DETECTION OF COLIFORM ORGANISMS IN DRINKING WATER BY RADIOMETRIC METHOD

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ABSTRACT

The radiometric method has been used for detection of coliform bacteria in water. The method is based on measuring the released metabolic 14002 from 14C- lactose in growth media containing coliform organisms incubated at 37°C under continuous shaking. This rapid and sensitive radiometric method permits the detection of even single coliform organisms within 6 hours of incubation. Using this automated method, a total of 102 samples (in duplicate) collected from different areas in and around Rawalpindi and Islamabad were assessed for coliform bacteria. Of these 102 samples, 50 were tap water samples, 40 from wells and 6 each were from Rawal and Simly dams. About 47% and67% tap water samples, while 62% and 74% well water samples were found unsatisfactory from around Islamabad and Rawalpindi areas, respectively. About 83% and 66% water samples from Rawal dam and Simly dam respectively were found to be unsatisfactory (JPMA 41:157, 1991).

INTRODUCTION

Drinking water is the main source of spreading diseases in developing countries[1,2]. The drinking water quality varies in different areas according to the hygiene and living standards. WHO survey suggested that 80% of all human illness in the developing countries are caused by biological contamination[2,3]. This contamination can occur directly or indirectly by sewage waste, or by human and animal excreta[4,5]. It can occur at the source, between the source and storage points or due to insufficient disinfection practices, distance from the filtration plant to the locality or due to low water pressure. Defective joints, rusted pipes and crossing over of the sewage lines may also cause this contamination. Pakistan is no exception in this regard. Most of the population in Pakistan is using untreated and unsafe water for drinking. In Pakistan, WHO recorded 315 cases of water borne diseases per 10,000 population due to consumption of polluted water[6]. Additional figures from WHO show that in 1981 there were 50,000 cases of typhoid and paratyphoid fever in Pakistan[7]. According to earlier estimates of WHO/World Bank contaminated water accounted for 30% of all reported diseases and 40% of all deaths in Pakistan[8]. In developing countries water borne infectious diseases mostly affect children resulting in high mortality, disability and retarded growth and thus creating a burden on health care system[9]. The bacteriological control is of utmost importance due to medical, ecological and economic considerations. This is usually based on the quantification of E.coli which serves as a marker for faecal contamination[10,11]. Ideally drinking water should not contain any pathogenic microorganism[12]; in treated tap water, 1 count per 100 ml is good, 2-4 satisfactory and above 4 counts is considered suspicious to unsatisfactory and above 10 as contaminated. In untreated water, presence of coliform organisms upto 10 counts is considered suspicious and 10-100 unsatisfactory to contaminated. The water quality has been tested in different Pakistani cities[13-15] including Rawalpindi and Islamabad[16] by conventional time consuming methods. The conventional methods provide an answer at least 24 hours after receiving the samples. With the increase in size and complexity of municipal water supply, there is a need for rapid detection of coliform organisms in water. This work describes an automated, rapid and

sensitive method using ^{14}C-lactose medium for detection of E. coli (a lactose fermenting bacteria) in water samples in and around Rawalpindi and Islamabad. This carbohydrate (14C-lactose) was selected since it permits the differentiation between E.coli and most of enterobacteriaceae within 6 hours of incubation. The method is also suggested to be useful by various workers[17-22].

