

# Modified H<sub>2</sub>S Paper Strip Test for Detection of Bacteriological Contamination in Drinking Water

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**Abstract** - The provision of safe drinking water to all is the priority item in the agenda of Government of India. To fulfill this requirement thousands of bore well installed in the villages. Despite of this, large population still depends upon the dug well water; shallow bore well and untreated surface waters for their drinking water needs. Hence there is need to monitor the bacteriological quality of water using field oriented test which needs to be inexpensive, fast and can be performed by unskilled person without the use of laboratory facilities. H<sub>2</sub>S paper strip test appear to meet this requirement. The objective of this paper to develop some additional characteristics of H<sub>2</sub>S paper strip test by using the chemicals of cystine, amino acid. To improve the performance of the test by adding cystine, an amino acid to the nutrient medium. The sensitivity improved as well as there was reduction in time to get the results. it was obtained 8 hours earlier than original H<sub>2</sub>S paper strip. Modified test became more sensitive, it correlated with fecal coli form 91% of the times while the original test 87% of the times. This study focused to modify the H<sub>2</sub>S strip by adding chemical cystine to the bacteriological media to improve its sensitivity and generate the correlation among total coliform, fecal coliform and H<sub>2</sub>S paper strip tests (original and modified).

**Keywords** - Coliform , Modified H<sub>2</sub>S Paper Strip, Water Quality.

## I. INTRODUCTION

According to guidelines for drinking water, water intended for human consumption should be safe, palatable and aesthetically pleasing. 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 diarrhea, 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. 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. One such method is H<sub>2</sub>S Strip Test.

The presence of coliforms in drinking water is consistently associated with hydrogen sulphide producing bacteria. Many pathogens also produce hydrogen sulphide. For the reason in hydrogen sulphide bacteria act as indicators of faecal pollution. Manja et al.(1982) 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 three report (1990) recommends the use of H<sub>2</sub>S Strip test for the testing of water in rural area and suggested that the investigation should be undertaken to improve its sensitivity. H<sub>2</sub>S Strip Test has been successfully used in small communities in South America, Indonesia and India, for assessing water quality (Castillo et al, 1994; Martins, et al, 1996; Kromoredjo and Fujioka 1991; Venkobachar et al, 1994). This method is based on an H<sub>2</sub>S strip test, which causes contaminated water to turn black, and hence provides a very effective visual mechanism for illustrating contamination of water supplies. A low-cost test for faecal contamination in drinking water which is simple to use and easy to interpret is the hydrogen sulphide (H<sub>2</sub>S) paper-strip test (Manja et al.1982).

## II. MATERIALS AND METHODOLOGY

In the present study a total 100 water samples from tube well, open well, dugwell, surface water analyzed by H<sub>2</sub>S test and modified H<sub>2</sub>S test, total coliform and fecal coliforms. The sampling locations were Loni In Ahmedabad district, Pravaranagar, S.T. stand and hostels of University of Poona. Table.1 presents the sampling location and source of samples.

Table.1 Sampling location and source of samples

| SN | Location                        | Source        | No. of samples |
|----|---------------------------------|---------------|----------------|
| 1  | Lake near Lontek, Loni area     | Surface water | 10             |
| 2  | Lake view Hostel                | Tap water     | 15             |
| 3  | Lake view Mess                  | Tap water     | 5              |
| 4  | Engineering College, New campus | Tap water     | 10             |
| 5  | Engineering College, Old campus | Tap water     | 10             |
| 6  | Staff quarters                  | Tap water     | 10             |
| 7  | Sadatpur Road                   | Bore well     | 10             |

|                             |               |           |            |
|-----------------------------|---------------|-----------|------------|
| 8                           | Near PMT      | Dug well  | 10         |
| 9                           | Anganwadi     | Open well | 10         |
| 10                          | Near ST stand | Open well | 10         |
| <b>Total no. of samples</b> |               |           | <b>100</b> |

For the H<sub>2</sub>S paper strip test, the 20 gm. Pepton, 1.5 gm. Diapotassium Hydrogen phosphate, 0.75 gm. Ferric Ammonium Citrate, 1 gm. Sodium Thiosulphate, 1 ml Teepol and 0.0125 gm. Cystine (for modified H<sub>2</sub>S strip test). The composition of H<sub>2</sub>S test for 50 ml of medium was prepared accordingly Manja et al. (2001). One mL of Manja’s H<sub>2</sub>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 in duplicate. The bottles were then incubated at room temperature and 37°C for 18, 24 and 48 h. The positive H<sub>2</sub>S test or contamination or fecal pollution in drinking water indicated by change in colour of the medium to black. Blacking in H<sub>2</sub>S medium was recorded after 24 and 48 h of incubation at room temperature and at 37°C, respectively. In the same manner modified H<sub>2</sub>S test were done with the use of cystine chemical of 0.125 gm. MPN test was performed by nine multiple tube dilution technique using double and single strength MacConkey(MC) medium.

