Advances in Bioresearch
Adv. Biores., Vol 9 (5) September 2018: 146-154
©2018 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.9.5.146154

Advances in Bioresearch

ORIGINAL ARTICLE

Development of Water Quality Field Testing Kit (WQFTKs)- A modified H_2S Strip Test Method for detection of Hydrogen Sulfide Producing Bacteria

Mona Kejariwal[1], Abhishekh Tiwari[2], Sunil Sahani[3]
[1]Assistant Professor, Department of Botany, R.D.&S.H. National College, Bandra (W), Mumbai-50.
[2]Department of Botany and Biotechnology, R.D.&S.H. National College, Bandra (W), Mumbai-50.
[3]Department of Botany and Biotechnology, R.D.&S.H. National College, Bandra (W), Mumbai-50.

ABSTRACT
Problems related to water is one of the major issues in India especially in a city like Mumbai. Large number of population suffers from various water borne diseases due unavailability of potable water. The polluted water contains fecal coliforms as the primary contaminant. Hence, detection of fecal coliforms is essential for prevention of this crisis. However, detection of fecally contaminated requires sophisticated equipment's, expertise in scientific techniques, time etc. resulting in the increase in cost of testing the water for the fecal coliforms. As majority of the population could not afford the cost of testing the drinking water neither they have the necessary knowledge, expertise or the time to do the test, they are helpless and consume the polluted water. The present study aims at designing a Water Quality Field Testing Kits (WQFTKs) for detection of fecal coliforms which is easy, user friendly, cost effective and take less time for determining the results. This study insights on the development of WQFTK by studying various othermethods, their usability a field testing kit. The project also aims to provide guidance to the citizen of Mumbai through our institution about the usability and importance of the Water Quality Field Testing Kits (WQFTKs).
Keyword: *Coliforms, water borne diseases, WQFTKs.*

Received 30.05.2018 Revised 11.07.2018 Accepted 19.08.2018

How to cite this article:
Mona Kejariwal, Abhishekh Tiwari, Sunil Sahani. Development of Water Quality Field Testing Kit (WQFTKs)- A modified H_2S Strip Test Method for detection of Hydrogen Sulfide Producing Bacteria. Adv. Biores. Vol 9 [5] September 2018: 146-154.

INTRODUCTION

Water is essential for survival of life on earth. It is also a medium for dispersion of various diseases like diarrhea, dysentery etc.According to World Development Report [1], 80% of the diseases in the world are related to water. According to a book published by World Health Organization and Organization for Economic Co-operation and Development[2], around 2.2 million of the 3.3 million water related deaths are caused due to diarrhea. The major reason for this problem is the unavailability of potable water to the masses. Absence of harmful chemicals, pathogenic micro-organisms are some of the characteristic features of potable water. To reduce the risk of people falling ill due to water borne diseases, it is essential that monitoring system for checking the physical, chemical and microbiological quality of drinking water are in place.

As mentioned in the WHO guidelines[3], the main reason for water borne diseases like diarrhea, dysentery etc. are the presence of a class of bacteria called coliforms in drinking water. These bacteria belong to the family of *Enteobacteriacae*. Micro-organisms in this family are the organisms which are present in the gut of mammals. Hence, presence of these micro-organisms in drinking water is an indication of water being fecally contaminated. Most of the member species of *Enteobacteriacae* are Sulphate-reducing bacteria (SRB), mostly anaerobic. They use sulphate as a terminal electron acceptor for degradation of organic compound, resulting production of sulphide and other products and also H2S gas. H2S gas was one of the earliest products of bacterial decomposition of organic compounds[4, 5]. Tanner

[6] found that large number of bacteria produced H2S gas from peptone and sulphur containing amino acids and some thiourea and thiosulphate.

Over past decades, many methods have been developed by researchers for the identification of fecal contamination in drinking water. Dilution technique of the Most Probable Number (MPN) method is used to identify the presence of coliforms[7, 8, 9]. This method is based on the characteristic reactions given by the bacteria in different media. It is used to identify and estimate the amount of total coliforms present in the water sample. However, these methods were time consuming and tedious.

Alead acetate strip test was developed by Wilmet [10]which could detect the presence of H2S producing micro-organisms. In this test, the media contained basic organic compounds like peptone etc. and sulphur containing amino acids.

