

6. ENUMERATION OF *ESCHERICHIA COLI* AND THE COLIFORM BACTERIA

(Most Probable Number technique - 3 tube)

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Escherichia coli (*E. coli*), a rod-shaped member of the coliforms group, can be distinguished from most other coliforms by its ability to ferment lactose at 44°C in the fecal coliform test. *E. coli* are mainly of fecal sources of animal and human. Other coliform bacteria will appear as thick, slimy colonies, with non-fermenters being colorless, and weak fermenters being pink.

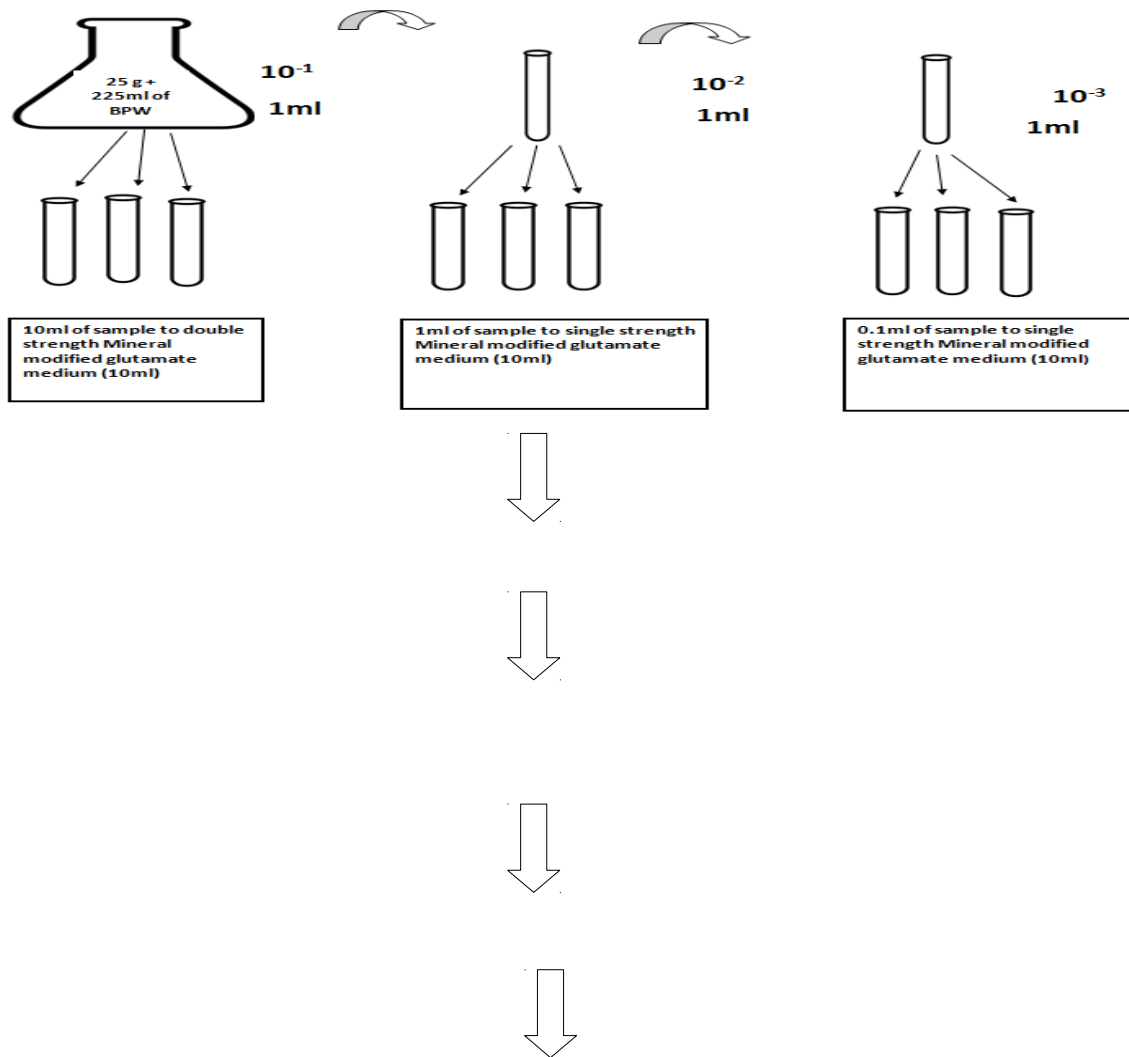
A **fecal coliform** is a facultatively anaerobic, rod-shaped, gram-negative, non-sporulating bacterium and is mainly from the intestines of warm-blooded animals. Fecal coli forms are capable of growth in the presence of bile salts, oxidase negative and utilize produce lactose(produce acid and gas) at 44 ± 0.5°C within 48 hours. Because of its growth at 44 ± 0.5°C called as "thermotolerant coliform".

