

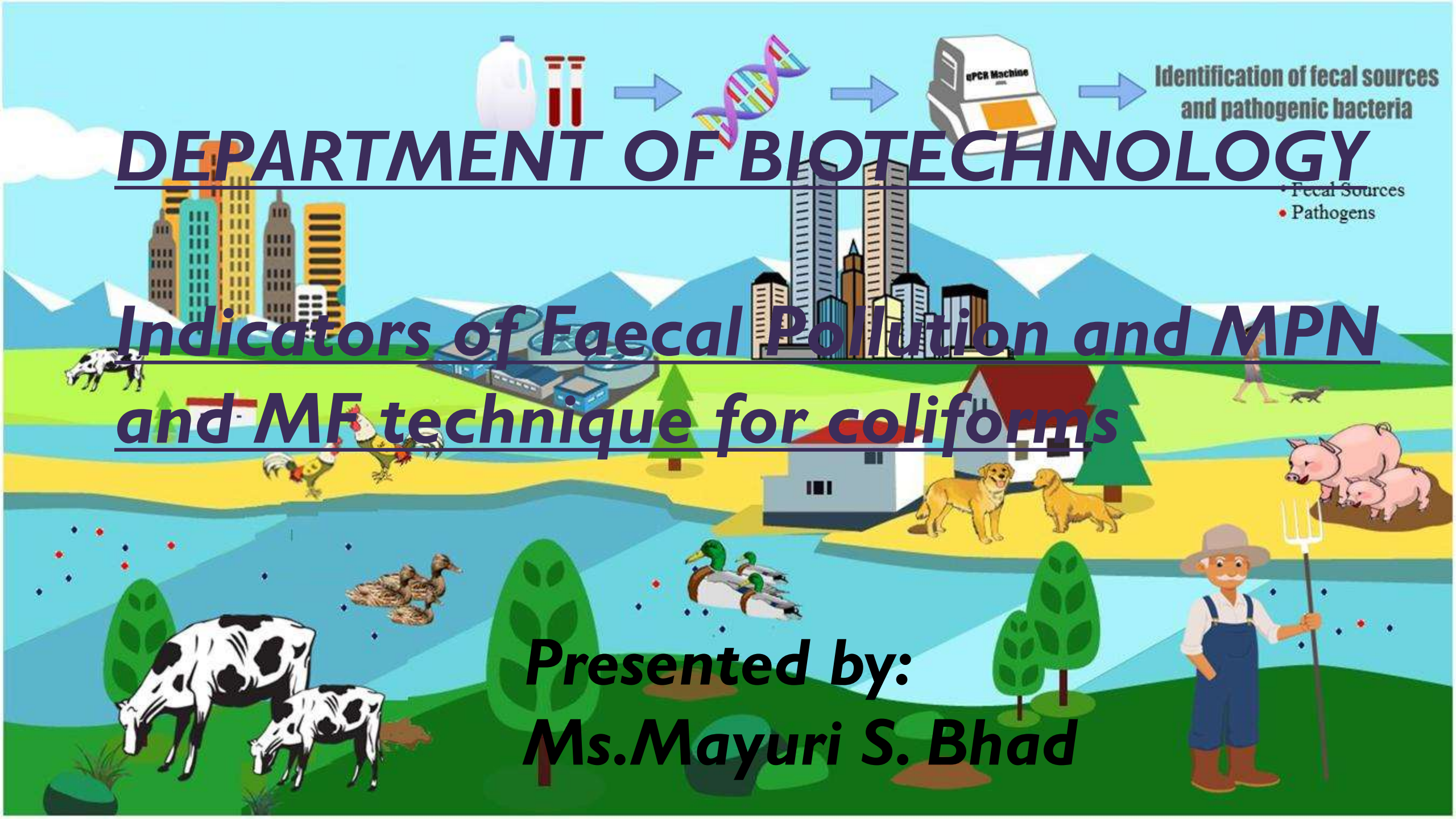
Identification of fecal sources
and pathogenic bacteria