Manja *et al* [11] developed a presence/absence (P/A) method which can be used for field testing of water. This method was based on hydrogen sulphide production by the H2S producing coliforms bacteria. In this method, the positive results were indicated by the blackening of the tissue paper impregnated with the new-medium. A modified P/A test was carried out by Venkobachar, C., Kumar, D., Talereja, K., Kumar, A. and Lyengar, L. [12], S.P Pathak and K. Gopal [13]. In this method, L-cysteine, a sulphur containing amino acid was used in the medium for enhancing the growth rate of the organisms and sensitivity of the test thus, improving the rate of reaction. In another assay, similar tests were performed at different temperatures and concentration of the fecal coliforms [14]. This study was focused on the effect of different temperatures on the growth rate of the fecal coliforms and the sensitivity of the method.

Manish and Abhishek [15]carried out a test using this modified medium. In this test, water samples from different sources of Loni in Ahmedabad, Pravaranagar, ST stand and University of Pune were taken. In this study, water samples were tested at varied incubation temperature and for different time period. Comparison between total coliforms, fecal coliforms, original H2S strip test and the modified H2S strip test were carried out.

D.H. Tambekar and N.B. Hirulkar [16, 17]carried out a comparative study of the methods of detecting coliforms in drinking water. The methods compared were MPN method, MFT (Membrane Filtration Technique), method developed by Manja *et al* [11]and a modified version of Manja *et al*[11]medium. Since the modified medium contained bile salts instead of teepol thus making the medium specific for gram negative enteric bacteria. Various other studies were carried out comparing the H2S strip test and other methods [18, 19]. Several other studies [20, 21, 22]also showed good correlation of the H2S test with fecal coliforms.

The present study aims at developing a water quality field testing kit. The kit should be cost effective, shouldn't require any expertise in handling, less time consuming and simple enough for a common man to understand. The objective was achieved after the studying various methods like Most Probable Number (MPN), lead acetate strip test, H2S broth method one after the other and understanding its usability as a field testing kit. Since these methods failed to fulfill all the criteria of the proposed kit we modified the H2S broth method. In our modified strip test method, cystine was replaced by the mixture of proline, leucine and lysine and phenol red was added as a pH indicator. These modifications aids in the detection of not only the H2S producing micro-organisms of fecal origin but also the non H2S producing ones. However, the presence of non H2S producing coliforms can only be confirmed after further analysis by IMViC tests etc. This kit is simple, more accurate, easy for the local population to understand, user friendly and gives results in less amount of time.

MATERIAL AND METHODS

Development Strategy:

Assessment of various methods for their usability, cost effectiveness and time consumption was carried out. Various methods that was studied are given below.

- **Most Probable Number (MPN) Method:**

Sample: Test Water sample.

Method: For presumptive test, water samples of volume 10 ml, 1 ml, 0.1ml were taken and inoculatedin test tubes containing Lauryl-tryptose broth of single strength and double strength media. Tubes were incubated at 37^0C for 24 hours. After 24 hours, if the test tubes shows production of gas then the test is positive, MPN index is calculated and a confirmatory test is performed.

For confirmatory test, a loopful of culture is taken from the test tubes of presumptive test showing positive result and is streaked on Eosin Methylene Blue Agar plate. Plates are incubated at 37^0 C for 24 – 48 hours. Presence of greenish metallic shine indicates presence of coliforms in the water sample [23].

- **Lead acetate paper strip test:**

Cultures: Pure cultures of *Escherichia coli, Enterobacter aerogenes, Lactobacillus sporogenes, Salmonella typhi and Salmonella paratyphi B* were used as standards for the test.

Method: For lead acetate strip test method, a saturated solution of lead acetate was prepared and was impregnated on strips made of Whattman filter paper No.1 (7mm×5cm), by submerging the strips in the lead acetate solution and keeping at 45°C in the oven for 30±5 minutes. During the test the test sample is inoculated in 5-10 ml of Peptone water (with little amount of Sulphur containing amino acid). The impregnated paper strip is placed in the test tube such that the strip do not touch the media (Insert lead acetate strip paper between the plug and inner wall of tube, above the inoculated media), and incubate at 35-37 °C for 18-24 hours. The H_2S gas produced by bacteria, comes in contacted with lead acetate paper strip turning the strip black indicating positive result [10].

- **H_2S broth method:**

Cultures: Pure cultures of *Escherichia coli, Enterobacter aerogenes, Lactobacillus sporogenes, Salmonella typhi and Salmonella paratyphi B* were used as standards for the test.