MATERIALS AND METHOD

Presterilized stoppered sample collection bactec vials, sterilized syringes of iml and 10 ml, sterilized 100 ml pipettes, ^{14}C-lactose (lUci/ml), lactose broth ($2x10^{-5}$M). E.coli strain, membrane filters of 0.45 um pore 47mm, Laminar flow and Bactec 460 instrument were used in the present (control) in which 0.45u m filter paper was added through which 100 ml of sterilized distill water was passed instead of standard bacterial dilutions. The vials were incubated at 37°C and tested for growth after every hour upto 7 hours and the results obtained were plotted as shown in Figures 1 & 2. The metabolic ^{14}CO2 produced by E. coli within 6 hours of incubation with 1 C-lactose nutritive media was detected by the automated instrument Bactec 460. The ^{14}C02 produced during the incubation period is aspirated into the ionization chamber, the electrometer present in Bactec 460 unit then measures the current produced in the ionization chamber in terms of Growth Index (GI) which is an arbitrary linear scale related to the amount of radioactivity in the ionization chamber. The amount of 14CO2 liberated is proportional to the amount of bacteria growth in the nutrient media. A threshold of 30 GI is set for aerobic bacteria and above this threshold level the reading is considered as positive and indicates the presence of bacteria. The collected water samples were also tested in the same way as described above. After passing 100 ml of each sample through 0.45um filter paper which were then incubated in bactecvials containing 0.1 ml ^{14}C-lactose (luci/mi) and 30 of lactose broth ($2x10^{-5}$M). The vials were then sealed and incubated at 37 °C. The samples were checked for bacterial growth after every hour upto 7 hours. All the above experimental work was carried Out under laminar flow unit.

RESULTS

The standard detection curves (Figures 1 and 2)

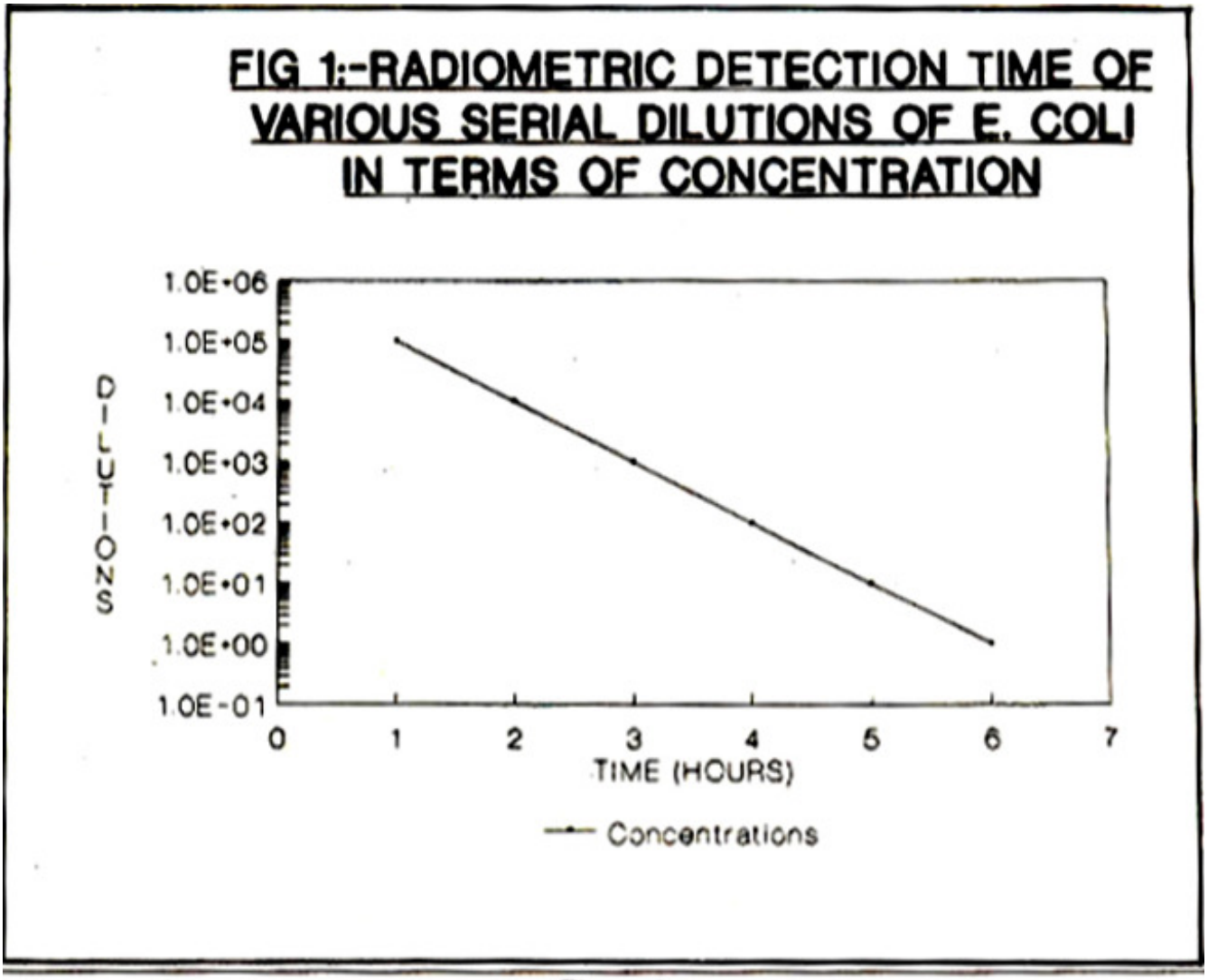
FIG 1:-RADIOMETRIC DETECTION TIME OF VARIOUS SERIAL DILUTIONS OF E. COLI IN TERMS OF CONCENTRATION
1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
1.0E+01
1.0E+00
1.0E-01
DILUTIONS
0
1
2
3
4
5
6
7
TIME (HOURS)
Concentrations

