	0	1	1	> 23	10	10	6	22
> 23	10	10	4	0	2	0	1	1	> 23	10	10	6	23
> 23	10	10	4	2	0	0	1	1	> 23	10	10	6	24
3.6	3	10	2	2	0	1	2	2	6.9	5	10	5	25
3.6	3	10	3	1	1	0	2	2	6.9	5	10	5	26
6.9	5	10	5	0	0	0	2	2	12	7	10	5	27
3.6	3	10	3	1	1	0	2	2	6.9	5	10	5	28
<1.1	0	10	0	0	0	5	0	2	< 1.1	0	10	5	29
2.2	2	10	0	3	2	0	2	2	5.1	4	10	5	30
< 1.1	0	10	0	0	0	5	0	2	< 1.1	0	10	5	31
< 1.1	0	10	0	0	1	4	0	2	1.1	1	10	5	32
< 1.1	0	10	0	4	1	0	1	2	2.2	2	10	5	33
> 23	10	10	5	0	0	0	2	2	>23	10	10	5	34
16.1	8	10	5	0	0	0	2	2	16.1	8	10	5	35
16.1	90	350	59	31	35	46	33	52		175	350	171	Total

others showed 5 CFU/100 ml or more (Grant and Ziel, 1996). In addition, the blacking of samples will be expected to be functions of microbial population and ability of bacteria for producing H_2S gas in the water samples. As shown in Figure 1 incubation period may have direct correlation with colour changes, because producing hydrogen sulfide bacteria can thrive in samples.

The best correlation of H_2S colour development time with other bacteria levels was for faecal coliforms, faecal streptococci and *Clostridium perfringens* (Mosely and Sharp, 2005).

Most of similar studies were either at room temperature or at a constant incubation temperature of 37°C (Pillai et al., 1999). Pillai et al. (1999) found that the faecal contamination could be detected by the H_2S method at a temperature range of 20 - 44°C. They also noticed that at a lower temperature of 14°C the bottles required more than 120 h (5 days) to blacken. From the present study it

Table 3. Effect of temperature on incubation period.

Temperature	H_2S Result (Time of colour change-h-)				P/A* Method (after 24 h)
	6	12	24	48	P/A
22	-	-	+	+++	P
35	-	+	+++	+++	P
45	+	++	+++	+++	P

(–), no change; (+), slight change, the water was turned gray; (++), the water was partially black; (+++), the water samples itself were noticeably black; *P, Presence; A, Absence.

