Chapter 7

Summary
SUMMARY

The provision of safe water and the management of wastewater have a central role in reducing the incidence of many waterborne or water-related communicable diseases. The diseases associated with contaminated water, remain serious public health problem. Consumption of unsafe water continues to be one of the major causes of diarrhoeal disease deaths annually in the world, mostly in children (WHO, 2002). Thus to reduce tremendous loss of health and wealth due to waterborne infection, the water quality and surveillance in most developing countries is essential for testing all drinking water sources regularly. But this is not possible, due to inadequate laboratory facilities, widely spread water sources and resource crunch. In such a scenario, a reliable and easy to use field test can help in effective monitoring of water sources by user themselves.

The requirements for laboratory resources or field analysis kits for standard bacteriological tests of fecal contamination in drinking water are major barriers to their accessibility in many parts of the world. The resources and infrastructure are simply not available to allow for routine bacteriological testing of drinking water using the standardized methods for fecal indicator bacteria analysis. The need for sterilized bacteriological materials (media, sample bottles, sterile diluent, culture tubes, bottles or plates, membrane filters, pipettes or other volumetric dispensing devices, etc.), temperature controlled incubators, required use of aseptic technique by trained individuals, and relatively high costs make it difficult, impractical or impossible to perform these tests in many places.

The lack of availability of standard bacteriological tests for drinking water quality highlights the great need for a rapid, simple, inexpensive test for detecting the microbial quality of drinking water. This need is especially great for small
community and household water supplies that lack access to and cannot afford conventional bacteriological testing of drinking water.

To overcome this problem, in 1982 Manja and his co-workers investigated a simple method for detecting evidence of fecal contamination in drinking water. The test was intended to meet the need for a simple, reliable field test for use by village public health workers to detect fecal contamination in drinking water. The study reported that the presence of coliform bacteria in drinking water was consistently associated with organisms that produce hydrogen sulfide ($H_2S$).

Over the last two decades, various investigators have verified this method and compared it to traditional bacterial indicators of fecal contamination in drinking water (Ratto et al., 1989; Kromoredjo and Fujikawa, 1991; Kaspar et al., 1992; Castillo et al., 1994; Venkobachar et al., 1994; Martins et al., 1997; Genthe and Franck, 1999). $H_2S$ test deserve evaluation as accessible alternatives to conventional bacteriological tests for fecal contamination of drinking water. Key issues to be addressed are, whether $H_2S$ tests are sufficiently reliable and adequately developed as tests of fecal contamination of drinking water to be recommended for widespread and routine use, and if, so under which conditions what caveats and cautions should be applied. So the present study has been undertaken for the assessment of $H_2S$ method as alternative to conventional bacteriological tests for detection of fecal contamination in drinking water and determination of their reliability and predictability in detecting fecal contamination of drinking water. Thus the specific objectives were:

- To promote sustainability of safe drinking water systems;

- To assess the efficiency of $H_2S$ test with the standard methods currently in use i.e. standard MPN, membrane filtration techniques and Eijekman’s test (for thermotolerant coliform) at various incubation temperature and time.

- To determine the effect of different sources of drinking water on efficiency of the $H_2S$ test.
To determine the efficiency of test at various incubation temperature and
time period with various medium composition (e.g. labolene and bile salt)
and the efficiency of test by some modification.

To detect at which extent the test measurements are or are not indicative of
fecal contamination and under what conditions are the basis for and
likelihood of false positive and negative results.

To finalize the current state of the methodology with respect to reliability,
uniformity, practicality, availability and cost.

To determine the extent to which the tests fulfill the ideal criteria of an
indicator of fecal contamination and recommendations for future actions
and their directions.