Figure 1.

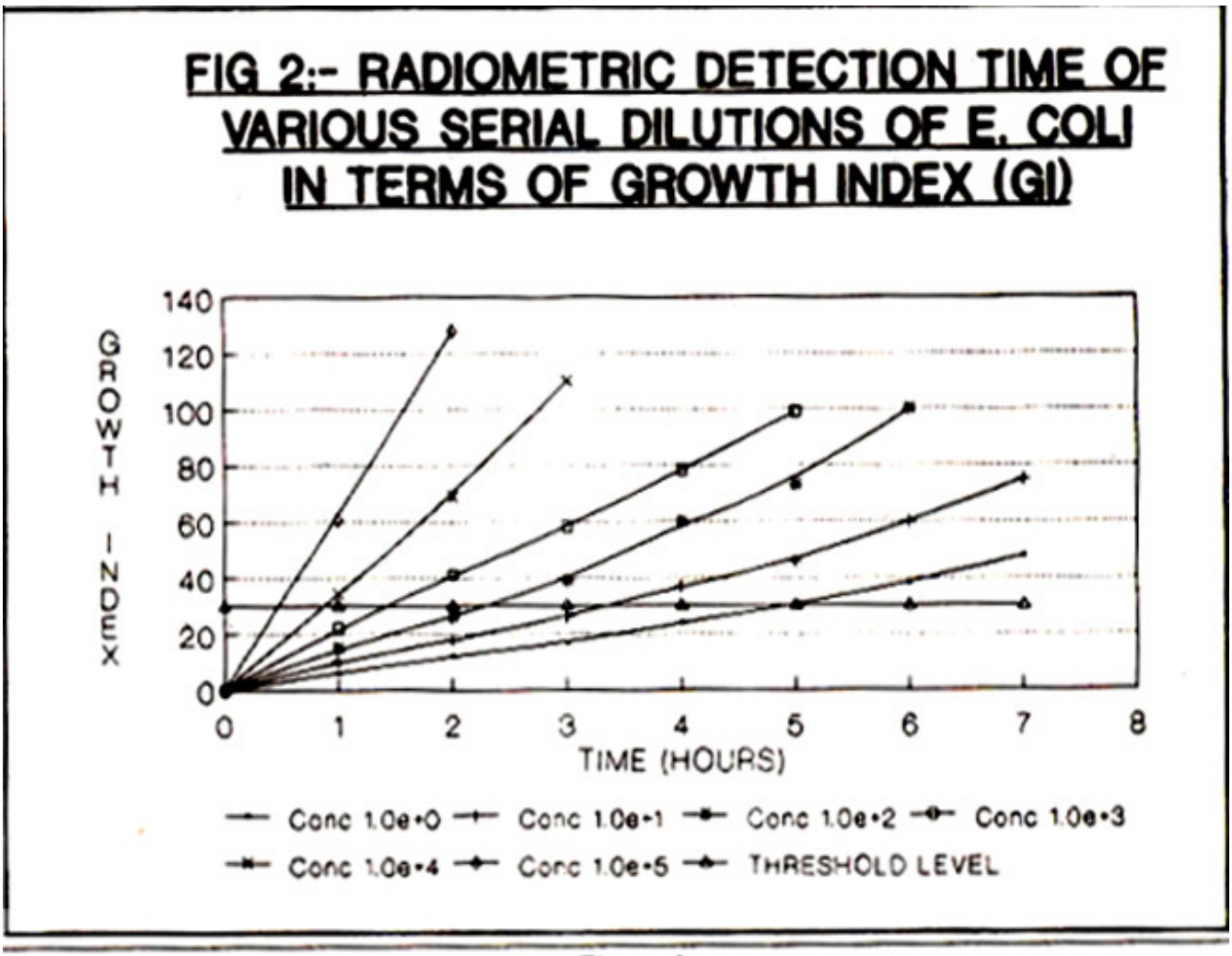

Figure 2.

of growth index versus detection time showed that the presence of colonies of E. coli were detected in 1 hours and 1 colony in 6 hours. The results of 23 tap water samples collected from Islamabad area are presented in Table 1

TABLE I. Results of Radiometric testing of tap water samples of Islamabad region in terms of growth index.

	TIME IN HRS						
SAMPLE NO.	1	2	3	4	5	6	7
1.	7	13	27	41	-	-	-
2.	6	12	25	38	-	-	-
3.	4	12	25	37	-	-	-
4.	6	12	20	28	46	-	-
5.	3	6	12	24	36	-	-
6.	2	6	11	23	35	-	-
7.	3	5	10	22	34	-	-
8.	4	8	16	24	38	-	-
9.	4	8	16	23	38	-	-
10.	4	9	14	26	38	-	-
11.	3	8	13	24	37	-	-
12.	2	5	10	18	27	40	-
13.	2	6	9	17	26	40	-
14.	1	5	8	16	26	41	-
15.	3	6	9	15	24	38	-
16.	0	5	9	16	23	38	-
17.	2	4	8	16	22	37	-
18.	0	4	8	16	21	37	-
19..	2	5	10	16	21	39	-
20.	2	6	12	18	28	47	-
21.	2	4	6	10	19	23	25
22.	1	3	6	9	18	22	25
23.	2	4	5	8	17	21	24
Threshold GI	30	30	30	30	30	30	30
Control	0	2	3	3	4	6	8

*The results shown are mean of two sample readings

**Above 20 GI, threshold level the reading is considered as positive

in terms of detection time against growth index. It was found that E. coli was detected between 4-6 hours in all the samples except in 3 samples (13%) in which it were detected after 7 hours, 9 samples (39%) in 6 hours, 8 samples (34%) in 5 hours and 3 samples (13%) were detected as positive in 4 hours. The results of bacterial contamination in terms of GI in 27 tap water samples collected from

Rawalpindi are given in Table II,

TABLE II. Results of Radiometric testing of tap water samples of Rawalpindi in terms of growth index.