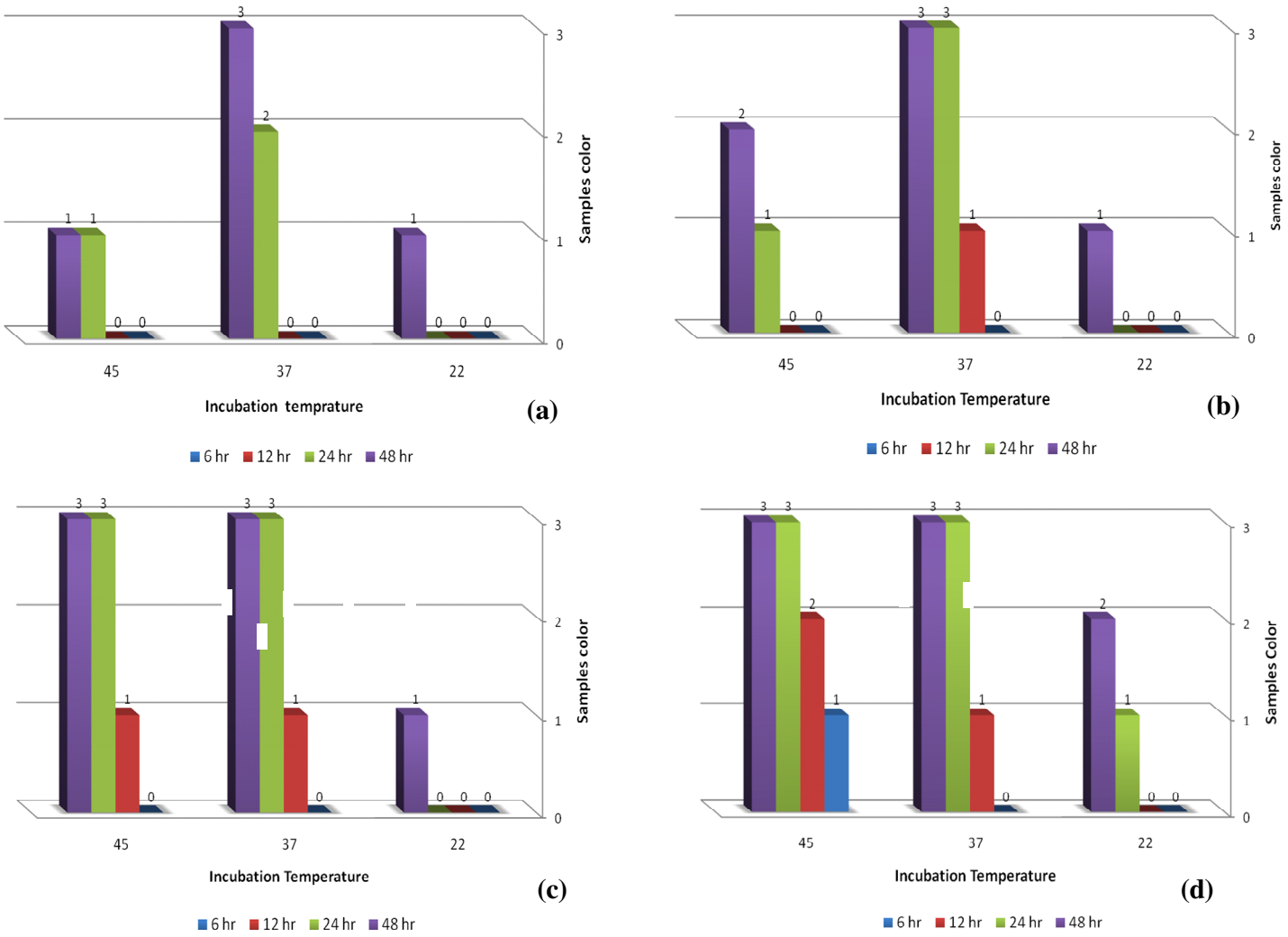

Figure 1. Incubation period for four concentrations of faecal coliforms at different temperatures in H_2S test. (0) = no change; (1) = slight change, the water was turned gray; (2) = the water was partially black; (3) = the water samples were noticeably black.) a = ≤ 2cfu/100 ml; b = 3 cfu/100 ml; c = 4 cfu/100 ml; d = ≥ 5 cfu/100 ml.

is evident that the H_2S technique could be done at temperatures of 22 - 45°C. As shown in Figure 1, the best temperature is 35°C, because most of the true results were obtained at this temperature.

It is evident that the blackening time will increase if the incubation temperature is increased from 37 to 45°C especially for lower concentration of H_2S producing bacteria. Pillai and coworker (1999) noticed that room temperature, which varied between 20 - 24°C in conventional rooms, required 60 h incubation period at lower concentration of faecal bacteria, while at 22°C incubation at constant temperature it took 90 h (Pillai et al., 1999)

Table 4. Comparison of H_2S test with P/A and MPN(Most probable number) tests as a reference tests for microbial analysis of drinking water.

H_2S vs. MPN test (%)	H_2S vs. P/A test (%)	Parameter
92.06	96.43	Sensitivity
81.82	72.41	Specificity
93.54	87.10	Predictive value for +ve
78.26	91.30	Predictive value for - ve
89.41	88.23	Accuracy

On the basis of statistical analysis, the comparison of H_2S technique with MTF technique as a reference method for microbial analysis of drinking water is summarized in Table 4.

All previous studies were in concordance that incubation period has significant effects on the efficiency of H_2S technique. Hirulkar and Tambekar (2006) showed that as incubation period increased from 24 to 48 h, the efficiency also increased from 47 to 95% at room temperature and from 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 (Hirulkar and Tambekar, 2006).