In present study, total 1050 samples were collected in sterilized bottles from
different sources e.g. tube wells (355), open wells (355) and hotels and
restaurants (340) in Amravati District. All water samples were subjected to
bacteriological examination by using H$_2$S method, standard most probable number
(MPN) technique, membrane filtration technique (MFT) and Eijkeman's test
(APHA, 1998). The composition of H$_2$S test for 50 ml of medium was prepared
accordingly Manja et al. (2001) that content labolene and modified H$_2$S medium by
using bile salt. One mL of each H$_2$S 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. The bottles were then
incubated at room temperature and at 37°C after 18 h, 24 h and 48 h of
incubation. The positive H$_2$S test or contamination or fecal pollution in drinking
water was indicated by change in colour of the both medium to black. A
comparative study was performed at various temperatures such as room
temperature and 37°C and for various incubation time period for assessment of
efficiency of H$_2$S test with different H$_2$S medium such as H$_2$S (BS) and H$_2$S (LB).
At the same time all water samples were also tested by MPN, MFT and Eijekman’s test. MPN test was performed for total coliform and fecal coliform by nine multiple tube dilution technique using double and single strength MacConkey medium. Water samples those were positive by MPN tests were inoculated in tryptone broth (Indole test) for confirmation of E. coli and in brilliant green lactose bile broth (BGLB) for thermotolerant fecal coliform by Eijekman’s test at 44.5°C for 24hr. Membrane filtration techniques (MFT) test was done by using M-EC test agar. All the bacteriological culture media were procured from Hi-media Laboratories Pvt. Ltd., Mumbai. The data was analyzed by SPSS software and various statistical methods.

Results indicated that out of total 1050, 326 (31%) sample found potable (MPN index <10) by MPN. Out of these 326, 185 (18%) water samples found coliform density 0 and 141 water samples had coliform density 3-9, whereas 724 (69%) drinking water samples found contaminated by MPN (MPN positive).

Out of total 1050, 283 (27%) water samples were potable and 767 (73%) water samples were non-potable by MFT, whereas 869 (83%) water samples were free from thermotolerant E. coli. (TTC) and 181 (17%) water samples were confirmed fecal contaminated with TTC by Eijekman’s test at 44.5°C. At the same time all drinking water samples were analyzed by H₂S test (LB) and modified H₂S test (BS) at room temperature and at 37°C for 18 h, 24 h and 48 h of incubation period. The 888 (85%), 708 (67%), 446 (42%) water samples were potable at room temperature and 685 (65%), 564 (54%) and 371 (35%) water samples were potable at 37°C. On the other hand 162 (15%), 342 (33%) and 604 (57.5%) water samples were non potable at room temperature while at 37°C, 365 (35%), 486 (46%) and 679 (65%) water samples were non potable by H₂S test (BS) after 18 h, 24 h and 48 h of incubation period respectively. Furthermore 889 (85%), 686 (65%) and 428 (45%) water samples were potable at room temperature and 742 (71%), 602 (57%) and 405 (39%) water samples were potable at 37°C, whereas 161 (15%), 364 (35%) and 622 (59%) samples were non potable at room temperature and 308 (29%), 448 (43%) and 645 (61%) water samples were non
potable at 37°C by H₂S test (LB) after 18 h, 24 h and 48 h incubation period respectively. These findings were similar with that of Genthe and Franck (1999).

The present study also aimed to assess the effect of various sources on efficiency of H₂S test. Total 355 open well water samples were analyzed for detection of fecal contamination in drinking water. Results indicated that out of total, 98 (28%) water samples were potable and 257 (72%) water samples were non-potable by MPN technique. Whereas out of 355 open well water samples, 101 (29.5%) water samples were potable and 254 (71.5%) water samples were non-potable by MFT while 280 (79%) water samples were potable and 75 (21%) water samples were non-potable by Eijekman’s (TTC) indicated confirmed TTC contaminated. When all those water samples were analyzed by H₂S test (labolene) at various temperature and incubation time period, results indicated that out of 355 water samples, 305 (86%), 235 (66%) and 139 (39%) water samples were potable at room temperature and 225 (63%), 177 (50%) and 118 (33%) water samples were potable at 37°C by H₂S test.