Method: For the H_2S broth method, the original composition of H_2S medium, which is designed by Manja *et al* [11] was used. The composition of the medium is given in the table below.

Table no. 1: Composition of Manja *et al*[11] H_2S medium.

Sr. No.	Components	Concentration (g)
1	Peptone	4 g
2	Dipotassium hydrogen phosphate	0.30 g
3	Ferric Ammonium Citrate	0.15 g
4	Sodium Thiosulphate	0.2 g
5	Teepol	0.025 g
8	Distilled water	20 ml

- **Modified H_2S paper strip method:**

Cultures: Pure cultures of *Escherichia coli, Enterobacter aerogenes, Lactobacillus sporogenes, Salmonella typhi and Salmonella paratyphi B* were used as standards for the test.

Making the Modified H_2S paper strip: For the H_2S paper strip, a modified H_2S medium was prepared. The composition of the medium is given in the table below.

Table no. 2: Composition of modified H_2S medium.

Sr. No.	Components	Concentration (g)
1	Peptone	4 g
2	Dipotassium hydrogen phosphate	0.30 g
3	Ferric Ammonium Citrate	0.15 g
4	Sodium Thiosulphate	0.2 g
5	Teepol	0.025 g
6	Amino acids (proline: leucine: lysine in the ratio 1: 1: 1)	0.9 ml
7	8 % Phenol red*	0.9 ml
8	Distilled water	20 ml

* The concentration of phenol red solution should be according to the grade of the chemical used. The color of the solution should be dark red.

The media was prepared and was impregnated on strips made of Whattman filter paper No.1 (1cm×5cm), by submerging the strips in the media and keeping at 65°C in the oven for 30-40 minutes. Dried strips were then put into the appropriate clean test tubes/ sample bottles and was sterilized at 121°C for 15 minutes.

For the positive test, tubes/ bottles were inoculated with the suspension culture of *Escherichia coli, Enterobacter aerogenes, Lactobacillus sporogenes, Salmonella typhi and Salmonella paratyphi B.* Negative controls were also prepared by inoculating distilled water. The tubes were kept at room temperature (25±5°C) for 14-24 hours and the results were recorded.

The protocol for performing the water quality test using this modified H_2S kit is given in annexure 1.

RESULT AND DISCUSSION

The main aim of this present study was to develop a water quality field testing kit (WQFTKs). In this study, usually used methods like MPN test were analyzed for their usability as a field testing kit. The results of the MPN test are given in Table no. 3

Presumptive Test

Table no. 3 : Presumptive test

Sample No.	Double Strength (10 ml)	Single Strength (1 ml)	Single Strength (0.1 ml)	MPN Index
1	5	5	4	1600

Confirmed Test

Greenish metallic shine was observed on Eosin methylene blue agar plate.

After the MPN test was carried out, it was understood that this method cannot be used as field testing kit. This conclusion was made because the MPN method is a long, tedious method and is also very expensive, requires expertise in handling and testing and is very much time consuming.

To overcome these difficulties, another method – lead acetate paper strip test, used for detection of H_2S producing fecal coliforms was analyzed. The results of this tests were negative as no black coloration of strip was observed. This can be due to the different grade of chemicals used in the test. Table no. 4shows the results of lead acetate test.

Table no. 4 : Lead acetate test

Organisms	No. of tubes	Result
		Black Coloration
Escherichia coli	3	–
Enterobacter aerogenes	3	–
Lactobacillus sporogenes	3	–
Salmonella typhi	3	–
Salmonella paratyphi B	3	–

Key: – indicates no black coloration.

After the failures of lead acetate paper strip test, another method - H_2S Broth method was studied for its usability as field testing kit. The composition of H_2S media is given by Manja *et al* [11]. In this test black precipitation by fecal coliform was observed, Table No. 5 shows the result of this test.

Table no. 5 : H_2S Broth Method

Organisms	No. of bottle	Result
		Black precipitation
Escherichia coli	3	–
Enterobacter aerogenes	3	+
Lactobacillus sporogenes	3	+
Salmonella typhi	3	–
Salmonella paratyphi B	3	+

Key: + indicates pink black precipitation.
– indicates no black precipitation.

As this test shows positive result only for H_2S Producing bacteria, this test is not conclusive for some strains of commonly found bacteria like *Escherichia coli* and *Salmonella* spices. This test also requires expertise in handling and is also expensive. Thus, this method cannot be used as WQFTKs.

