Asian Journal of Chemistry Vol. 17, No. 4 (2005), 2705–2709

Development of a Simple and Rapid Field Test for Monitoring Bacteriological Quality of Water

M.K. BHUTRA* and AMBICA SONI
Department of Chemistry, J.N.V. University, Jodhpur-342 005, India

A rapid method for examination of faecal coliforms in water using a new chemically well defined culture medium (BS medium I & II) stable under extreme climatic conditions of desert is described. The presumptive test requiring 3 to 12 h incubation at 44°C for faecal contamination is qualitative, quicker and simpler than existing standard methods. The confirmative test for faecal coliforms *(Escherichia coli)* on agar plates using BS agar (II) requires incubation for another 8–10 h at 40°C. An attempt has also been made to evaluate the efficiency of BS medium for the detection of faecal as well as non-faecal origin in 18 h. It was observed that BS medium could be used for rapid isolation of coliforms even under the field conditions (37°C) with greater efficiency than the conventional media used in standard methods. The technique using new culture medium could be a good supplement to the existing methods in health centres towars control of water borne diseases like typhoid, cholera and dysentery.

Key Words: Coliforms, Monitoring, Quality of water.

INTRODUCTION

The microbiological examination of water[1–6] enjoys a special status in pollution studies, as it is a direct measurement of deleterious effects of pollution on human health. It is routinely conducted to ensure the safety of potable water, to monitor the water quality for recreational, industrial and agricultural uses and also to evaluate prospective water resources for drinking purposes. Natural water supplies such as river, lakes and streams contain sufficient nutrient to support growth of various pathogenic and non-pathogenic bacteria which enter the water supply in several different ways.

Pathogenic bacteria[7, 8] may be present in water bodies contaminated by domestic sewage and other pollutants. The detection and estimation of these bacteria is a tedious work because of the presence of very small numbers and use of complicated techniques for their isolation. Hence, the coliform group of bacteria (non-pathogenic) is used worldwide as an indicator of the microbiological quality of drinking water. The group mainly consists of several genera of bacteria belonging to the Enterobacteriacae family, mostly *Escherichia, Klebsiella, Enterobacter, Citrobacter* and *Seratia;* out of them *E. coli* is entirely

of human origin. The detection of coliforms in tap water is supposed to indicate to utilities and health authorities that a breach has occurred in treatment or in the distribution system, allowing microbial (faecal) contamination of water to occur and thereby rendering it inappropriate for drinking.

Several standard methods like multiple tube fermentation technique (MTFT) and millipore filter technique etc. are available for monitoring bacteriological quality of water and its related health risks but they have the disadvantages of being costly, time consuming, involving use of unstable culture media and requiring trained personnel. Such methods are also difficult to employ in the field. These restrictions have made it difficult to attempt regular drinking water quality monitoring or to carry out any extensive survey of water source and to classfiy them. This is particularly true for developing nations, where financial and man-power resources are often limiting and where a significant number of communities and their water sources are often isolated and difficult to reach. The development of an alternative method[9] which could be used more easily in the field as well as being relatively cheap and easy to carry out would be of great advantage to such nations.

The present paper reports a rapid and simple field test using a stable chemically well defined culture medium and compare its performance with other existing methods for assessing bacteriological quality of water under extreme tropical conditions, particularly of desert region.

EXPERIMENTAL

Keeping in view the WHO[10]/ICMR[11] limits of two *Escherichia coli* or 10 coliforms per 100 mL of untreated water, a technique was developed which completes in two stages requiring BS medium I during presumptive test and BS medium II for completed test.

(a) Presumptive test: The test involves the dissolution of about 2 g BS medium (I) in 10 mL of sample water in a specially designed presterilized inoculating tube (height 9 cm, diameter 5.5 cm with loose lid) having graduation at 10 and 110 mL. The dissolution of medium is done by gentle heating, cooling and making up the volume by sample water to 110 mL. The pH is adjusted at 7. It is then incubated under facultative anaerobic conditions for maximum 12 h at 44°C. The facultative anaerobic conditions are created by covering the inoculating tube with a little loosely fitted inverted petridish. The change in colour from blue-green to yellow with turbidity and gas formation shows presence of faecal coliforms in the water sample. Absence of any such symptoms indicates that the sample water is free from bacteria.

(b) Confirmative Test: If required, it is carried out on standard agar plates. The plate is prepared from BS II agar medium (about 70 mg for each plate) and streaked with fermented broth of positive presumptive test obtained from the above tests. It is then incubated at 40°C for 8 to 10 h under facultative anaerobic conditions. The positive completed test is characterized by typical, discrete colonies (2–3 mm diameter) of *E. coli* with dark red centres.

The composition of BS I and II media is given below:

Lactose AR (oxoid)	1.5 g
L-Valine GR (Loba Chem)	0.3 g
Glycine GR (Loba Chem)	0.2 g
KH_2PO_4	0.2 g
Urea	0.1 g
Sodium citrate	0.01 g
Sodium lauryl sulphate AR (BDH)	0.01 g
Neutral red/Bromothymol blue*	0.001 g
Agar powder, No. 3 (oxoid)	1.5 g
Distilled water	100 mL

* Neutral red and bromothymol blue indicators are used in agar plate and broth media respectively.

RESULTS AND DISCUSSION

Studies on different standard culture media[12–14] indicate that none of the media are suitable for examination of faecal coliforms in water under extreme climatic conditions of the arid areas (Table-1) because either they are unstable or require more number of ingredients and incubation time.