SAMPLE NO.	TIME IN HRS 1	2	3	4	5	6	7
1.	6	13	27	51	-	-	-
2.	6	11	25	40	-	-	-
3.	4	10	25	47	-	-	-
4.	5	11	24	49	-	-	-
5.	3	6	12	24	39	-	-
6.	2	6	11	24	38	-	-
7.	3	5	10	23	37	-	-
8.	4	8	16	22	38	-	-
9.	3	7	15	23	41	-	-
10.	5	8	16	21	40	-	-
11.	4	8	15	22	40	-	-
12.	5	9	18	28	51	-	-
13.	6	10	19	27	50	-	-
14.	7	11	19	26	49	-	-
15.	5	10	18	23	47	-	-
16.	6	9	17	22	45	-	-
17.	6	10	19	23	41	-	-
18.	7	11	20	28	57	-	-
19.	2	5	10	18	44	48	-
20.	2	6	9	17	26	40	-
21.	1	5	8	9	16	26	41
22.	3	6	9	15	24	38	-
23.	0	5	9	15	23	39	-
24.	2	4	8	14	22	47	-
25.	0	4	8	15	21	47	-
26.	2	5	10	16	21	39	-
27.	2	6	12	17	26	42	-
Threshold GI	30	30	30	30	30	30	30
Control	0	1	2	2	3	4	4

*The results shown are mean of two sample readings

**Above 30 GI threshold level the reading is considered as positive

showing bacterial detection in 9 samples (33%) in 6 hours, 14 samples (52%) in 5 hours and in 4 samples (15%) in 4 hours. The results of 21 and 19 well water samples obtained from suburbs of Islamabad and Rawalpindi are presented in Tables III and IV,

TABLE III. Results of radiometric testing of well water samples of suburbs of islamabad in terms of growth index.

	TIME IN HRS						
SAMPLE NO.	1	2	3	4	5	6	7
1.	4	10	21	41	-	-	-
2.	6	12	24	48	-	-	-
3.	4	13	19	37	-	-	-
4.	6	13	24	38	-	-	-
5.	3	8	16	34	-	-	-
6.	2	7	16	33	-	-	-
7.	3	6	12	32	-	-	-
8.	4	8	16	34	-	-	-
9.	4	8	15	33	-	-	-
10.	4	7	14	30	-	-	-
11.	3	8	16	34	-	-	-
12.	4	8	16	38	-	-	-
13.	3	6	14	30	-	-	-
14.	1	4	8	15	26	41	-
15.	2	5	10	16	28	58	-
16.	2	5	9	16	23	39	-
17.	2	4	8	16	22	44	-
18.	1	4	7	14	20	39	
19.	2	5	10	16	25	49	--
20.	2	4	12	17	28	47	-
21.	2	4	6	10	20	37	-
Threshold GI	30	30	30	30	30	30	30
Control	0	2	3	3	4	6	6

*The results shown are mean of two sample readings

**Above 30 GI threshold level the reading is considered as positive

TABLE IV. Results of Radiometric testing of well water samples of of Rawalpindi in terms of growth index.

	TIME IN HRS						
SAMPLE NO.	1	2	3	4	5	6	7
1.	6	13	28	46	-	-	-
2.	6	11	25	48	-	-	-
3.	4	12	25	47	-	-	-
4.	5	11	23	46	-		-
5.	4	9	18	34	-	-	-
6.	3	8	19	38	-	-	-
7.	3	5	12	30	-	-	-
8.	4	8	17	34	-	-	-
9.	4	8	16	33	-	-	-
10.	4	9	18	30	-	-	-
11.	3	8	13	30	-	-	-
12.	3	6	12	30	-	-	-
13.	4	8	16	32	-	-	-
14.	5	10	20	36	-	-	-
15.	3	7	9	16	34	-	-
16.	1	4	8	16	33	-	-
17.	2	5	8	15	32	-	-
18.	1	3	6	12	33	-	-
19.	2	6	12	16	27	38	-
Threshold GI	30	30	30	30	30	30	30
Control	0	2	3	3	4	4	6

*The results shown are mean of two sample readings

**Above 30 GI threshold level the reading is considered as positive

respectively. The 13 samples (62%) of well water from Islamabad suburbs showing growth after 4 hours, whereas 8 samples (38%) showing growth after 5 hours. The 5 samples (26%) of Rawalpindi well water showed positive growth after 5 hours, whereas 14 samples (74%) after 4 hours.