DEPARTMENT OF BIOTECHNOLOGY

- Fecal Sources
- Pathogens

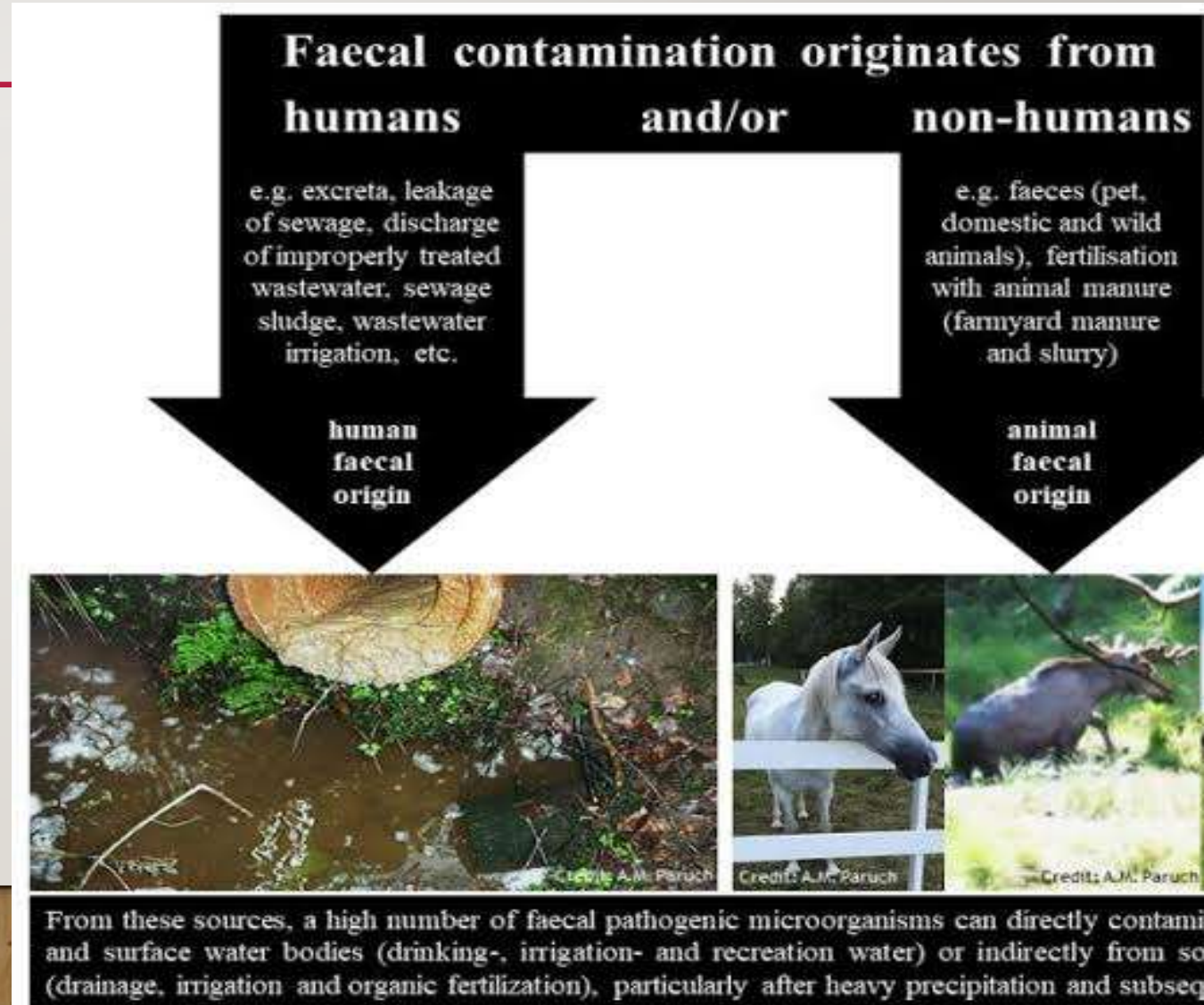
Indicators of Faecal Pollution and MPN and MF technique for coliforms

