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H_2S Strip Test, a User-Friendly Method to Find Faecal Coliforms in Water Quality Analysis – a Case Study

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Abstract: Faecal contamination of drinking water is the main cause of the outbreaks of epidemics. According to WHO (1996) for drinking water to be safe, a 100 ml sample should not contain any coliform bacteria. The standard methods currently used for routine testing for detection of e-coli bacteria have many limitations especially when applied in remote areas. The H_2S strip technique has been found to be an on-site, inexpensive and easy to use method to test drinking water, especially for remote and rural areas.

Even though many substrate configurations are available with several modifications, in the present work, the one suggested by Manja et al., (1982), has been adopted. These E-coli detecting vials were prepared in thousands, locally in an Environmental Engineering Laboratory itself making it highly economical and have been extensively used in a Government of Karnataka-assigned project which involved the analysis of water from of existing drinking water sources in selected rural villages for fourteen water quality parameters including e-coli detection.

The use of these vials in the project indicated that the test is quite simple and user-friendly, without any high skill requirement. The results are reliable, time saving and highly economical with more suitability to rural areas, where sophisticated laboratory facilities are not available.

Key Words: Coliforms, H_2S Strips, Vials, Anaerobic, Faecal pollution, MPN Test

INTRODUCTION

According to guidelines for drinking water, water intended for human consumption should be safe, palatable and aesthetically pleasing WHO. This means that the water used for domestic purposes should be free of pathogenic microorganisms and other substances that may present a health risk. Around 2.2 million die of basic hygiene related diseases, like diarrhoea, every year. The provision of drinking water of acceptable microbiological quality and low infectious disease risk requires a number of essential elements within a water safety plan (Sobsey and Faender, 2002).

The applications of measuring the microbial quality of water for educational as well as health aspects, especially of rural population. Teaching people about the microbial quality of water and the fundamentals of germ theory, within the context of education and outreach programs for water, sanitation and hygiene at the individual, household, community and regional levels is a continuing and long-term task in the global health initiative. In conveying these educational messages, the availability of simple, practical, accessible and affordable tests for faecal contamination of drinking water are extremely useful and potentially powerful tools. In some situations the best tests to accomplish these goals are those that are the simplest to use, understand, visualize and interpret. This is because such tests can be widely disseminated both directly by the primary educators and then subsequently via communications within households, families, schools and communities and by other means (educational materials such as leaflets, signs and labels). All over the world numerous attempts are being made to find out simple and reliable method which does not need elaborate set up, inexpensive and can be performed by common man in rural area where people have compelled to drink highly contaminated water.

The presence of coliforms in drinking water is consistently associated with hydrogen sulphide producing bacteria under anaerobic conditions. Many pathogens also produce hydrogen sulphide. For this reason in hydrogen sulphide producing bacteria act as indicators of faecal pollution. One such, low-cost, presence–absence test for faecal contamination in drinking water, simple to use and easy to interpret is the hydrogen sulphide (H_2S) test, also called paper-strip test suggested by Manja et al., 1982. They have successfully employed this method for isolating various localities affected by infectious hepatitis in Gwalior, M.P(India). The outbreak of hepatitis in one of military units in central India was effectively handled using this method. International Development Research Centre (IDRC), Canada, eight countries and their report (1990) recommends the use of H_2S Strip test for the testing of water in rural area and suggested that the investigation should be undertaken to improve its sensitivity (Manish Nigam and Abhishek Dixit, 2006). H_2S Strip Test has been successfully used in small communities in South America, Indonesia and India, for assessing water quality (Martins, et al,

1997; Kromoredjo and Fujioka 1991; Venkobachar et al, 1994). Amarthya Saha and Jaqueline Thomas (2016) have used the H2S strip test as a rapid test for assessing the bacterial contamination in Tanzania through a case study. This method as well as the various modifications of it does not consistently measure the presence of either total coliform bacteria, specific groups of faecal bacteria (e.g., faecal coli forms) or a specific faecal bacterium (E. coli). The test is based on measuring bacteria that produce hydrogen sulphide 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 H2S. The test measures the production (actually, the presence) of H2S by its reaction with iron to form an insoluble, black precipitate of iron sulphide. Given the low solubility product of iron sulphide, the test can detect even small amounts of sulphide formation or presence. Any source of H2S in the sample can lead to a positive result. Sulphides also can be formed by abiotic chemical reactions. Many different bacteria, from a variety of habitats, including many of enteric origin, can release sulphide from proteins, amino acids and other reduced sulphur compounds by reduction reactions. Therefore, there are many possible sources of a positive result in the H2S test.

