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Course Title : Microbiology

Course Code : BM24AC4

Unit-III

Enumeration of Bactria by MPN method

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ENUMERATION OF BACTERIA BY MPN METHOD

MPN :

- Most Probable Number (MPN) method used to estimate the concentration of viable microorganisms in a sample by means of ten-fold dilutions.
- MPN is most commonly applied for quality testing of water.
- Coliform bacteria act as an indicator of contamination of water.

PRINCIPLE :

- Water to be diluted serially and inoculated in lactose broth,

coliforms if present in water utilizes the lactose present in the medium to produce acid and gas.

- The presence of acid is indicated by the color change of the medium .

presence of gas is detected as gas bubbles collected in the inverted Durham tube present in the medium.

- The number of total coliforms is determined by counting the number of tubes giving positive reaction

MPN method is performed in three ways :

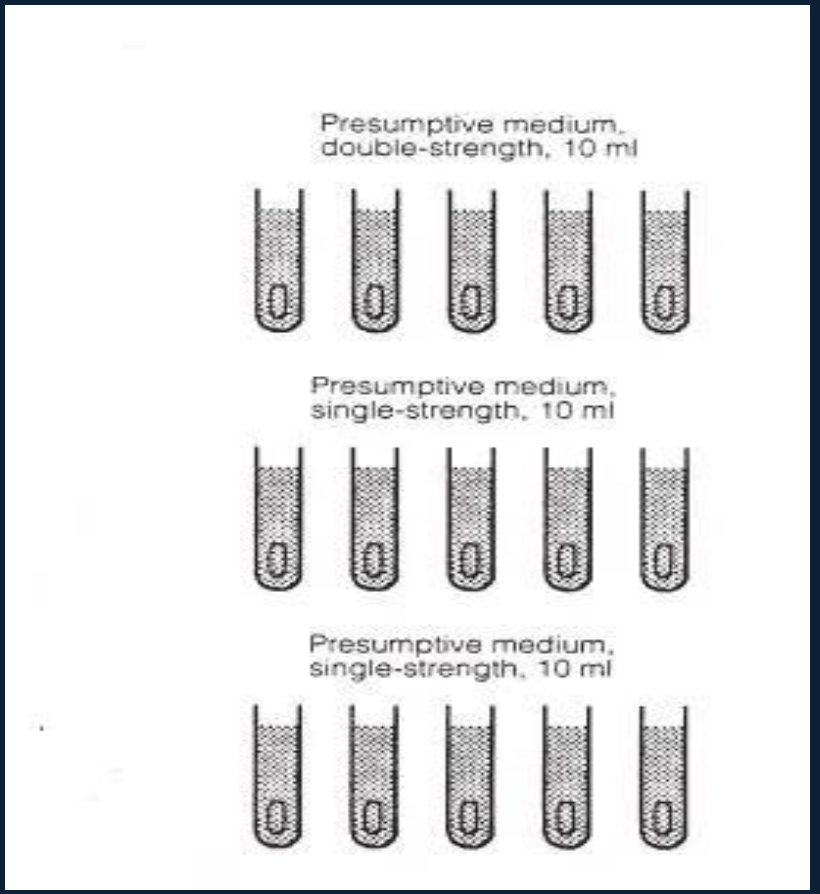
- PRESUMPTIVE TEST
- CONFIRMATORY TEST
- COMPLETED TEST

Requirements

- Medium: Lactose broth or MacConkey broth or Lauryl tryptose (lactose) broth ,both single and double strength . Brilliant green eosin methylene broth EMB agar .
- Glasswares: Test tubes of various capacities (20ml,10ml,5ml) Durham tube
- Others: Sterile pipettes

PREPARATION OF THE MEDIUM :

- Prepare medium (either MacConkey broth or lactose broth) in single and double strength concentration.
- Take 5 test tubes with double strength A
- Take 10 test tubes (5+5) of single strength B
- Place the Durham tube in each test tubes.
- Sterilize by autoclaving at (121ºc) for 15 min.