**Presented by:
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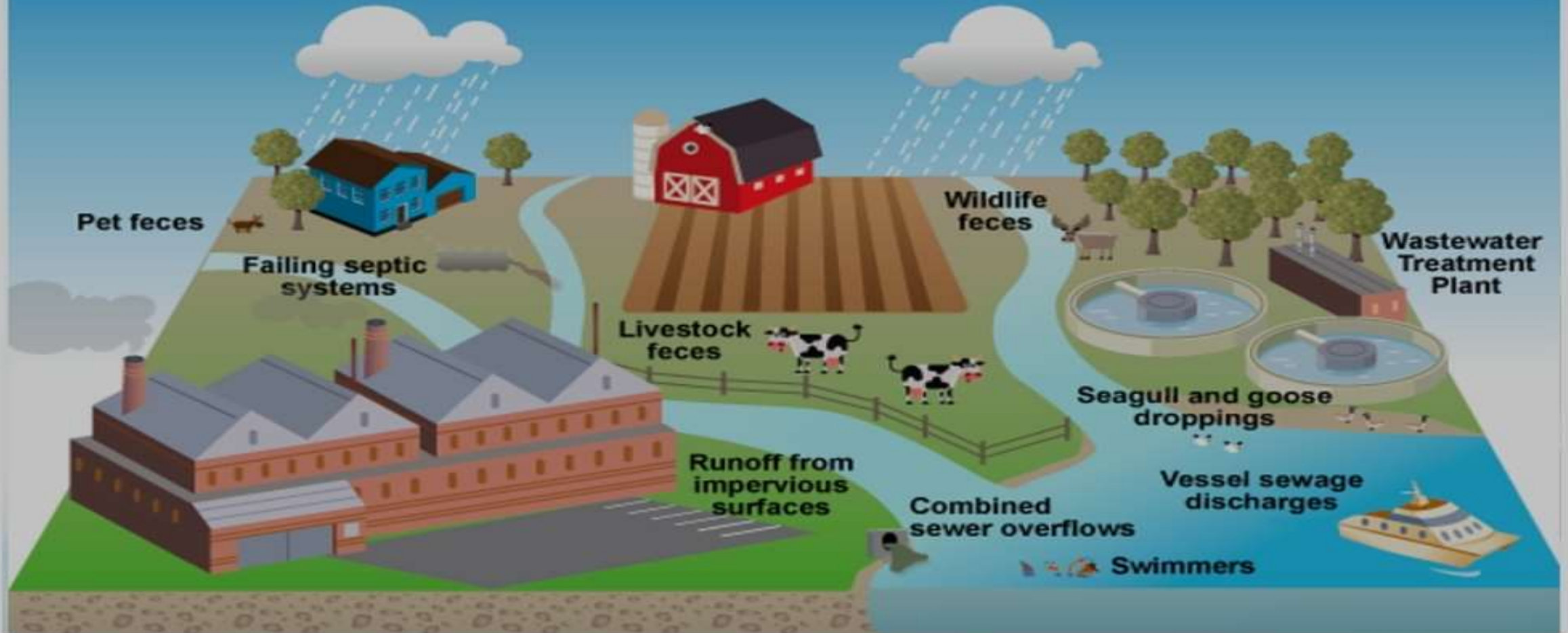


WHAT IS FAECAL POLLUTION ?

- Faecal pollution of water from a health point of view is the contamination of water with disease-causing organisms (pathogens) that may inhabit the gastrointestinal tract of mammals, but with particular attention to human faecal sources as the most relevant source of human illnesses globally.



Identify Sources of Fecal Contamination



WHAT IS AN INDICATOR MICROORGANISM?