**III. RESULT AND DISCUSSION**

Over the last two decades, several investigators have assessed the H<sub>2</sub>S test with various modifications such as medium composition, incubation period and temperature in different tropic and temperate regions including Indonesia, Peru, Chile, Nepal and South Africa, and compared it to conventional water bacteriological methods. Ratto et al. (1989) evaluated the H<sub>2</sub>S test at 22°C and 35°C and compared it to MPN and fecal coliform tests for 20 potable water samples and concluded that H<sub>2</sub>S test was a more sensitive test than total coliform (TC) and fecal coliform (FC) tests.

**Reduction in time of reaction:**

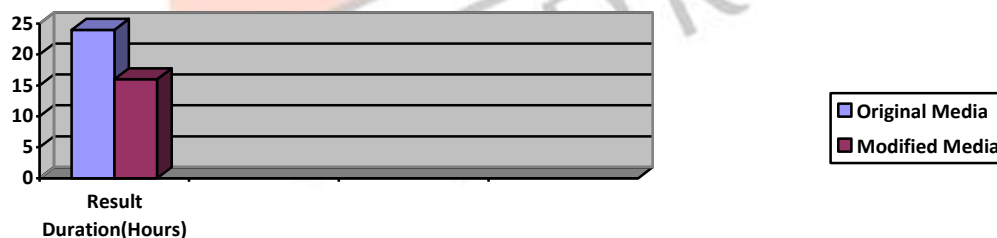
Before attempting the correlation between conventional bacteriological tests and field oriented H<sub>2</sub>S paper strip, efforts were directed to improve the sensitivity and also to reduce the time of the getting final result by was the H<sub>2</sub>S paper strip test. The basic for the modification of the test was as following

The presence of the coliform in the drinking water was consistently associated with organisms that produce hydrogen sulphide. Other enteric bacteria like salmonleea also produce H<sub>2</sub>S. In the bacterial growth media, one of the ingredients provided in the sodium thiosulphate which is used electron acceptor in the anaerobic environment of the test bottle, thus producing H<sub>2</sub>S.

It was contemplated to incorporate cystine (0.25 gm./1000 ml.) a sulphur containing amino acid in the media suggested by Manja et al.(1982). It was thought cystine being amino acid besides the growth of microbes. Might also supply sulphur for producing H<sub>2</sub>S. H<sub>2</sub>S paper strip bottles were prepared incorporating cystine in nutrient medium and its effect on the reduction in time. Table.2. presents the chemical reaction time for different media.

Table.2. Chemical reaction time for different media

| Type of Media | Chemical reaction time for result (hours) |
|---------------|---|
| Original      | 24  |
| Modified      | 12  |



**Fig.1 Graphical presentation of the chemical reaction time for different media.**

The improvement in a modified test appeared due to the fact that Cystine enhance the growth of the bacteria in the test bottle rather than providing sulphur for production of H<sub>2</sub>S. Due to complexity of the Cystine molecule, sulphur is not broken easily.

**Correlation among Total, Fecal coliform and both (original and modified) H<sub>2</sub>S strip test:**

About 100 samples were collected from different water sources and subjected to total and fecal coliforms tests using MacConkey(MC) medium and also to H<sub>2</sub>S strip tests both original and fecal coliforms were also evaluated using multiple tube techniques of MacConkey(MC) medium. The number of sample analyzed from different sources and locations were presented in Table.3.The results shows that there is a good correlation between total, fecal and H<sub>2</sub>S paper strip tests. H<sub>2</sub>S original and modified tests correlate better with fecal coliform than total coliform. Fecal coliform correlates 87% and 91% with H<sub>2</sub>S original and modified tests respectively. The figures 2 to 7 depict the correlation between different tests.

**Sensitivity of Modified H<sub>2</sub>S paper strip test:**

In order t evaluate the modified test with respect to the original H<sub>2</sub>S paper strip test, only the contaminated water samples were considered. The total number of samples from tap water, dug well, bore wells and surface water sources considered were 65. The permissible total coliform as per table.3. for un-piped water supplies is 10 numbers/ 100 ml. At this level of coliform, the

modified H<sub>2</sub>S test gives more than 75% positive results while the original H<sub>2</sub>S test gives 58% positive results. In developing countries providing water with 5 fecal coliform/100 ml is a great achievement for un-piped water supply schemes. At the fecal coliform level, the modified H<sub>2</sub>S gave results 60% while original tests yielded 35% positive.