TABLE-1
COMPARATIVE STUDIES OF DIFFERENT CULTURE MEDIA AT 37°C

Culture medium	No. of ingredients	Incubation period (d)	Size of colonies (mm)	Stability
Desoxycholate lactose agar medium	6	16–17	2–3	Unstable
MacConkey's agar medium	7	20–24	2–3	Unstable
Casienate agar medium	6	17–20	1–2	Unstable
Eijkman agar medium	7	20–24	1–2	Unstable
Glucose peptone agar medium	7	22–24	1–2	Unstable
Simmons citrate agar medium	6	25–28	1–2	Stable
Lauryl tryptose agar medium	6	12–15	2–3	Unstable
Glutamic acid peptone agar medium	6	24–28	1–2	Unstable
MF endo medium	12	20–24	2–3	Unstable
EMB agar medium	6	20–24	1–2	Unstable
EC medium	6	20–24	1–2	Unstable
BS medium	8	10–12 (at 37°C) 8–10 (at 40°C)	2–3	Stable

A perusal of Table-1 reveals that BS media (I & II) are more suitable for rapid growth of faecal coliforms. So in new technique for analysis of water sample these media were used. The results have also been found in agreement with conventional tests carried out side by side.

TABLE-2
COMPARISON OF NEW TECHNIQUE WITH STANDARD TECHNIQUES

Technique	Number of stages	Culture medium	No. of ingredients	Incubation		Stability	Remarks
				Period (h)	Temp. (°C)		
Multiple tube fermentation technique	Stage I: Presumptive phase	Lactose broth	12	24–48	35	Unstable	The technique requires 5 days for complete test. It also needs IMViC test for confirmation of *E. coli*
	Stage II: Confirmative phase	Brilliant green lactose bile broth EMB agar	4	24–48	35	Unstable	
	State III: Completed		6	24	35	Unstable	
Membrane Filter Technique	One stage	MF endomedium	12	18–24	35	Unstable	It requires imported filtration unit and culture media and it is also a costly technique
New proposed technique	Stage I: Presumptive phase	BS media (broth) (I)	7	3–12	44	Stable	Temp. 44 ± 0.5°C is the best temperature exclusively for faecal coliform and chemically defined culture media are used which are highly stable
	Stage II: Confirmative phase	BS Agar (II)	8	8–10	40	Stable	

TABLE-3
COMPARISON OF IMViC AND BS DIFFERENTIAL TESTS

Test	No. of ingredients	Incubation		Stability	Organisms		
		Temp (°C)	Time (h)		*E. coli*	*Citrobacter freundii*	*Klebsiella*
Indols	4	35	24	Unstable	+ or –	–	+ or –
Methyl red	5	35	120	Unstable	+	+	–
Voges-Proskauer	6	35	48	Unstable	–	–	+
Citrate	6	35	72–96	Unstable	–	+	+
BS	7	40	18–24	Stable	+	–	–

Water becteriologists have developed a series of simple biochemical tests, *viz.*, indole, methyl red, Voges-Proskauer, citrate (IMViC) reactions for distinguishing faecal and non-faecal coliforms. The new technique (using BS media) requiring maximum 24 h has proved most effective and simpler for differntiation of faecal coliforms than standard IMViC tests which need about 5 days and 4 different culture media.

Conclusion

Comparison of the new technique with conventional standard techniques reveals that the new technique has the following advantages:

1. The test is specific for faecal coliforms.
2. It utilizes chemically defined culture media which are non-perishable and have long shelf life.
3. The test is simple, which can be performed by a person of normal scientific temper.
4. The proposed technique is rapid and economical too.
5. It can also be safely used in place of IMViC test for differentiation of faecal and non-faecal coliforms.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. G.L. Bhutra for providing valuable suggestions. One of the authors, Ambica Soni, is thankful to CSIR for providing financial assistance.

REFERENCES

1. J. Rodier, Analysis of Water, Keter Publishing House Jerusalem Ltd. (1975).
2. S. Takizawa, T.V.V. Tran and L.Fu, *Chem. Abstr.*, **134,** 1150 (2001).
3. A.P. Dufor, ASTM Special Technical Publications, Philadelphia, Pennsylvania, pp. 48-58 (1977).
4. G.F. Graun and J.L. McCabe, *J. Am. Water Works Assoc.*, **65,** 65 (1973).
5. A.L. Nair, D. Bhuyan and H.B. Das, *AFMJ*, **28,** 231 (1972).
6. J.H. Strandridge and J.J. Delfino, *Appl. Environ. Microbiol.*, **42,** 918 (1981).
7. R. Hoffman and M. Marshall, *J. Am. Water Works Assoc.*, **96,** 66 (2004).
8. M.C. Besner, V. Gauthier, P. Servais and A. Camper, *J. Am. Water Works Assoc.*, **94,** 95 (2002).
9. S.E. Hruday and S. Rizak, *J. Am. Water Works Assoc.*, **96,** 110 (2004).
10. WHO, WHO Geneva report, pp. 1–37 (1994).
11. ICMR, Manual of Standards of Quality for Drinking Water Supplies, 2nd ed., Govt. of India, New Delhi (1977).
12. R. Gopal, T.C. Tak and M.K. Bhutra, Indian Patent No. 153880 (1980).
13. R. Gopal, T.N. Bhargava, O.P. Bhati, T.C. Tak, P.K. Ghosh, S. Rai and M.K. Bhutra, *J. Indian Water Works Assoc.*, **15,** 59 (1983).
14. S.C. Lenore, E.G. Arnold and D.E. Andrew, Standard Methods for the Examination of Water and Wastewater, APHA, AWWA & WPCF, 20th Edn., (1998).

(*Received*: 5 February 2005; *Accepted*: 25 July 2005) AJC-4314