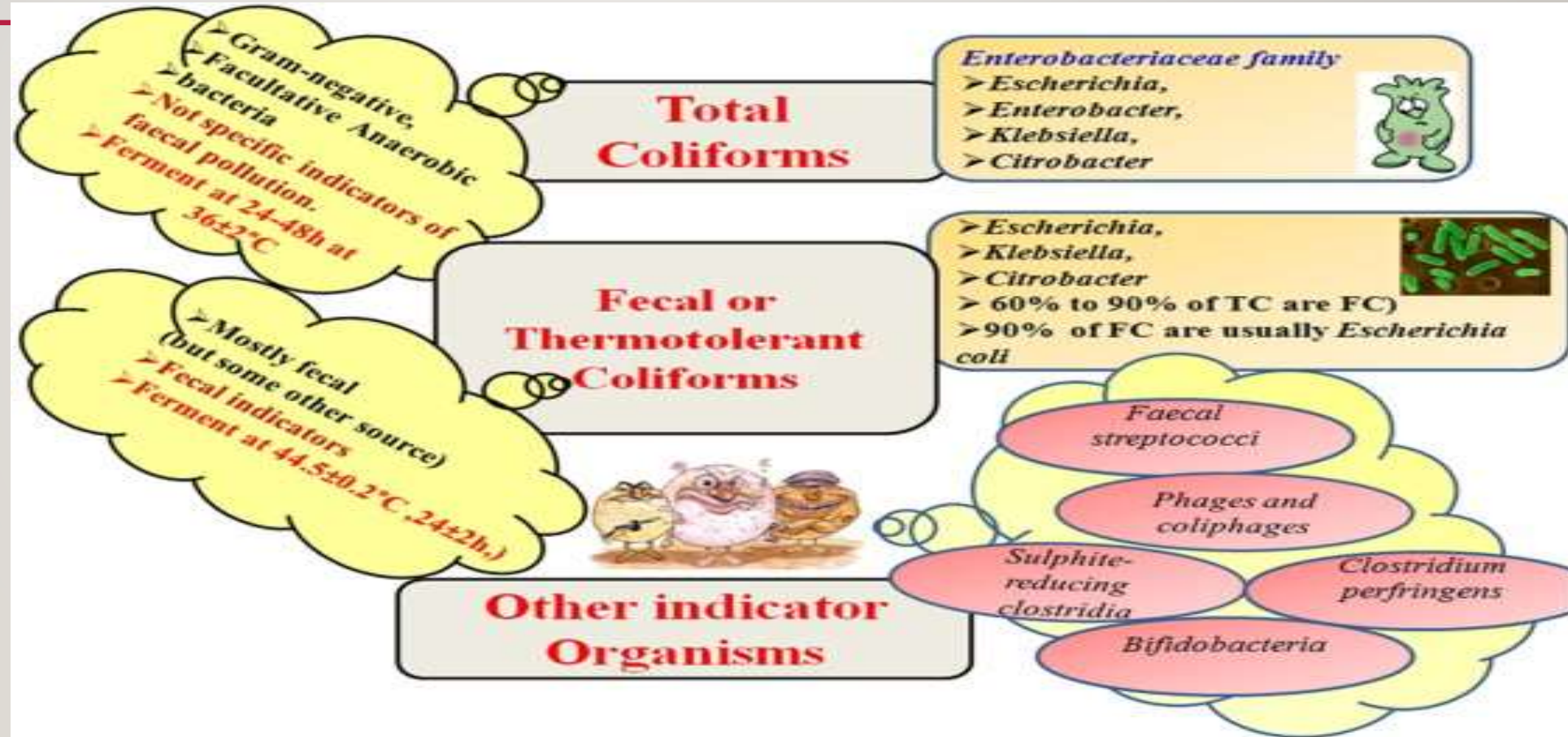
- Indicator organisms are organisms used as a sign of quality or hygienic status in food, water, or the environment.
- Indicators organisms are used because pathogens themselves are frequently difficult to detect in drinking water and waste water.
 1. Low number [but still above MID]
 2. Difficult, time consuming, or expensive to culture

CRITERIA FOR AN IDEAL INDICATOR ORGANISM

- Should be useful for all types of water (drinking water, wastewater, recreational water, sea water)
- Should be present whenever enteric pathogens are present, and absent when pathogens are absent
- Should survive longer in the environment than the toughest enteric pathogen
- Should not grow in water
- Detection protocols should be easy and inexpensive
- Density of indicator microorganisms should correlate with the degree of fecal pollution
- Should be a member of the normal intestinal microflora of warm-blooded animals