To overcome this difficulty, we modified the composition of the media and made modifiedH_2S strip method.H_2S medium was modified by replacing L-cystine with a mixture of L- proline, L-leucine and L-lysine in the ratio of 1: 1: 1 and addition of phenol red pH indicator. Phenol red indicates change in pH caused due to base produced before H_2S production. This modified method not only detects coliforms that produce H_2S but also other coliforms that do not produce H_2S as these micro-organisms will cause change in pH resulting in change in color of the strip. Table no. 6 shows the result of the test.

Table no. 6 : Modified H_2S strip test

Organisms	No. of Kit	Result					
		Pink coloration			Black precipitation		
Escherichia coli	3	+	+	+	–	–	–
Enterobacter aerogenes	3	+	+	+	+	+	+
Lactobacillus sporogenes	3	+	+	+	+	+	+
Salmonella typhi	3	+	+	+	–	–	–
Salmonella paratyphi B	3	+	+	+	+	–	+

Key: + indicates pink coloration/ black precipitation.
– indicates no pink coloration/ black precipitation.

H_2S as these micro-organisms will cause change in pH resulting in change in color of the strip. However, this cannot be the only criteria for confirming the presence of coliforms. Thus, further analysis of the water sample should be carried out using biochemical tests like IMViC tests.

The modified H_2S method is a better candidate for being used for field testing of water sample when compared with other methods like MPN, Lead acetate paper strip test and H_2S broth method as it fulfills the criteria of a WQFTK mentioned earlier.