It was observed that out of 355 water samples, 305 (86%), 235 (66%) and 139 (39%) water samples were potable at room temperature and 225 (63%), 177 (50%) and 118 (33%) water samples were potable at 37°C by H₂S test (BS) whereas 50 (14%), 120 (34%) and 216 (61%) at room temperature and 130 (37%), 178 (50%) and 237 (67%) water samples were non-potable at 37°C by H₂S test (BS) after 18 h, 24 h and 48 h of incubation period respectively. The 300 (84.5%), 219(62%) and 139 (39%) water samples were potable at room temperature and 242 (68%), 189 (53%) and 129 (36%) water samples were potable at 37°C whereas 55 (15.5%), 136 (38.7%) and 216 (61%) water samples were non-potable at room temperature and 113 (32%), 166 (47%) and 226 (64%) water samples were non-potable at 37°C by H₂S test (labolene) after 18 h, 24 h and 48 h of incubation with concordances to findings of Anwar et al. (1999).

A total of 355 tube well water samples were analyzed for detection of fecal contamination in drinking water and results indicated that, out of total (355),
122 (34%) water samples were potable and 233 (66%) water samples were found to be non-potable by MPN technique. Out of total 355, 91 (26%) water samples were potable and 264 (74%) water samples were non-potable by MFT and 311 (88%) potable and only 44 (12%) water sample were non-potable by Eijkman's test (TTC) whereas 304 (86%), 258 (73%) and 177 (50%) samples were potable at room temperature and 257 (72%), 258 (73%) and 177 (50%) water samples were potable at 37°C. On the other hand 51 (14%), 97 (27%) and 178 (50%) water samples were non-potable at room temperature and 98 (28%) 97 at 37°C by H₂S test (bile salt) after 18 h, 24 h and 48 h of incubation period respectively. Moreover 310 (87%), 261 (74%) and 169 (48%) water samples found to be found potable at room temperature and 169 (48%), 274 (77%) and 235 (66%) water samples were potable at 37°C, while 45 (13%), 94 (27%) and 186 (52%) non-potable at room temperature and 81 (23%) 120 (34%) and 192 (54%) water samples were non-potable at 37°C by H₂S test (labolene) after 18 h, 24 h and 48 h of incubation period respectively (Pathak and Gopal, 2005).

When 340 hotels and restaurants water samples were analyzed the results indicated that out of total, 106 (31%) water samples were potable and 234 (69%) water samples were non-potable by MPN. Out of 340, 91 (26%) water samples were potable while 249 (73%) water samples were non-potable by MFT, while 278 (82%) potable and only 62 (18%) water samples were non-potable by Eijkman's test (TTC). Whereas 279 (82%), 215 (63%) and 130 (38%) water samples were potable at room temperature and 203 (60%) 168 (50%) and 105 (31%) water samples were potable at 37°C while 61 (18%), 125 (37%) and 210 (62%) water samples were non-potable at room temperature and 137 (40%) 172 (51%) and 235 (69%) water samples were non-potable at 37°C by H₂S test (BS) after 18 h, 24 h and 48 h of incubation period respectively. The 279 (82%), 206 (61%) and 120 (35%) water samples were potable at room temperature and 226 (66%), 178 (52%) and 114 (34%) water samples were potable at 37°C while 61 (18%), 134 (39%) and 220 (65%) non-potable at room temperature and 114 (33%) 178 (52%) and 226 (66%) water samples were non-potable at 37°C by H₂S test (LB) after 18
h, 24 h and 48 h of incubation period respectively as that of the findings of Nair et al. (2001).

The observations concluded that for total water samples, H₂S test (BS) showed higher efficiency 83% at room temperature and 93% efficiency at 37°C whereas H₂S test with (LB) showed 86% efficiency at room temperature and 89% efficiency at 37°C. It was concluded that both types of surfactants (BS and LB) were suitable for H₂S test and showed good correlation with standard methods. H₂S test (BS) showed higher efficiency at both incubation temperature and for all incubation time periods. The assessment study also concluded that for open well, tube well and hotels and restaurants water samples both types of surfactants (BS and LB) are suitable for H₂S test showed good correlation with standards methods.