MATERIALS AND METHODS

Preparation of medium and ready-to-test vials

Preamble: The Government of Karnataka entrusted on consultancy basis, the project on "Water Quality Analysis in Rural Taluks of Karnataka" during 2000-2001, to several organisations and educational institutions. In this project, National Institute of Engineering, Mysore, was also allotted the villages in four taluks of Mysore district. For this project, the corresponding author was the chief coordinator. The main objective of the project was to analyse the water from the existing sources (hand pump or bore well) in the given villages for 14 water quality parameters of which E-coli count was also a parameter.

The medium used in the present study conformed to Manja et al., (1982) who have suggested the following substrate-configuration mixed in 50 ml distilled water:

peptone – 20 g; di-potassium hydrogen phosphate – 1.5 g; ferric ammonium citrate – 0.75g; sodium thiosulphate – 1g; teepol or any equivalent liquid soap as flow agent – 1 ml.

This mixture was kept in an incubator along with the cleanly washed 25 ml glass vials with aluminium caps for about 15 minutes under a pressure of 1.5 kg/cm^2. The medium was made to be absorbed by about 80 cm^2 area of tissue paper (approximating 1 ml of medium) which is inserted in to the sterilised vials and sealed with the air-tight cap. These vials, when dried up at about 50^{o}C are ready-to-use.

Sampling Technique and Use of E-coli testing vials in the field

Going to each village, samples were collected from the existing sources of water in well-cleaned two litre plastic cans. These samples were tested in the Environmental Engineering Laboratory for 14 water quality parameters including the most probable number (MPN) of E-coli bacteria.

For E-coli testing, samples were collected in the vials directly from the source of water in the field itself, on daily basis. The results pertaining only to e-coli detection are presented in this paper.

It is interesting to note that, out of the collected samples in each taluk, some samples showed +ve indication of E-coli presence by becoming black. For these samples, MPN test using membrane filtrate technique was conducted to determine the bacterial count which is indicated in the Table 1.

Membrane filtration Technique

100 ml of the water sample which has answered the H_2S strip test in glass vial is passed through a pressure filter with a 47 mm, 0.45 µm pore size cellulose ester membrane filter (Millipore make) that retains the bacteria present in the sample. The filter paper is placed on a ready-made absorbent pad saturated with 2-3 ml Mac Conkey agar broth, and the plate is incubated at 35°C for 24 hours. The bacterial colonies that grow on the plate are inspected for the presence of yellowish-white colour dots on the pink medium, keeping it on a colony counter.

RESULTS and DISCUSSION

Soon after preparing the vials, they were put to control tests by adding variety of waters from various sources as follows:

	Source	Result
1.	Distilled water	Solution not turning black even after 48 hours
2.	Diluted Sewage	Solution turning black after 6 hours indicating E-coli
3.	Municipal Tap water	Solution not turning black after 24 hours.
4.	Bore well water	Solution not turning black even after 48 hours

These results indicated that the vials responded correctly as per the test and are ready for use.

For the Government project, the water quality analysis was done for the existing water sources in villages of four taluks in Mysore district, namely, Mysore, Nanjangud, T.Narasipur and H.D Kote. The results were submitted to DPMU, Mysore in a record time of 5.5 months. A sample format for showing the results pertaining to E-coli bacterial analysis only for 25 numbers of samples are given in Table 1 below:

Table 1 Sample result sheet for the analysis of E-coli bacteria in villages

Sl.No	Can No.	Date	G.P.	Village	Location	SCH	B.No
1	104	08-21-00	Alanahalli	Alanahalli	Back to Water Tank	H.P	0
2	105	08-21-00	Alanahalli	Alanahalli	Infront of Ramakrishan House	H.P	0
3	106	08-21-00	Alanahalli	Alanahalli	Infront of Animation Centre	H.P	0
4	107	08-21-00	Alanahalli	Alanahalli	Infront of Sourabh Nilaya	H.P	0
5	108	08-21-00	Alanahalli	Alanahalli	in front of Laxmi Krupa	H.P	0
6	109	08-21-00	Alanahalli	Alanahalli	infront of Laxmi Krupa	H.P	0
7	110	08-21-00	Alanahalli	Alanahalli	Infront of Balaji Kushion Works	H.P	0
8	111	08-21-00	Alanahalli	Alanahalli	On left to Lingaraju's House	H.P	0
9	112	08-21-00	Alanahalli	Alanahalli	On main road (left side)	H.P	0
10	113	08-21-00	Alanahalli	Alanahalli	On Main road (right side)	H.P	0
11	114	08-21-00	Alanahalli	Alanahalli	Near S.M. Landry (right side)	H.P	0
12	115	08-21-00	Alanahalli	Alanahalli	On right side of the road	H.P	0
13	116	08-21-00	Alanahalli	Alanahalli	On right side (near oak tree)	H.P	0
14	121	08-21-00	Alanahalli	Lalithadripura	Infront of Govt. Primary School	H.P	0
15	122	08-21-00	Alanahalli	Lalithadripura	Inside the Govt. Primary School	H.P	0
16	123	08-21-00	Alanahalli	Lalithadripura	Right to Rammandira	H.P	**16**
17	124	08-21-00	Alanahalli	Lalithadripura	Near Amasayya's House	H.P	0
18	125	08-21-00	Alanahalli	Lalithadripura	Infront of Nanjunda's House	H.P	0

