

A new technique for monitoring bacteriological quality of water in relation to water-borne diseases

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ABSTRACT

A rapid method for examination of faecal coliforms in water using a new chemically well-defined culture medium stable under extreme climatic conditions of desert is described. The presumptive test requiring 3 to 10 hr incubation at 40°C for faecal contamination is qualitative, quicker and simpler than existing standard methods. The confirmative test for *Escherichia coli* on agar plate requires incubation for another 8 to 10 hr at 40°C. The technique could be a good supplement to the existing methods in health centres towards control of water-borne diseases like typhoid, cholera and dysentery.

INTRODUCTION

The existing techniques for bacteriological examination of water viz., the multiple tube technique, the membrane filter method and the GEL technique employ the culture media (AWWA, 1980) which are unstable and hence unsuitable in the extreme climatic conditions of the Indian desert. The present paper reports a rapid and simple field test using a stable culture medium and compares its performance with other existing methods for assessing bacteriological quality of water under extreme tropical conditions, particularly of desert region.

MATERIAL AND METHODS

Keeping in view the WHO limits of two *Escherichia coli* or 10 coliforms per 100 ml untreated water (Anon., 1971), a

technique was developed using 2 g CFL medium for one stage test (Gopal *et. al.*, 1980).

The test involves the dissolution of CFL medium in 10 ml of sample water in an inoculating tube (height 9 cm, diameter 5.5 cm with loose lid and a durham tube) especially designed for this test, having graduation at 10 ml 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 in an incubator under facultative anaerobic conditions for maximum 10 hr at 40°C. The facultative anaerobic conditions are created by covering the inoculating tube with a little loosely fitted inverted petridish. The change in colour from orange-red to violet 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. Confirmatory test, if required, is carried out on standard agar plates. The plate is prepared from CFL agar medium (Gopal *et al.*, 1980) (about 70 mg) 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 hr under facultative anaerobic conditions. The positive completed test is characterised by typical, discrete colonies (2-3 mm diameter) of *E. coli* with dark red centres.

RESULTS AND DISCUSSION

Studies on different culture media indicate that none of the media are suitable for examination of *E. coli* in water under extreme climatic conditions of the arid areas. Conventional methods (Table 1) are complex requiring number

of stages and skilled operators. These tests cannot assess water quality in one stage too. The present technique, after undertaking testing of over 1500 water samples, indicated positive presumptive tests in the first broth stage. All such results have been found in agreement with conventional tests carried out side by side (Table 1).

Studies at 37°C, 40°C and 44°C were also carried out in order to study the behaviour of *E. coli* at these temperatures in CFL medium (broth). It is observed from Fig. 1 that maximum period required at 37°C varied between 3-12 hr; at 40°C, between 3-10 hr and 44°C, between 3-8 hr. The maximum incubation period studied in all three sets of experiments was at concentration of two *E. coli* in 100 ml of water as per limits laid down by ICMR and WHO (Anon, 1971).

Completed test, if required, is carried out using CFL agar medium. Results of the comparative studies undertaken on

Table 1. Comparison of CFL technique with other standard techniques

Technique & Stages	Culture medium	No. of ingredients	Incubation temp. (°C)	Time (hr)	Stability
Multiple tube technique (3 stages)	(a) Lactose broth	9	37	72-96	Unstable
	(b) Brilliant green bile broth				
	(c) EMB agar				
Millipore filter technique	(a) MF Endo agar	12	37	18-24	Unstable
CFL technique	(a) CFL broth	7	40	3-10	Stable
	(b) CFL agar	8	40	8-10	Stable

