# Detection and Rapid Enumeration of Fecal Coliforms in Drinking Water: A Comparison of New Technique with Established Techniques

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## ABSTRACT

***The major aim of the present study is introduction of a new technique for assessment of fecal coliforms in drinking water. The indiscriminate disposal of industrial and municipal wastes makes the ground water susceptible to pollution. Although there are standard conventional techniques and rapid analytical techniques for detection of fecal coliforms in drinking water but there are some inherent limitations with them. The present results show that the proposed technique may serve as an emergency test for detecting fecal contamination in drinking water. The developed test/technique is sensitive, easy to use, rapid, economic and gives reproducible results. This technique can be incorporated in existing water testing field kits.***

***Keywords: Fecal coliforms, indicator organisms, E. coli, colonies***

## 1. INTRODUCTION

Water is one of the essential components for the sustenance of life on earth. The indiscriminate disposal of industrial and municipal wastes makes the ground water susceptible to pollution. It is reported that two thirds of all the illnesses in India are related to water-borne diseases.

Various standard techniques and rapid analytical techniques are available for detection of fecal coliforms in drinking water. Paul Kabler W [1] discussed about coliform group of organisms. A new culture medium 'Violet Red Bile 2-Agar' has been proposed [2] for enumeration of stressed coliforms. Use of potentiometric measurement of lipoic acid reduction [3] for detection of fecal coliforms in water has been suggested. '$H_2S$ paper strip method' a bacteriological test [4] for fecal coliforms in drinking water was discussed. Although standard conventional techniques are serving us well over the years, yet there are some inherent limitations with them. Keeping in view the limitation of conventional methods, the aim of present study is development of *a new rapid technique for detection and enumeration of fecal coliforms in drinking water*.

***Materials and Methods: Description of New Technique (based on MTFT technique)***

The technique employs newly formulated BR culture medium and it completes in two stages

**i) Presumptive Phase:** BR broth is used

Composition of medium (BR broth)

| | |
|---|---|
| Lactose ..... | 15.0 g |
| L-Asparagine ..... | 5.0 g |
| Urea ..... | 1.0 g |
| Disodium hydrogen orthophosphate..... | 2.0 g |
| Sodium desoxycholate ..... | 2.0 g |
| Sodium lauryl sulphate ..... | 0.1 g |
| Methylene blue dye ..... | 0.01 g |
| Distilled water ..... | 1 L |

**Procedure:** About 2.5 g of above medium is dissolved in 10 ml water sample in a specially designed sterilized inoculating tube (graduated at 10 ml and 110 ml) by gentle heating using spirit lamp/burner. The contents are cooled and volume is made up to 110 ml by the inoculum (water sample under test). It is then covered with a loose lid as shown in fig and incubated at 44 ± 0.5$^{o}$C in incubator, with occasional stirring by a pointed glass rod provided inside the tube. The positive test is indicated by change of colour with simultaneous liberation of gas and appearance of turbidity in the fermented broth. This shows the presence of fecal coliforms (E. coli) and also possible presence of pathogens.

**ii) Completed phase:** BR agar is used.

Composition of BR agar medium:

| | |
|---|---|
| Lactose ..... | 1.5 g |
| L-Aspargine ..... | 0.5 g |
| Urea ..... | 0.1 g |
| Disodium hydrogen orthophosphate..... | 0.2 g |
| Sodium desoxycholate ..... | 0.2 g |
| Sodium lauryl sulphate ..... | 0.01 g |
| Agar ..... | 1.5 g |
| Neutral red dye..... | 0.001g |
| Distilled water ..... | 100 ml |

200 mg BR agar medium is used for making standard plate using 5 ml water and diluted fermented broth (from presumptive phase) is poured over and spread evenly. Excess material is discarded. It is then kept in incubator in inverted position under facultative anaerobic conditions. Typical and

discrete colonies (2-3 mm diameter) with dark red centres confirm the presence of fecal coliforms, especially E. coli in water.

## 2. RESULTS AND DISCUSSION

Selection of indicator organism: For our study, E. coli is the proposed indicator organism. For the New Rapid Technique, studies were carried out in following steps

**I) Formulation and Selection of New Chemically Defined Culture Medium:** Various chemically defined culture media have been formulated and tried for assessment of bacteriological quality of water, keeping in mind that they should be stable, cheap and give the results within shortest period of time. Based on their response, finally BR culture medium has been selected for use in new proposed technique.

**II) Selection of Suitable Dye to be used with Newly Formulated BR Culture Medium**

*i)* *For Presumptive Phase:* Studies were carried out using eight different dyes and results were recorded. Finally, methylene blue dye was selected because it shows sharp colour change with early bacterial growth. So this was finally incorporated with BR medium for further studies.