The statistical analysis of obtained data showed that the "true-positive" and "true-negative" result will be 87.1 and 91.3% for H_2S technique; respectively, If the H_2S technique is compared to P/A technique as a references point. But they will be 93.54 and 78.26%, respectively, if the H_2S technique is compared to MTF technique standard as reference points. In addition results showed that the "false-positive" and "false-negative" result will be 12.9 and 8.69% for H_2S test; respectively, If the H_2S test is compared to P/A test as a references point. But they will be 6.45 and 21.74%, respectively, if the H_2S test is compared to MTF technique standard as a reference point.

It could possibly be due to naturally-occurring sulphide-reducing bacteria being present (Leclerc and Moriamez, 2001), but we obtained similar results which were also reported by Mosely and coworkers. According to Mosely, the conditions needed for these bacteria to thrive are anaerobic waters with high organic matter and sulphate content. In this research, none of the tested water samples had these characteristics and therefore, we considered these results as unlikely to be false-positives in the sense of a natural H_2S producer being present.

Mosely and co-workers reported that total and faecal coliform contamination of water samples was 2 and 6%, respectively (false-positive). Indeed this likely was due to this fact that some H_2S reducing bacteria (e.g. *Clostridium* sp.) persist in the environment longer than coliform bacteria (Mosely and Sharp, 2005).

Chandrashekara et al. (2001) tested 686 samples by standard MTF technique and H_2S test. They noticed that the Sensitivity, Specificity, Predictive value for +ve, Predictive value for -ve and Accuracy of H_2S test are 91.32, 89.1, 91.8, 88.5 and 90.4%, respectively (Alonso et al., 1996). These values compared with values obtained in our experiment were different because the main difference in the number of samples is tested. On the other hand research conducted by Genthe and Franck (1999) on 413 water samples from various sources showed 82 and 86% agreement with faecal coliform results with test incubation temperatures of 35 and 22°C, respectively.

Data Analysis showed significant different for correlations between "H_2S vs MTF" and "H_2S vs P/A" techniques. The present study showed 75.4 and 71% correlation for "H_2S vs P/A" and "H_2S vs MTF", respecttively. As shown in Table 5, the correlation between P/A test and standard MTF technique was equal to 60.9% ($P < 0.001$).

In addition, data analysis showed that between all analyzed methods, the standard MTF technique showed more correct results for detecting faecal coliform, because the highest agreement was found for "MTF vs EC-M". The EC-M showed 65.1, 56 and 62.3% agreement with standard MTF, H_2S and P/A tests, respectively.

Higher correlation for "H_2S vs MTF", (ave. 89%) had been reported in the previous researches (Tambekar et al., 2007) which they are different with data obtained in present study. The reason for this difference is that unlike them, we continued MTF tests up to Confirmation phase.

Data Analysis showed that the correlation is dependent on the number of bacteria in samples. Higher correlation was measured at higher number of faecal coliform bacteria.

Incubation temperature had a significant effect on the correlation between all methods.

Previous studies had also confirmed that the correlation for "H_2S vs MTF" test would increased, if the incubation temperature is increased. This study showed 22, 47 and 95% correlation at room temperature and 47, 63 and 96% correlation at 37°C of H_2S technique with MTF test (Hirulkar and Tambekar, 2006). A maximum correlation of 88% was reported for "H2S vs MTF" by Sivaborvorn (1998) and Tambekar et al. (2007).

Fecal contamination of water resources are associated with high concentration of obligate anaerobes ($>10^{10}$ /g), which can produce H_2S on anaerobic conditions. Therefore, if the water source was contaminated by faecal bacteria, hydrogen sulfide method can be used to deter-

Table 5. The correlation of H_2S test with standard MPN (Most probable number) technique, P/A (Presence/Absence) and EC-M (*E. coli* Medium) test.