Presumptive test:

- At first three set of 5 test tubes are taken.
- 10 ml of double strengthen liquid media (MacConkey broth) is placed in each test tubes of 1st set
- Similarly, 10 ml of single strengthen liquid media is placed in each test tubes of 2nd set and 3rd
- double strengthen broth refers to broth made up using twice the normal amount of broth powder.
- Lactose broth or lauryl sulphate broth is used as liquid media for the test.

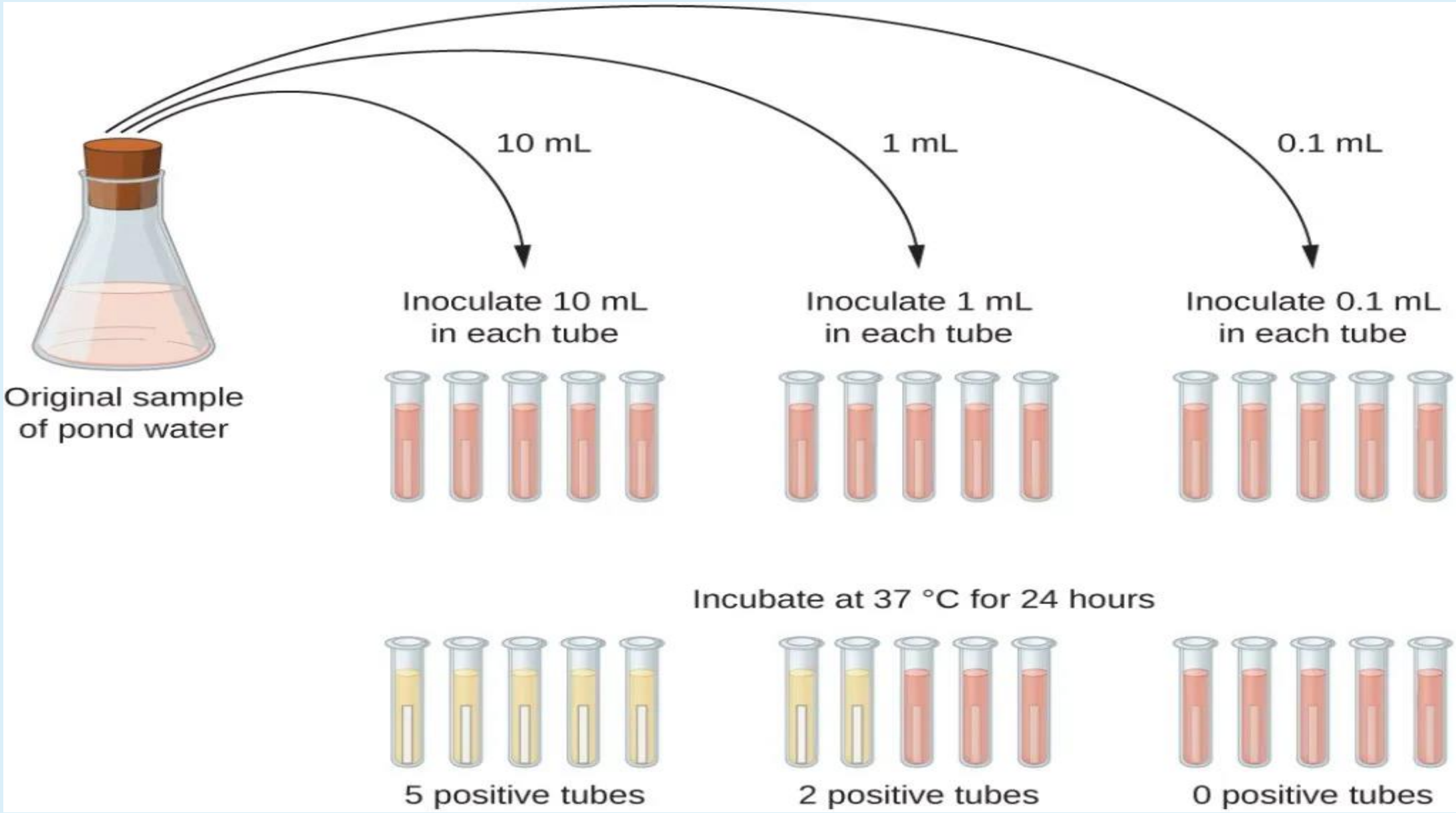
- Then, Durham's tube is inserted in inverted position in each test tubes of all sets.