*ii)* *For Completed Phase:* Studies were carried out using three different dyes and results were recorded. Finally neutral red dye was selected. Colonies about 2-3 mm in diameter with clear and dark centres are shown in the figure below.

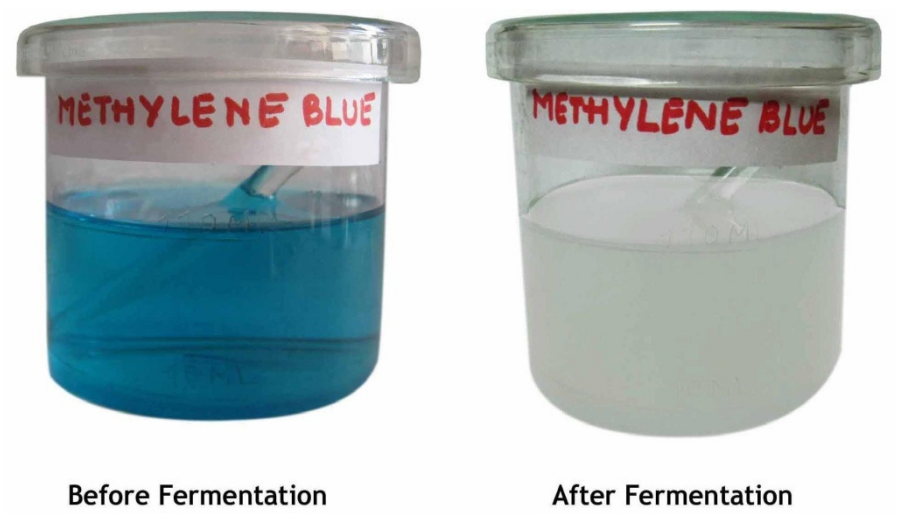



**Figure 1: Presumptive phase**

**Figure 2: Completed Phase**

## 3. TYPICAL COLONIES OF E. COLI WITH NEUTRAL RED DYE

**III) Selection of Optimum Incubation Temperature (during presumptive phase):** Studies were also carried out in laboratory to find out the behaviour of bacteria (especially fecal coliforms) in different temperatures (using BR culture medium). Results have been are summarised as follows;

**Table 1: Time-Temperature relationship w.r.t. Bacterial Counts**

| E.coli MPN*/100 ml | Incubation period [hours] | | | |
|---|---|---|---|---|
| | at 37±0.5Oc | at 40±0.5 $^{o}$C | at 42±0.5$^{o}$C | at 44±0.5$^{o}$C |
| ≥ 2400 | 10.0 | 8.0 | 6.0 | 4.0 |
| 1600 | 11.0 | 8.5 | 6.5 | 5.0 |
| 900 | 12.5 | 9.0 | 7.0 | 5.5 |
| 500 | 14.0 | 9.5 | 7.5 | 6.0 |
| 280 | 16.0 | 10.0 | 8.0 | 7.0 |
| 170 | 20.0 | 11.0 | 9.0 | 8.0 |
| 50 | 22.0 | 12.0 | 10.0 | 9.0 |
| 22 | 24.0 | 14.0 | 12.0 | 10.0 |
| 9 | 28.0 | 17.0 | 14.0 | 12.0 |
| 4 | 33.0 | 21.0 | 17.0 | 14.0 |
| 2 | 40.0 | 25.0 | 20.0 | 16.0 |

The table reveals that at temperature 44±0.5°C, minimum incubation time is required (even for lower MPN value) so further studies were carried out at this temperature. For completed phase, 40°C is most suitable because at higher temperature medium gets dried up.

After all the above observations, results were as follows;

i) *Presumptive Phase:* The positive test is indicated by change of colour from blue to dirty white incubated at 44 ± 0.5°C with simultaneous liberation of gas and appearance of turbidity in the fermented broth within 16 hours. This shows the presence of fecal coliforms (E. coli) and also possible presence of pathogens.

ii) *Completed Phase:* Typical and discrete colonies (2-3 mm diameter) with dark red centres within 8-10 hours at 40 ± 0.5°C under facultative anaerobic conditions confirm the presence of fecal coliforms, especially E. coil in water.

To check the suitability of proposed method as field test, studies were carried out in following steps and results were recorded:

**1) Check Stability / Shelf Life of BR Medium:** Small pouches were prepared by using aluminium foil lined with polythene. Weighed dried BR medium (separately for presumptive and completed phase) were filled in these pouches and sealed. In field for single sample, contents from single pouch can be used. After regular interval of time, stability/shelf life of medium was checked and found to be one year.