19	126	08-21-00	Alanahalli	Lalithadripura	Infront of Sri Ramamandira	H.P	0
20	127	08-21-00	Alanahalli	Lalithadripura	Left to Javaraiah's House	H.P	0
21	128	08-21-00	Alanahalli	Lalithadripura	Near Linganna's House	H.P	0
22	129	08-21-00	Alanahalli	Lalithadripura	Near Basaveshwara Temple	H.P	0
23	130	08-21-00	Alanahalli	Lalithadripura	On left side inside the village	M.W.S	0
24	131	08-21-00	Alanahalli	Lalithadripura	On right side (Near Bridge)	M.W.S	0
25	132	08-21-00	Alanahalli	Lalithadripura	On right side (inside the village)	M.W.S	0

HP – Hand Pump; M.W.S. Municipal Water Supply; B.No. – Number of E-coli per 100ml.

The consolidated figures of E-coli analysis in all the four taluks are shown in Table 2.

Table 2 Summary of the statistics of E-coli bacteria analysis in water sources of villages

Sl.No.	Taluk Name	Total No. of Samples	E-coli +ve samples	% age of E-coli +ve samples
1	Mysore	1343	36	2.68
2	T.Narasipura	1078	140	14.30
3	Nanjangud	1270	182	13.55
4	H.D.Kote	985	48	4.87

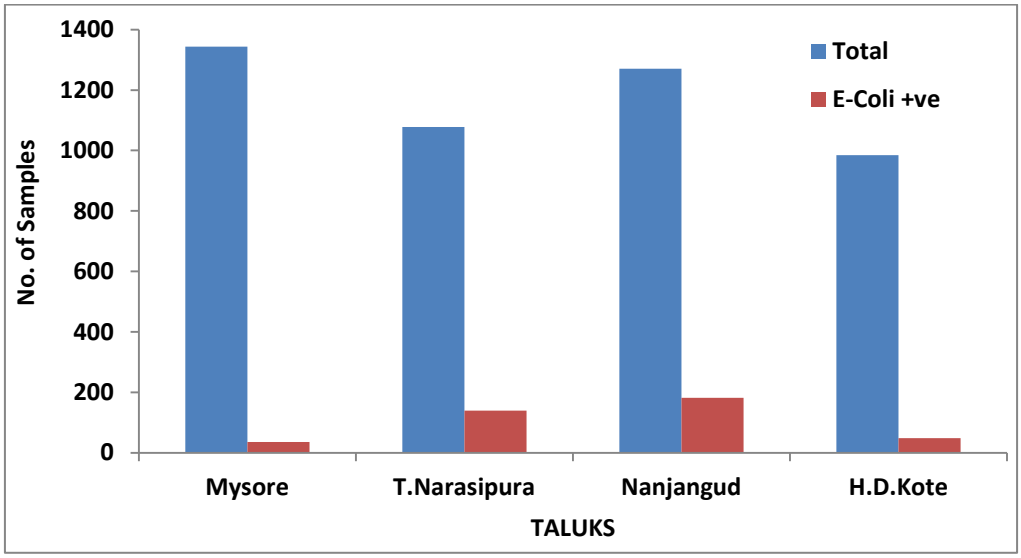


Fig 1 Summary of E-Coli analysis in various Taluks

The percentage of samples having E-coli bacteria are shown as sector graphs in Figures 1 to 4.