- All the test tubes are then cotton plugged and sterilized using autoclave for 15 minutes at 15 lb/inc pressure at 121 °

- After cooling water sample is added in each test tubes as follows;
  - Add 10 ml water sample in each test tubes of 1st set
  - Add 1 ml water sample in each test tubes of 2nd set
  - Add 0.1ml water sample in each test tubes of 3rd

- Then incubate all test tubes at 35.5 °C for 24 hours. After incubation gas production in Durham's tubes is observed.

- Tubes in which gas production is 10% or more is recorded as positive tube and tubes in which gas production is less than 10% is recorded as doubtful.


10 mL
1 mL
0.1 mL
Original sample of pond water
Inoculate 10 mL in each tube
Inoculate 1 mL in each tube
Inoculate 0.1 mL in each tube
Incubate at 37 °C for 24 hours
5 positive tubes
2 positive tubes
0 positive tubes

- Doubtful test tubes are further incubated for 24 hours and again gas production is noted. If gas production is still less than 10%, then tube is recorded as negative and are discarded

- doubtful result is given by other gas producing lactose fermenting bacteria other than coliforms such as *Lactobacillus, Streptococci, Bacillus, Clostridium* produces more than 10% gas but only after 48 hours of incubation.

- All the positive test tubes are taken for confirmatory test

- For example, a water sample tested shows a result of 3–2–1 (3 × 10 ml positive, 2 × 1 ml positive, 1 × 0.1 ml positive) gives an MPN value of 17, i.e. the water sample contains an estimated 17 coliforms per 100 ml

MPN values per 100 ml of sample and 95% confidence limits for various combinations of positive and negative results (when five 10-ml, five 1-ml and five 0.1 ml test portions are used)

No. of tubes giving a positive reaction :			MPN (per 100 ml)	95% confidence limits	
5 of 10 ml	5 of 1 ml	5 of 0.1 ml		Lower	Upper
0	0	0	<2	<1	7
0	1	0	2	<1	7
0	2	0	4	<1	11
1	0	0	2	<1	7
1	0	1	4	<1	11
1	1	0	4	<1	11
1	1	1	6	<1	15
2	0	0	5	<1	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46

CONFIRMATORY TEST :

- For confirmation of coliform, **brilliant green lactose bile (BGLB)** broth is used as culture media, because BGLB broth inhibits growth of gram positive bacteria such as lactobacillus, Streptococci, Bacillus and Clostridium

- Coliforms can grow in this BGLB medium

- For confirmation, one loopful of sample from each positive tubes obtained from presumptive test is inoculated in respective tubes containing Brilliant green lactose bile broth and incubated for 24 hours at 35.5 °

- Gas production 10% or more are recorded as positive while less than 10% is recorded as doubtful. Doubtful tubes are again incubated and the result is recorded.

- All the positive test tubes are now confirmed for presence of coliforms.