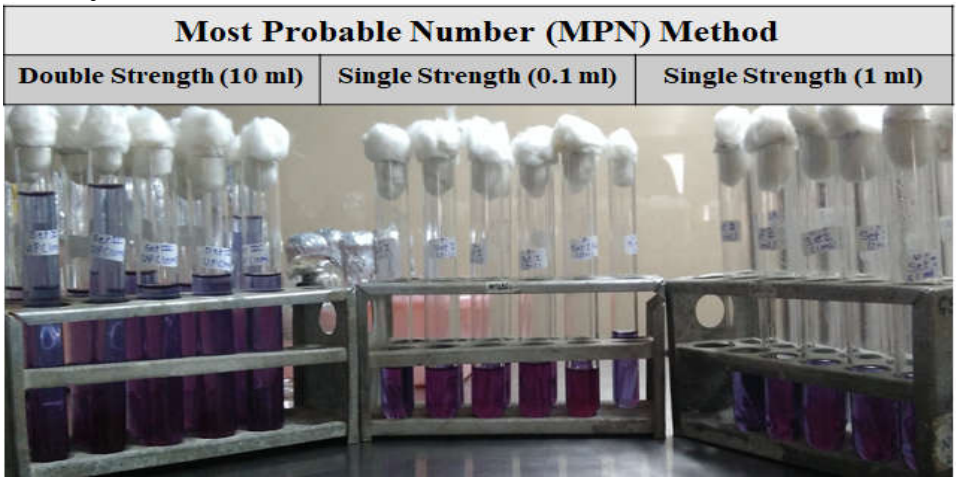

Figure no. 1 : Presumptive test of MPN Method

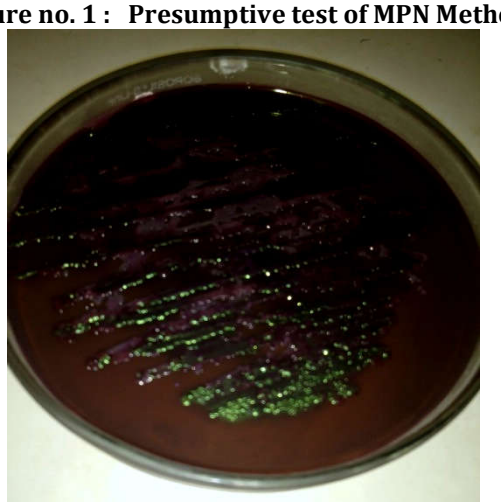

Figure no. 2 : Confirmed test of MPN

Figure no.3 : Lead acetate strip test

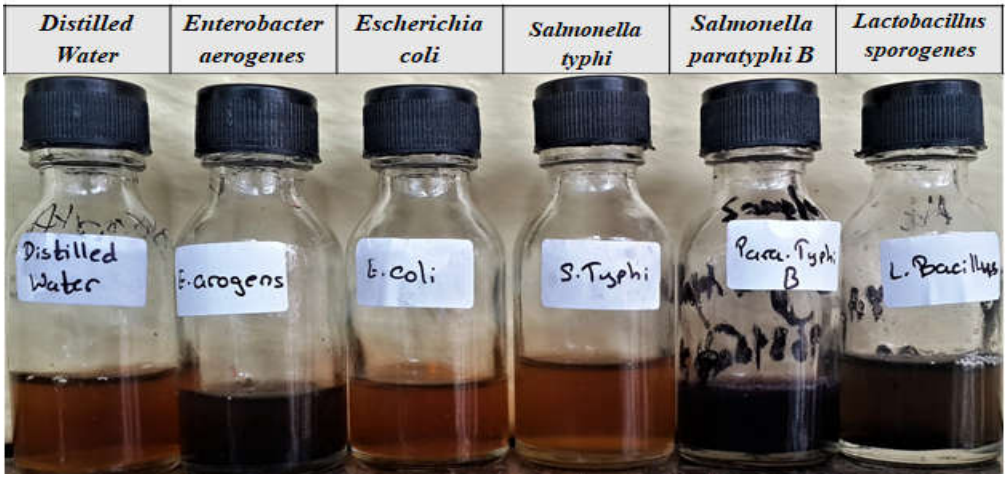

Figure no.4 : H_2S Broth Method

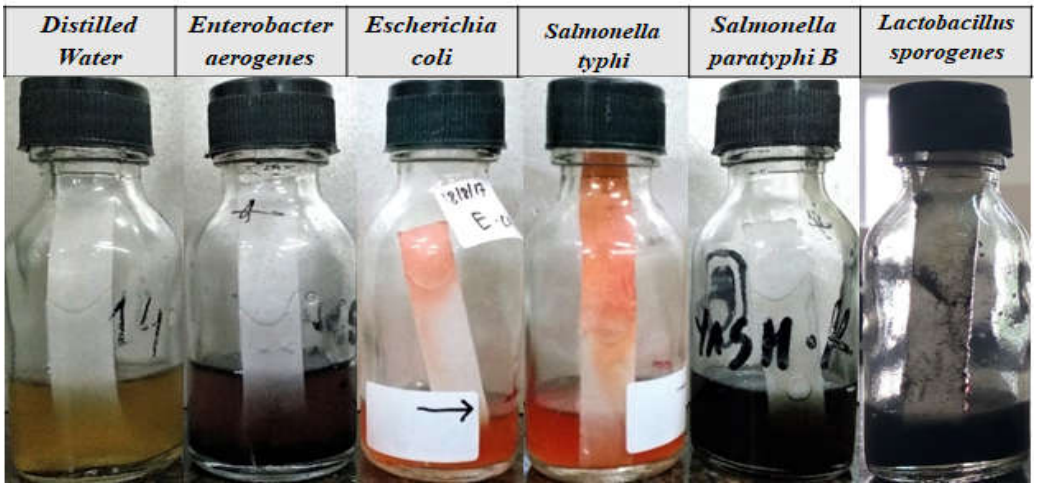

Figure no.5 : Modified H_2S strip test method

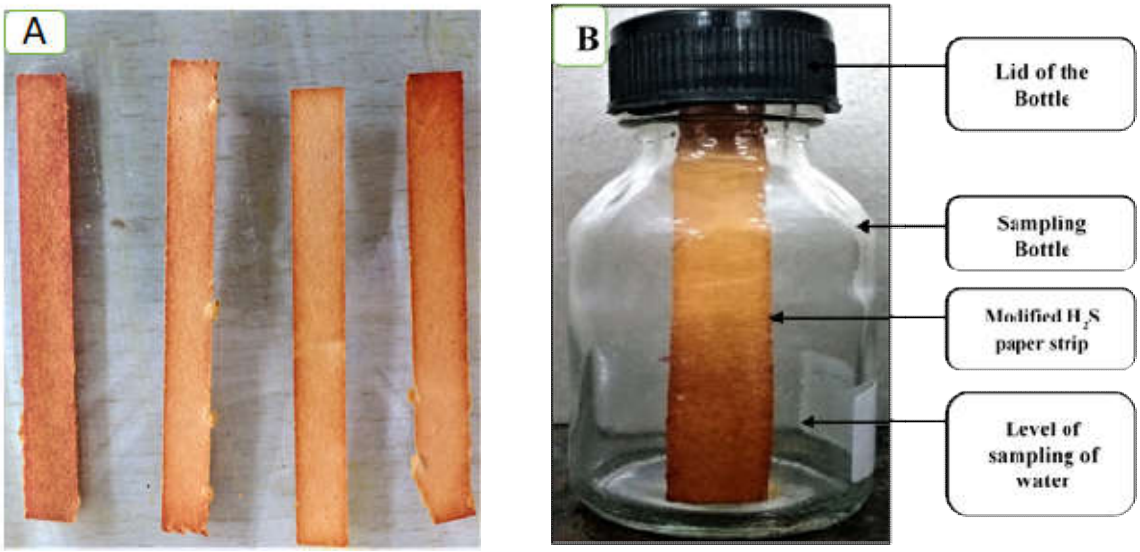

Figure no 6: (A) Modified H_2S Media Impregnated Paper Strip, (B) Water Quality Field Testing Kit (WQFTK)

CONCLUSION

This research insights on the development of water quality field testing kit (WQFTKs). Different method like MPN, Lead acetate paper strip test and H_2S broth method, were studied for their usability as water quality field testing kit (WQFTKs).As these methods failed to fulfill the criteria of WQFTK, we modified

Manja *et al* [11] H_2S medium and designed a modified H_2S strip test method. This study gives a proper understanding of efficiency and accuracy of this modified H_2S strip test method. This method is cost effective, less time consuming and requires less expertise, and can be used as water quality field testing kit (WQFTKs). This user-friendly water quality field testing kit (WQFTKs) can be used in distant region like slums, rural areas and areas where it is difficult to establish water testing laboratories.

ACKNOWLEDGEMENTS

We would like to express our gratitude to the Principal of R.D. and S.H. National College and S.W.A. Science College – Mr. Dinesh Panjwani for his continuous support to this research work. We would also like to thank the Department of Biotechnology and the Department of Botany of R.D. and S.H. National College and S.W.A. Science College for providing us the required amenities. We would also like to thank the lab assistants of the Department of Biotechnology and the Department of Botany of R.D. and S.H. National College and S.W.A. Science College for their help as an when needed.