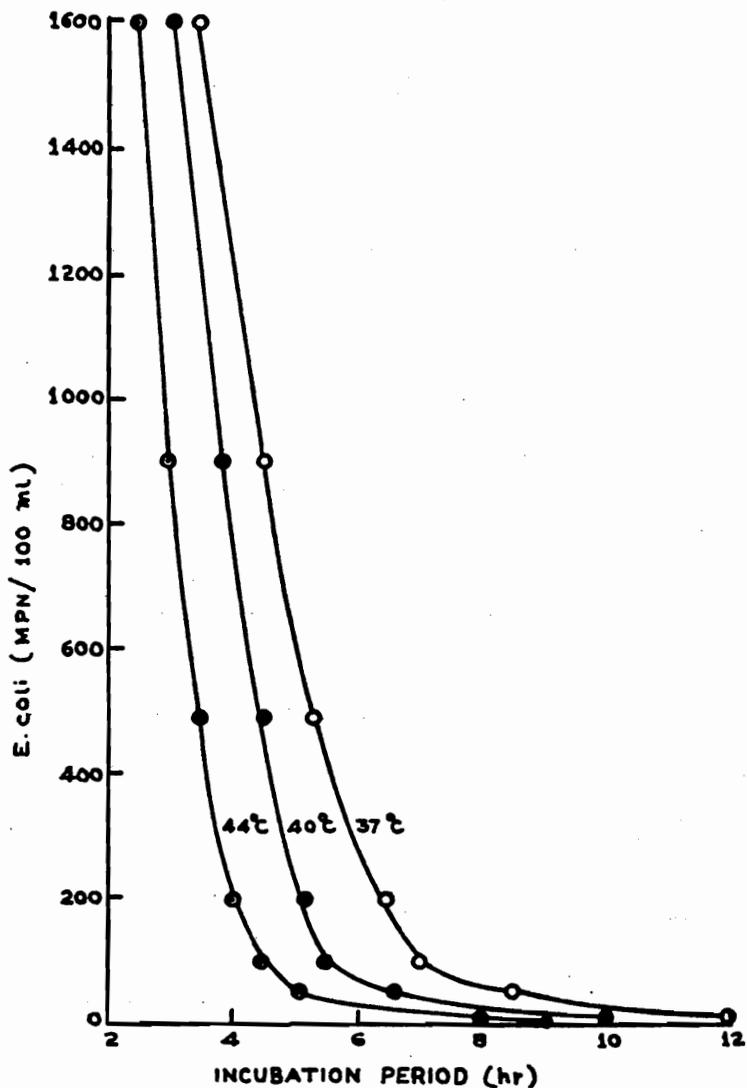


Fig. 1. Incubation period at varying concentrations of *E. coli* at 37, 40 & 44°C.

different agar media (Table 2) clearly indicate that CFL agar medium is the most suitable. The tests employing CFL medium is qualitative, quicker and simpler than others.

Water bacteriologists have developed a series of four simple bio-chemical tests, Indole Methyl-Red Voges - Proskauer Citrate (IMViC) reactions (AWWA, 1980) for distinguishing faecal and non-

faecal coliforms. The CFL technique requiring maximum 18 hr has proved most effective and simpler for differentiation of *E. coli* than IMViC tests needing about 5 days and 4 different culture media (Table 3).

Table 2. Comparative studies of different culture media at 37 °C

Culture Medium	No. of ingredients	Incubation period (hr)	Size of colonies	Stability
Desoxycholate Lactose agar medium	6	16-17	2-3 mm	Unstable
Mac Conkey's agar medium	7	20-24	2-3 mm	Unstable
Casinate agar medium	6	17-20	1-2 mm	Unstable
Eijkman agar medium	7	20-24	1-2 mm	Unstable
Glucose Peptone agar medium	7	22-24	1-2 mm	Unstable
Simmons citrate agar medium	6	25-28	1-2 mm	Stable
Lauryl Tryptose agar medium	6	12-15	2-3 mm	Unstable
Glutamic acid peptone agar medium	6	24-28	1-2 mm	Unstable
MF Endo medium	12	20-24	2-3 mm	Unstable
EMB agar medium	6	20-24	1-2 mm	Unstable
EC Medium	6	20-24	1-2 mm	Unstable
CFL Medium *	7	8-10	2-3 mm	Stable

* Studies carried out at 40°C.

Table 3. Comparison of IMViC and CFL differential tests

Tests	No. of ingredients	Incubation		Stability	Organisms		
		Temp. (°C)	Time (hr)		<i>E. coli</i>	<i>Citrobacter freundl</i>	<i>Klebsiella ent</i>
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	-	+	+
CFL	7	40	18-20	Stable	+	-	-

The CFL test has been incorporated in water testing field kit (Gopal *et al.*, 1983) developed by this Laboratory for examination of chemical as well as bacteriological quality of water. The chemicals and the media are stable for a period of over one year under ambient conditions of the desert. The kit weighing about 6 kg can undertake examination of 100 water samples. The reagents are replaced for further testing. This could be a good addition to the existing methods used in the health centres for monitoring water quality.

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