The results of Simly and Rawal dam water samples are presented as detection time versus bacteria growth in Table V.

TABLE V. Results of Radiometric testing of Rawal and Simly Dam water samples in terms of growth index.

	TIME IN HRS						
SAMPLE NO.	1	2	3	4	5	6	7
SIMLY DAM							
1.	7	14	28	41	-	-	-
2.	6	13	26	40	-	-	-
3.	4	12	24	39	-	-	-
4.	5	11	22	40	-	-	-
5.	3	7	14	28	46	-	-
6.	1	5	12	23	38	-	-
RAWAL DAM							
1.	4	12	26	42	-	-	-
2.	5	10	27	44	-	-	-
3.	4	13	28	43	-	-	-
4.	6	17	29	46	-	-	-
5.	8	17	28	44	-	-	-
6.	2	5	9	18	37	49	-
Threshold GI	30	30	30	30	30	30	30
Control	0	2	3	3	4	5	5

*The results shown are mean of two sample readings.

**Above GI threshold level the reading is considered as positive.

The 2 Simly dam samples (33%) showing growth after 5 hours and while other 4 samples (67%) showing growth after 4 hours. The 6 Rawal dam samples have indicated positive growth after 3-5 hours, only one sample (17%) after 5 hours whereas 5 samples (83%) showed high bacterial contamination after 4 hours.

DISCUSSION

The results of 23 tap water samples collected from Islamabad area, showed no colony in 3 samples (13%) were classified as excellent, 9 samples (39%) in which 1 colony was detected in 6 hours were classified asgood, 8 samples (34%) in which 1-10 colonies were detected in 5 hours as satisfactory to suspicious and 3 samples (13%) detected as positive in 4 hours (meaning 10-100 colonies) were classified as unsatisfactory for drinking. The results of 27 tap water samples collected from Rawalpindi showed a bacterial concentration of 1 in 9 samples (33%) were classified as good, that showing (1-10) colonies in 14 samples (52%) as suspicious to unsatisfactory and (10-100) colonies in 4 samples (15%) as unsatisfactory. The results 01 21 well water from Islamabad suburbs showing growth in 13 samples (62%) after 4 hours (10-100 colonies) were classified as unsatisfactory, whereas 8 samples (38%) showing growth after 5 hours (1-10 colonies) were classified as satisfactory. The 5 samples (25%) of Rawalpindi well water showed positive growth after 5 hours (1-10) colonies can be classified as

satisfactory according to untreated drinking water standards, whereas 14 samples (74%) have shown positive results after 4 hours (10-100) colonies and classified as contaminated. The 2 Simly dam samples (33%) showing growth after 5 hours (1-10 colonies) were classified as satisfactory and while other 4 samples (67%) showing growth after 4 hours (10-100 colonies) were thus classified as unsatisfactory for drinking. The 6 Rawal dam samples have indicated positive growth after 3-5 hours, 5 samples (83%) showed high bacterial contamination after 4 hours and only 1 sample (17%) was found satisfactory. The presence of such high number of coliform organisms in treated water of Rawalpindi and Islamabad region suggests either an inadequate treatment or post treatment contamination of water which could be due to rusted pipes and bad joints and low water pressure. It is therefore suggested that water pipes should be checked regularly for any leakage or cross contamination and sufficient chlorination should be done. The results of untreated drinking water clearly indicates that the quality of drinking water in Rawalpindi and around Islamabad needs attention. These results, besides indicating the quality of the drinking water, have also proved the worth of automated radiometric method used in this study as rapid and sensitive technique for the fast detection of coliform organisms in drinking water using ^{14}C-labelled lactose as medium. The screening of a large number of water samples can be carried out in a very short time using this technique and thus can save time and efforts used in tedious conventional procedures.

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