I. Presumptive test for coliforms, faecal coliforms and *E. coli*

- Weigh 25 g of food into sterile high-speed blender jar (for frozen samples can be softened by storing for <18 h at 2-5°C, but do not thaw)
- Add 225 mL of Buffer peptone water and blend for 2 min.
- Prepare decimal dilutions (1:10) with sterile Buffer peptone water diluents or equivalent in test tube.
- Shake all suspensions 25 times in 30 cm arc or vortex mix for 7s.
- Using at least 3 consecutive dilutions, inoculate 10ml of sample to double strength Mineral modified glutamate medium (10ml) and from test tube of serial dilution 10⁻² mL, added 1ml of sample to single strength Mineral modified glutamate medium (10ml), test tube of serial dilution 10⁻³ mL, added 0.1 ml added of sample to single strength Mineral modified glutamate medium (10ml).
- Incubate tubes at 37 °C for 24hrs and observe for yellow coloration

- Examine tubes and record reactions, i.e., For each tube showing colour change take a loop, streak on tryptone bile glucuronide agar (TBX) and incubate the plate at 44 °C for 24hrs
- Blue green colonies after at 44 °C for 24hrs
- No colour change in tube or no blue green colonies after incubation report result as 0 MPN/gm or 0 MPN/ ml

Flowchart -Isolation of *E. coli* by MPN method



3 Tube –MPN values for coliforms in food ,when three test portions of 1g (ml),three of 0.1g (ml) and three of 0.01 g (ml)

Number of positive results			MPN index ^a	Category ^b	Confidence limits (95 %) ^{a, c}	
					Lower limit	Upper limit
0	0	0	< 0,30		0,00	0,94
0	0	1	0,30	3	0,01	0,95
0	1	0	0,30	2	0,01	1
0	1	1	0,61	0	0,12	1,7
0	2	0	0,62	3	0,12	1,7
0	3	0	0,94	0	0,35	3,5
1	0	0	0,36	1	0,02	1,7
1	0	1	0,72	2	0,12	1,7
1	0	2	1,1	0	0,4	3,5
1	1	0	0,74	1	0,13	2
1	1	1	1,1	3	0,4	3,5
1	2	0	1,1	2	0,4	3,5
1	2	1	1,5	3	0,5	3,8
1	3	0	1,6	3	0,5	3,8
2	0	0	0,92	1	0,15	3,5
2	0	1	1,4	2	0,4	3,5
2	0	2	2,0	0	0,5	3,8
2	1	0	1,5	1	0,4	3,8
2	1	1	2,0	2	0,5	3,8
2	1	2	2,7	0	0,9	9,4
2	2	0	2,1	1	0,5	4
2	2	1	2,8	3	0,9	9,4
2	2	2	3,5	0	0,9	9,4
2	3	0	2,9	3	0,9	9,4
2	3	1	3,6	0	0,9	9,4
3	0	0	2,3	1	0,5	9,4
3	0	1	3,8	1	0,9	10,4
3	0	2	6,4	3	1,6	18,1
3	1	0	4,3	1	0,9	18,1
3	1	1	7,5	1	1,7	19,9
3	1	2	12	3	3	36
3	1	3	16	0	3	38
3	2	0	9,3	1	1,8	36
3	2	1	15	1	3	38
3	2	2	21	2	3	40
3	2	3	29	3	9	99
3	3	0	24	1	4	99
3	3	1	46	1	9	198
3	3	2	110	1	20	400
3	3	3	> 110			