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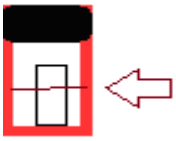
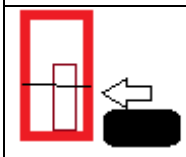
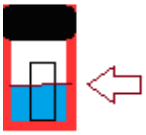
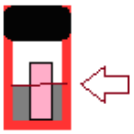
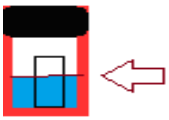
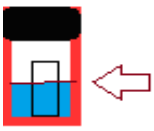
Annexure 1.

Potable Water Quality Field Testing Kit (WQFTK) Instruction

Procedure:

	1. Take the bottle having a strip , as provided in the kit
	2. Open the lid of the bottle and put water sample up to the marked level.
	3. Close the bottle and keep it for 12-16 hours.
	4. After 16 hours , if the water turns black and the strips becomes pink, the water is non-potable and not safe to drink.
	5. If the water does not turn black , the water is potable i.e. it is safe to drink.
	6. For Your further reference, same procedure from step no. 1-4 can be repeated with boiling water (after cooling) to ensure complete safety and efficacy of Kit.

Annexure 2.

R.D & S.H. National College of Commerce & Arts and
S.W.A. Science College

TESTING OF POTABILITY OF DRINKING WATER

ALL INFORMATION IN THE BOX BELOW MUST BE COMPLETED

NAME:-_____

CLASS:-_____ ROLL NO:- _____

CONTACT NUMBER:-_____ EMAIL ID:-_____

ADDRESS:-_____

AREA:-_____ PIN CODE:-_____

SOURCE: **OPEN AREA/TAP WATER/MUNCIPAL WATER/FILTER WATER/BOREWELL**

STUDENT'S SIGNATURE:

FORLAB USE ONLY

Date of Issued:-_____ Time:- _____

Date of Received:-_____ Time:-_____

Sample collected by:-_____

SAMPLE NO:-

ANALYSIS PERFORMED:

PHYSICAL PARAMETER	RESULT	H_2S KIT	RESULT	BIOCHEMICAL TEST	RESULT
COLOUR		**PINK COLOURATION OF STRIP**		INDOLE	
TURBIDITY				METHYL RED	
pH		**BLACK PRECIPITATION**		VP	
TASTE				CITRATE	
BACTERIA PRESENT:					

CONCLUSION:_____