Table.3. Correlation among Total coliform, fecal coliform, H<sub>2</sub>S strip test (original) and H<sub>2</sub>S strip test (modified).

|   | Total Coliform |    |    | Fecal Coliform |    |    | H <sub>2</sub> S strip test (original) |    |   | H <sub>2</sub> S strip test (modified) |   |    |
|---|----------------|----|----|----------------|----|----|--|----|---|--|---|----|
|   | A              | B  | C  | A              | B  | C  | A                                      | B  | C | A                                      | B | C  |
| <b>Total Coliform</b>                       | -              | -  | -  | 82             | 12 | 6  | 83                                     | 15 | 2 | 89                                     | 0 | 11 |
| <b>Fecal Coliform</b>                       | 82             | 6  | 12 | -              | -  | -  | 87                                     | 13 | 0 | 91                                     | 2 | 7  |
| <b>H<sub>2</sub>S strip test (original)</b> | 83             | 2  | 15 | 87             | 0  | 13 | -                                      | -  | - | 94                                     | 0 | 6  |
| <b>H<sub>2</sub>S strip test (modified)</b> | 89             | 11 | 0  | 91             | 7  | 2  | 94                                     | 6  | 0 | -                                      | - | -  |

Note: A- Total agreement (++, --)  
 B- Disagreement (+, -)  
 C- Disagreement (-, +)

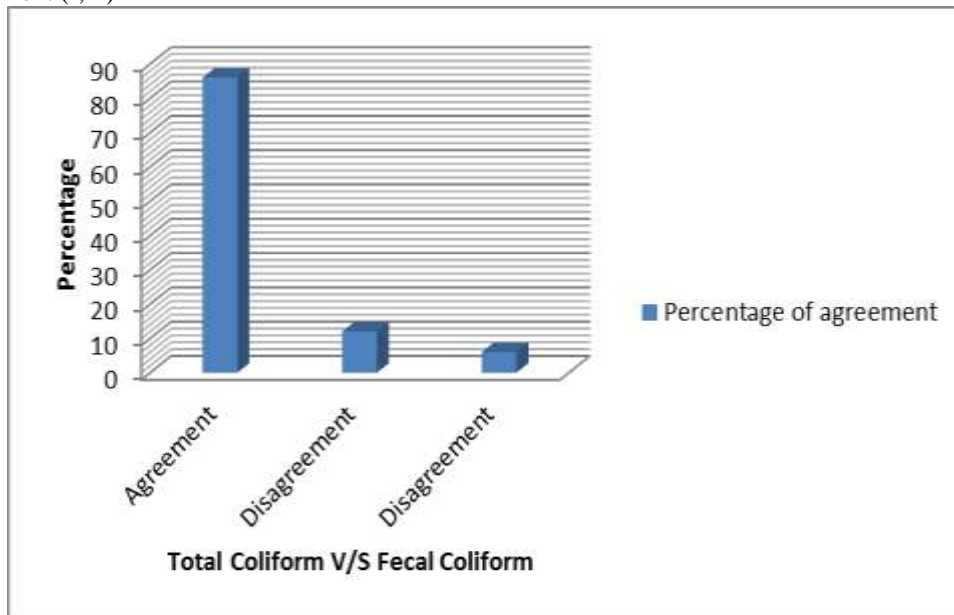


Fig.2. Correlation between Total and fecal coliforms.