			P/A Result	H2S	St.MPN	St.EC
Spearman's rho	P/A Result	Correlation Coefficient Sig. (2-tailed) N	1.000	0.754**	0.609**	0.603**
			-	0.000	0.000	0.000
			35	35	35	35
	H2S	Correlation Coefficient Sig. (2-tailed) N	0.754	1.000	0.710**	0.560**
			0.000	-	0.000	0.000
			35	35	35	35
	St.MPN	Correlation Coefficient Sig. (2-tailed) N	0.609**	0.710**	1.000	0.651**
			0.000	0.000	-	0.000
			35	35	35	35
	St.EC	Correlation Coefficient Sig. (2-tailed) N	0.623	0.560**	0.651**	1.000
			0.000	0.000	0.000	-
			35	35	35	35

**, Correlation is significant at the 0.01 level (2-tailed). (Note: MPN: Most probable number, P/A: Presence/Absence and EC-M: *E. coli* Medium test.).

mine the contamination (Pamtallon et al., 2005).

In fact in H_2S test, certain hydrogen sulfide (H_2S) producing enteric bacteria such as *Salmonella* sp. and *Citrobacter* sp., associated with coliforms, have been considered for rapid detection of recent faecal contamination in water (Pathak and Gopal, 2005).

Water quality study of Tanganyika Lake (Tanzania) using hydrogen sulfide method showed that the presence of H_2S-producing bacteria in the analyzed positive bottles may be from naturally occurring bacteria, not of faecal origin and introduces the possibility of false positives (Sobsey and Pfaender, 2002). There are different types of bacteria, which can participate in the experiment by producing H_2S gas. Recent researches have shown that *Aeromonas* spp. and *aeromonads* spp. which are found in environmental water samples can cause false-positive colonies on coliform media, evaluating the total coliform (TC) count (Alonso et al., 1996).

The main limitation of this method is false positive and negative results, which can be determined through screening tests. On the other hand, multiple advantages including low cost (estimated at 20% of the cost of coliform assays), simplicity and ease of application to environmental samples have been reported by many researchers. This method can be used in area with limited laboratory facilities. Minimally trained persons can do test and the results are easy to score as negative (no visual change in the water sample) or positive (appearance of a black colour in the water sample due to iron sulfide precipitation) (Lanakila, 2007). Another limitation reported for this method is its application as the presence or absence of faecal coliform. The numbers of indicator organisms in a water sample aids in indicating the degree of contamination and therefore relative risk to public health are not shown in H_2S test. The H_2S test only indicates whether or not there is a risk.

However, the degree of contamination (bacterial density) can be determined through reaction rate (time to change color).

Conclusion

Considerable effects of faecal coli-forms concentration and temperature on H_2S bottles incubation period was one of the most important results in this study. It was proved that H_2S test can detect the presence of faecal contamination at a temperature range within 22 to 45°C; and incubation temperature is not needed to be constant if the room temperature is within the mentioned range. At a lower contamination level (1 - 2 cfu/100 ml), more time is required for the bottle to turn black. It was also noticeable that the rate of blackening depended on the concentration and temperature. In addition to incubation period and temperature, the H_2S technique detects positives samples with small sample size (10 ml) versus the 100 ml sample size of the faecal coliform tests (Murcott and Lukacs, 2003).

The results from H_2S tests are visual and therefore it is simple for the operator to distinguish the contamination, as a black colour change occurs when bacteria levels in drinking water are high. This enables communities and community health workers with minimum training to safely test their own water supplies. The colour changes during specified incubation period can be used as a reference point to determine pollution degree. In fact the needed time for H_2S test to turn black shows a correlation with faecal levels so an indication of the risk that pathogenic organisms are present can be obtained. Therefore it can be concluded that: (1) H_2S test is a reliable and alternative indicator of faecal contamination in drinking water quality surveillance and screening of

large number of water samples in short duration in the field where laboratory facilities are limited. (2) H_2S test, a simple and versatile test, can be carried out in the field within a broad range of incubation temperature and is recommended for the routine monitoring of water for detection of faecal contamination.

ACKNOWLEDGEMENTS

This research project was jointly supported by Baqiyatallah University of medical sciences and Isfahan University of medical sciences in the frame of the research program. Results are published with their permission. The authors would like to express their gratitude to Department of Environmental Health Engineering, Medical Sciences University of Baqiyatallah and Isfahan, for their support for the theoretical and laboratory research program and for helpful discussions.

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