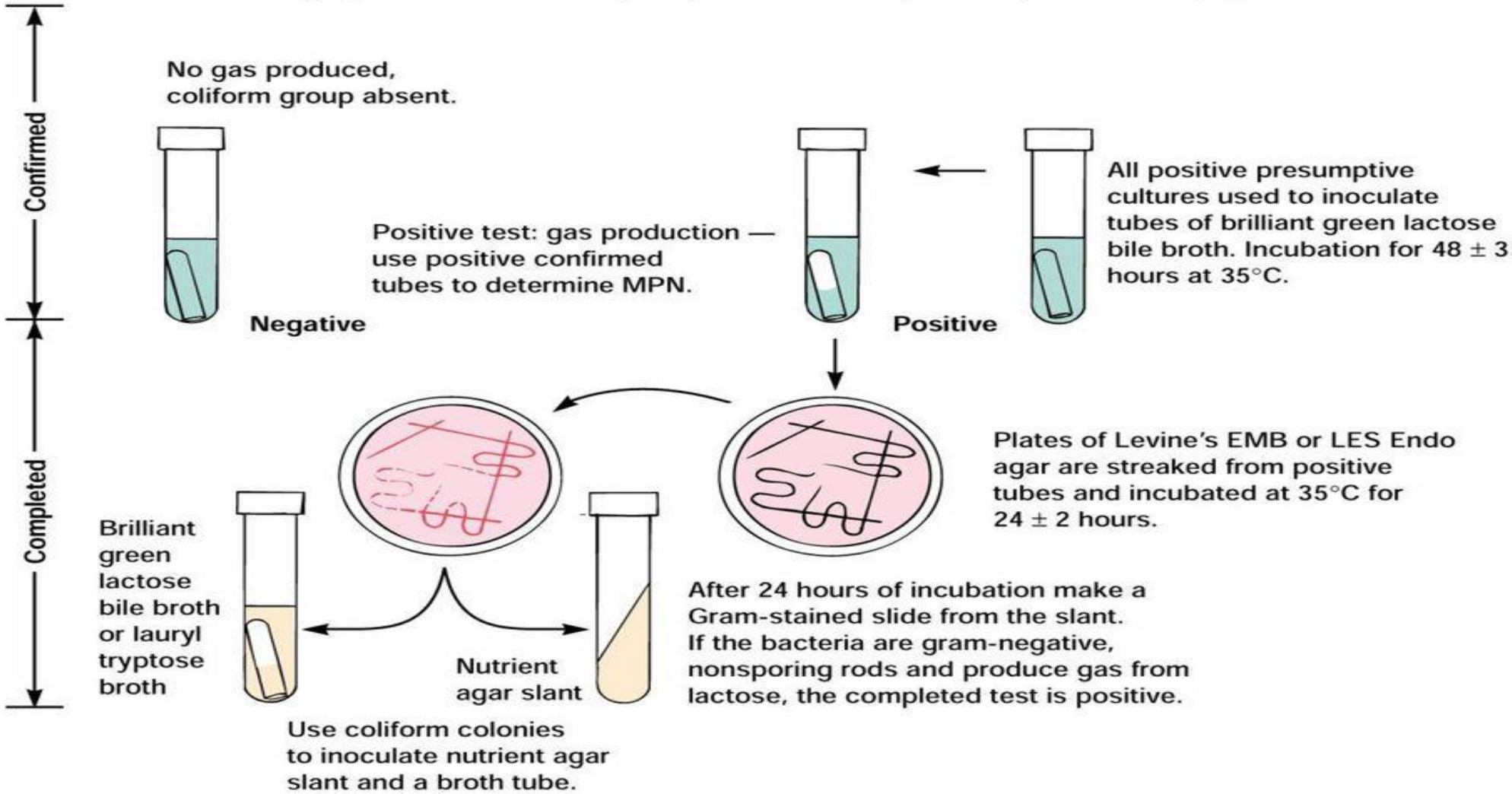
- Finally the number of bacteria present in water sample is determined from previous MPN chart.

COMPLETED TEST :

- It is a final test in which a loopful of sample from positive confirmatory tubes is streaked on Eosin methylene blue agar or M-endo agar and incubated for 24 hours.

- Three types of colonies are obtained in culture media;
  - Typical colony: they are pink colored with greenish metallic appearance or nucleated colony.
  - Atypical colony: they are pink and non-nucleated colony.
  - Non-typical colony: they are non-pink colony given by non-coliforms.

Flow chart of Confirmed and Completed MPN



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THANK YOU