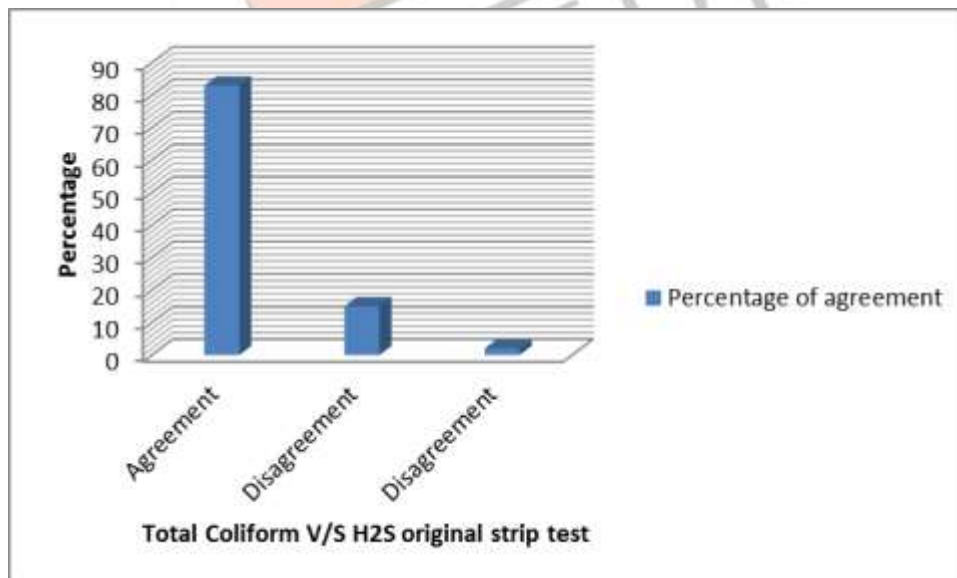
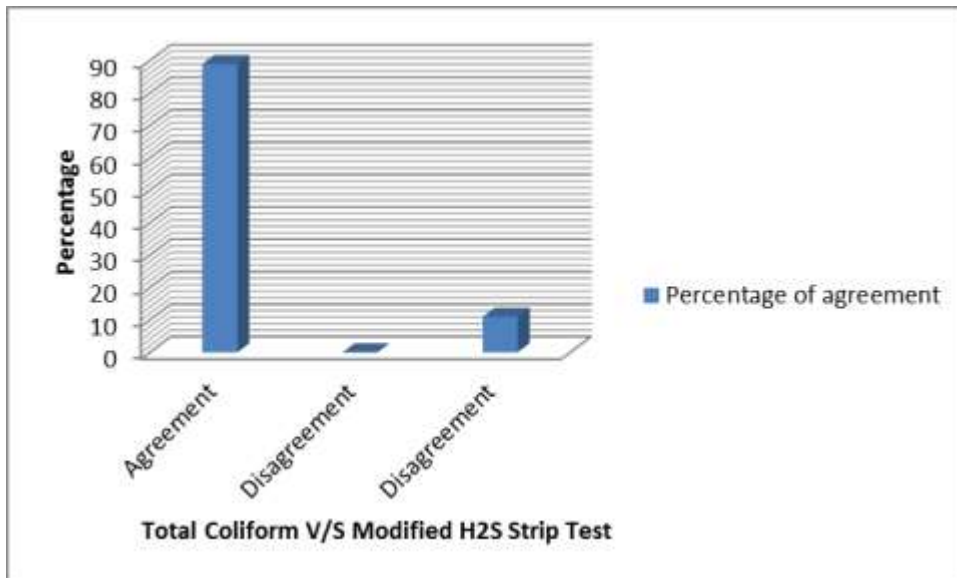
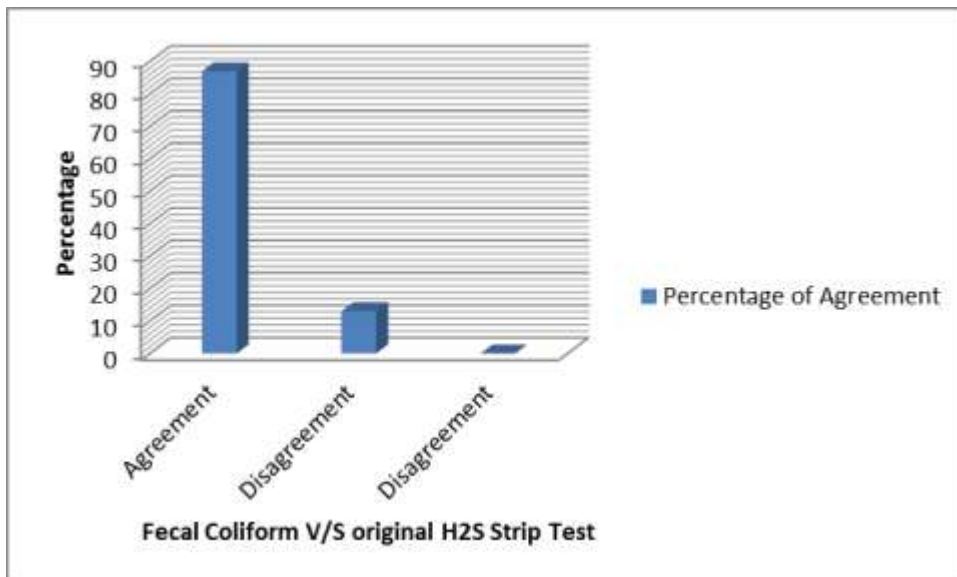