Fig. 1 Mysore

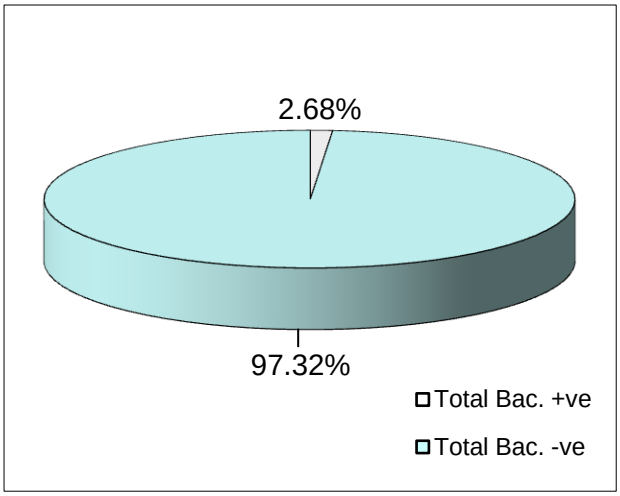


Fig. 2 T.Narasipur

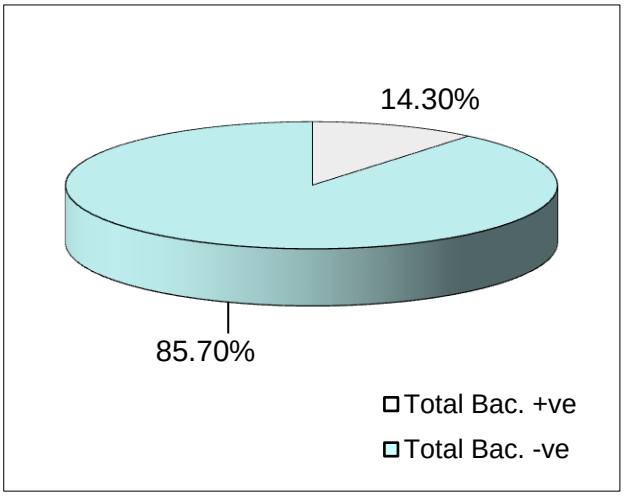


Fig 3 Nanjangud

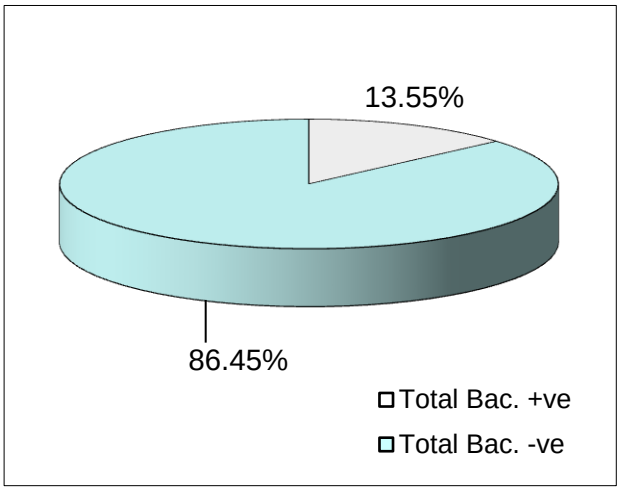
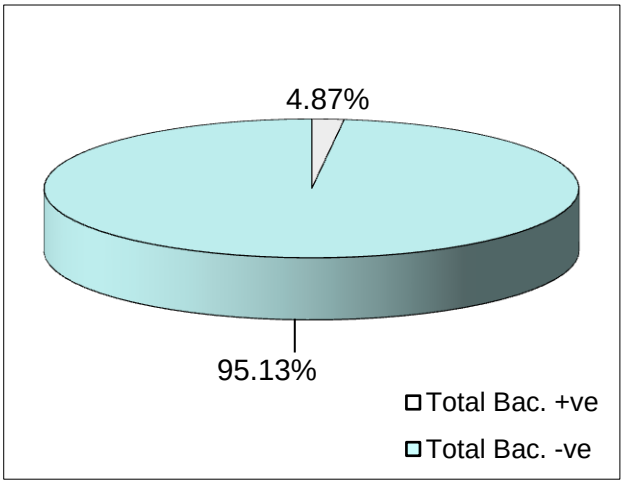


Fig 4 H.D.Kote



From the above project, using the laboratory prepared E-coli testing vials extensively, following conclusions could be drawn:

1. This methodology can make the bacteriological analysis very simple and highly economical, to a reasonably good degree of accuracy.
2. The glass vials can be prepared easily in an environmental laboratory without much expertise and complication.
3. These vials can be used for the qualitative measurement to begin-with.
4. For the samples answering as +ve, showing E-coli presence, turning black within 24 hours, the usual MPN test by microfiltration technique can be adopted to get the actual number of E-coli bacteria.
5. The vials can be used even by common people and arrive at the result, more useful to rural public.
6. However, careful and judicial use of the vials keeping in view, the limitations (Sobsey and Faunder, 2002) is advisable.

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