**2) Comparison of Proposed technique with IMViC test**: For ascertaining fecal coliforms, water bacteriologists have developed a series of four simple biochemical tests viz Indole, Methyl Red, Voges-Proskauer and Citrate reactions (IMViC) for distinguishing fecal and non-fecal coliforms. The effect of polluted water from Khan River on nearby drinking water sources [5]were analyzed.

The proposed technique requiring maximum 26 h. has proved to be most effective and simpler for differentiation of E.coli than IMViC tests which needs about 5 days and 4 different reagents/media.

**Table 2: Comparison of IMViC and Proposed Technique**

| Test | No. of ingred-ients | Incubation | | Stability of culture medium | Organisms | | |
|---|---|---|---|---|---|---|---|
| | | Temp [°C] | Time [h.] | | E.coil | Citrob-acter freundii | Klebsiella entero- bacter |
| Indole | 4 | 35 | 24 | Unstable | + or - | - | + or - |

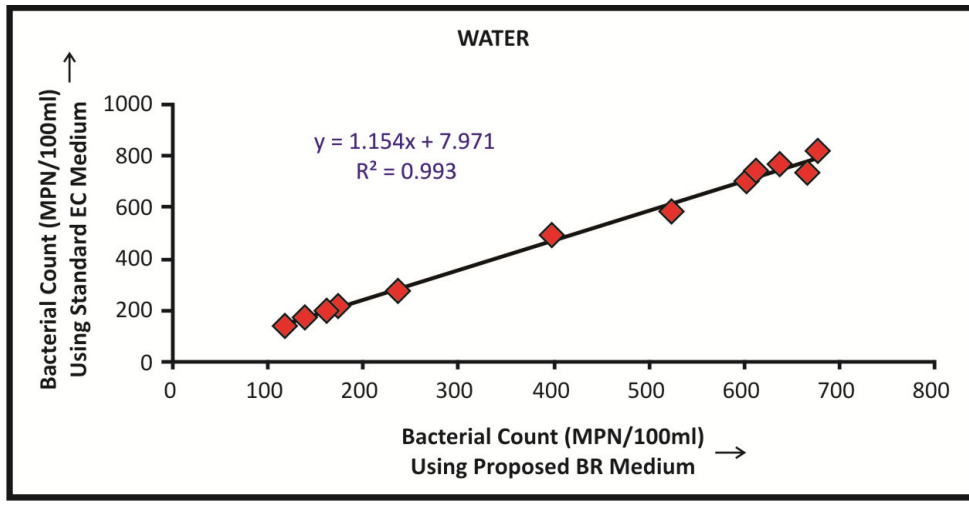
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Methyl red | 5 | 35 | 120 | Unstable | + | + | - |
| Voges proskauer | 6 | 35 | 48 | Unstable | - | - | + |
| Citrate | 6 | 35 | 72-96 | Unstable | - | + | + |
| New proposed technique | 8 | 44 | 4-16 | Stable | + | - | - |

**3) Survey Trials**: A study on the fecal contamination of Cagayan de Oro River [6] was done (Barangay Kauswagan) using MTFT method. The total counts (fecal coliforms) per 100 ml in various water resources in and around Jodhpur city were detected by using BR medium and EC medium (standard MTFT method) at $44^0$C. BR medium has been observed to be better in giving more counts of coliforms in short period of time.

**Table 3: Comparison of EC medium (MTFT) with BR medium**

| S. No. | Sample source | EC medium [max 48 h.] | BR medium [max. 26 h.] | Ratio of counts EC: BR |
|---|---|---|---|---|
| 1. | Tapi Baori | 238 | 274 | 100 : 115 |
| 2. | Nagadari | 120 | 140 | 100 : 116 |
| 3. | Baiji Ka Talab | 175 | 216 | 100 : 123 |
| 4. | Turji Ka Jhalra | 164 | 200 | 100 : 121 |
| 5. | Ranisar | 526 | 584 | 100 : 120 |
| 6. | Padamsar | 668 | 735 | 100 : 118 |
| 7. | Fateh Sagar | 615 | 740 | 100 : 117 |
| 8. | Balsamand | 400 | 490 | 100 : 122 |
| 9. | Kailana | 605 | 700 | 100 : 115 |
| 10. | Takhat Sagar | 640 | 765 | 100 : 119 |
| 11. | Umed Bhawan Talab | 680 | 820 | 100 : 120 |
| 12. | Akheraj Talab | 140 | 172 | 100 : 122 |

The graph (1) indicates linear correlation between behaviour of standard EC and proposed BR medium




## 4. CONCLUSION

The present results show that the proposed technique may serve as an emergency test for detecting fecal contamination in drinking water. The developed test/techniques are sensitive, easy to use, rapid, economic and give reproducible results. The techniques can be incorporated in existing water testing field kits.

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