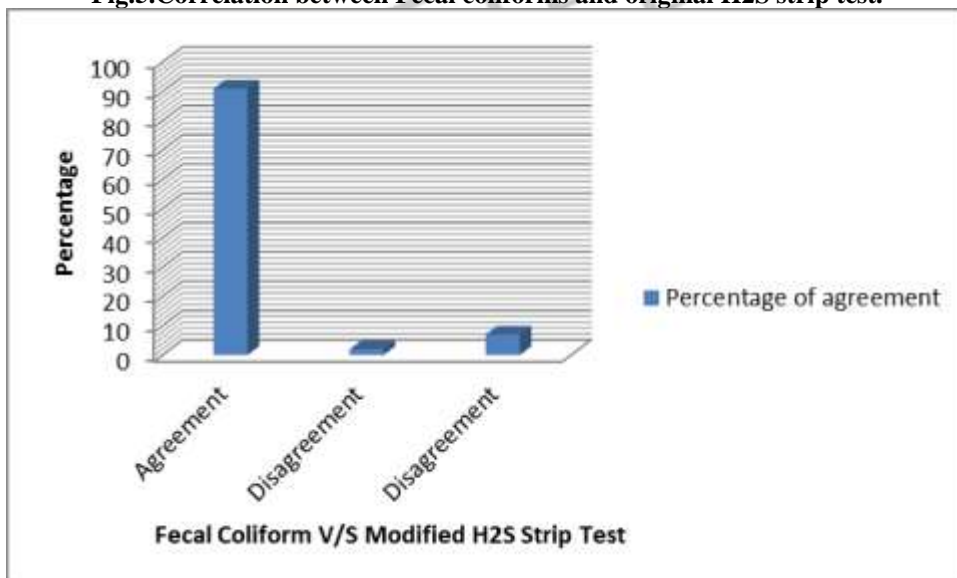
Fig.3. Correlation between Total coliforms and original H<sub>2</sub>S strip test.



**Fig.4.**Correlation between Total coliforms and Modified H2S strip test.



**Fig.5.**Correlation between Fecal coliforms and original H2S strip test.



**Fig.6.**Correlation between Fecal coliforms and Modified H2S strip test.

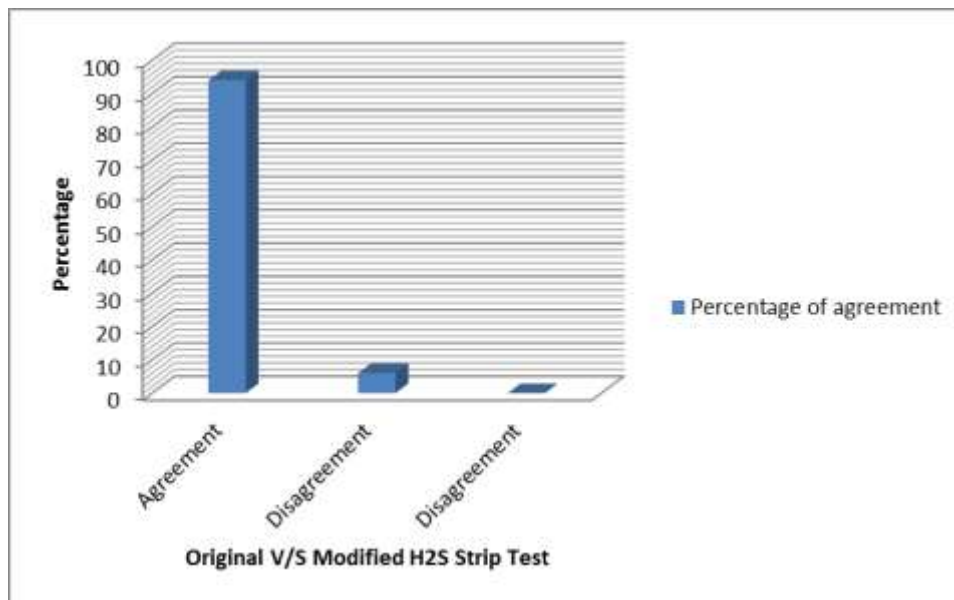


Fig.7. Correlation between original H2S strip test and Modified H2S strip test .

#### IV. CONCLUSIONS

On the basis of this study, following conclusions may be drawn:

1. Correlation among original H2S strip test, total and fecal coliform was quite high. Original H2S paper strip correlated with total coliform **83%** times while with fecal coliform 87% of times.
2. Addition of chemical cystine enhanced the rapidity of test. From original H2S paper strip test results could be obtained in **24** hours but modified was giving the result in **16** hours. So, final results could be obtained **8** hours earlier.
3. The test also exhibited improved sensitivity. Modified test correlated with total coliform **89 %** of times with fecal coliform **91%** of times.

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