It was concluded that 37°C incubation temperature was highly suitable for detection of fecal contamination in drinking water. The H₂S test was developed as field test and at room temperature also H₂S test exhibited good correlation with standard methods but 37°C incubation temperature recommended for its higher efficiency for detection of fecal contamination in drinking water for all type of drinking water sources. Thus it was confirmed that the efficiency of H₂S test increases with incubation temperature.

Study concluded that 37°C incubation temperature was most suitable for both types of H₂S test (BS and LB) for detection of fecal contamination in open well water source. For tube well water sources the conclusion was that H₂S test (BS) and H₂S test (LB) showed good correlation and efficiency at room temperature as well as at 37°C but for high efficiency 37°C incubation temperature is recommended for this type of water source. The study of hotels and restaurants water samples concluded that for these types of water sources 37°C incubation temperature was most suitable for both types of H₂S test for detection of fecal contamination in hotels and restaurants water. With reference to incubation temperature the overall conclusion was that the H₂S test is highly suitable for tropical and subtropical area where ambient temperature always high.
The present study concluded that the efficiency of both types of H₂S test (BS and LB) increased with incubation time. H₂S test showed higher efficiency after 48 h, while less efficiency at lower incubation time period i.e. 18 h for all types of water sources. Study concluded that 24 h-48 h was the most suitable incubation time for production of H₂S in contaminated drinking water.

It was concluded that both types of H₂S test (BS and LB) showed good correlation with standard methods but for hotels and restaurants water samples H₂S test showed higher efficiency as compared to open well and tube well water samples. Thus it was concluded that the efficiency of H₂S test is higher for treated water sources as compared to untreated water sources.

Over some of the disadvantages of standard methods for developing countries H₂S test have great advantage to such nations for detection of fecal contamination in drinking water. H₂S test is a simple, rapid and inexpensive field test for the screening of drinking water for faecal pollution and can be performed by layman with limited laboratory facilities. Some advantages of H₂S test are as follows:

- This test showed good agreement with the standard most probable number (MPN) test.
- The detection of H₂S producing organisms was found to be simple and feasible, the test was designed in such a way that the water of any pH range could be able to give satisfactory result.
- The H₂S test can be applied immediately after collection in the bottle unlike other methods. The H₂S test is less time consuming and correlated with traditional indicator bacteria, especially fecal coliform and requires little laboratory support.
- The test can be considered valuable as an educational and motivational tool for improved water sanitation.
Limitations of H₂S Test:

- The H₂S test has addressed groundwater specifically, and when it has, by this method false positive results have been observed in ground water, particularly those contaminated with human or animal wastes, or those containing reduced sulfur from natural or anthropogenic sources. Thus there is a high potential for anaerobic aquifers and the formation of sulfides by bacteria of non-human or non-animal origin.

Thus study recommended that H₂S test should be used as an alternative to conventional bacteriological tests in village, anganwadi, small schools, gram panchayat and remote area where no laboratory facilities and technical staff is available. The aim is to recommend effective guidelines for monitoring bacteriological contamination in drinking water.

- This test provides rapid results and therefore it should be used in the field for routine testing of water quality in field.

- This test is very cheap, less time consuming and can be performed by layman without the basic laboratory knowledge.

- The test should be used for testing of water during epidemics by water borne diseases to find the source and spread of water pollution.

- This test is not recommended as a replacement for conventional presumptive coliform test but can be used as an alternative to other laboratory-based tests.

- This test is recommended for use by community health workers to monitor water supply sources.

- The H₂S test is recommended in developing countries or in temperate region where there is ambient temperature between 25°C-44°C. Within this temperature, it does not need an incubator and gives results within 24-48 h.
Though the H₂S test showed maximum efficiency at 37°C but at room temperature also it showed good correlation with standard methods because it is developed as a field test. The 48 h of incubation time period is recommended to give more efficient results of H₂S test.

H₂S test (BS and LB) can be recommended as the most suitable test for detection of fecal contamination in treated water (Hotels and restaurants) as compared to untreated water (Open well and Tube well).

Bile salt in this test is recommended as a suitable surfactant as compared to labolene for untreated as well as treated water. The results of this test can be better interpreted with powder form of medium as compared to strip impregnated in the medium and thus reduces the cost of test.