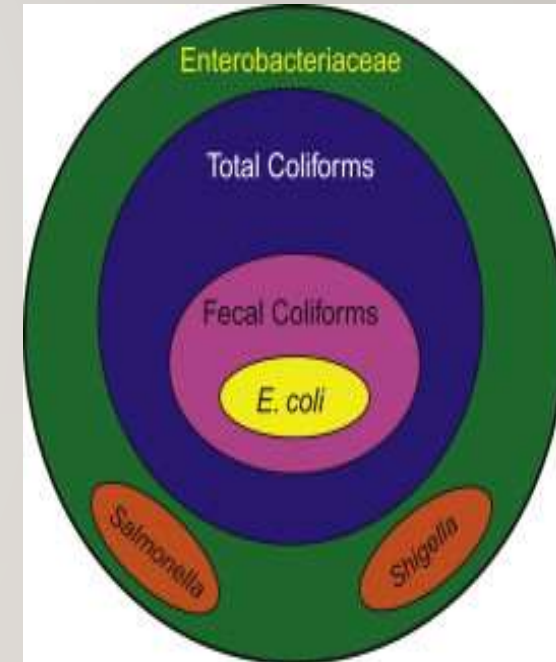
TYPES OF INDICATORS

1. Total coliforms
2. Faecal coliforms
3. Faecal streptococci
4. Anaerobic bacteria
5. Bacteriophage



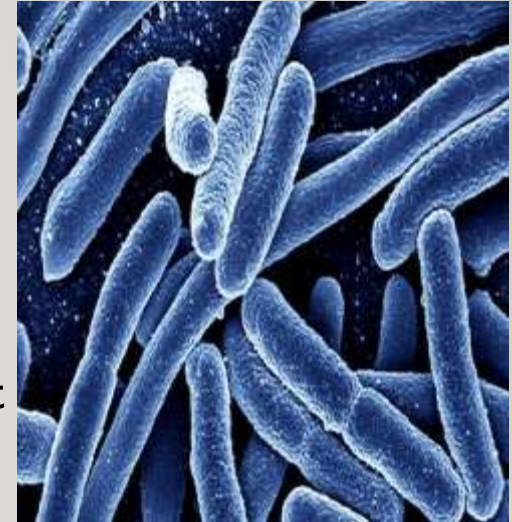
TOTAL COLIFORMS

- Coliform bacteria are defined as Rod shaped Gram-negative, non-spore forming and motile or non-motile bacteria which can ferment lactose with the production of acid and gas when incubated at 35–37°C. Due to the limited ability of certain coliform bacteria to ferment lactose, the definition has changed to bacteria containing the enzyme β -galactosidase.
- Coliforms can be found in the aquatic environment, in soil and on vegetation; they are universally present in large numbers in the faeces of warm-blooded animals.
- Typical genera include: *Klebsiella*, *Citrobacter*, *Enterobacter*, *Hafnia*, *Klebsiella*, *Escherichia*
- Unlike the general coliform group, E. Coli are almost exclusively of fecal origin and their presence is thus an effective confirmation of faecal contamination. Most strains of E. Coli are harmless, but some can cause serious illness in humans. Infection symptoms and signs include bloody diarrhea, stomach cramps, vomiting and occasionally, fever.



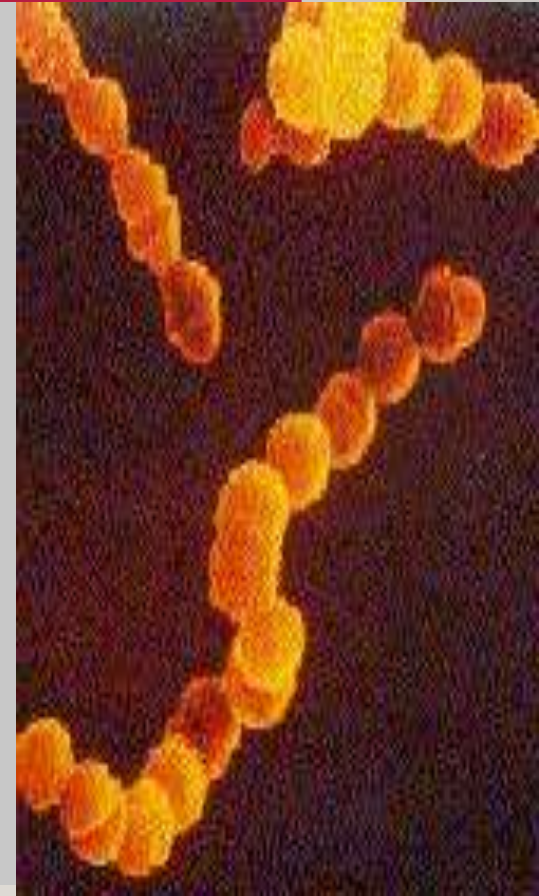
FAECAL COLIFORMS

- A faecal coliform is a facultatively anaerobic, rod-shaped, gram-negative, non-sporulating bacterium. Coliform bacteria generally originate in the intestines of warm-blooded animals. Faecal coliforms are capable of growth in the presence of bile salts or similar surface agents, are oxidase negative, and produce acid and gas from lactose within 48 hours at $44 \pm 0.5^{\circ}\text{C}$. The term “thermotolerant coliform” is more correct and is gaining acceptance over “faecal coliform”.
- Coliform bacteria include genera that originate in feces (e.g. *Escherichia*) as well as genera not of fecal origin (e.g. *Enterobacter*, *Klebsiella*, *Citrobacter*).
- Faecal coliform bacteria can enter rivers through direct discharge of waste from mammals and birds, from agricultural and storm runoff, and from human sewage.



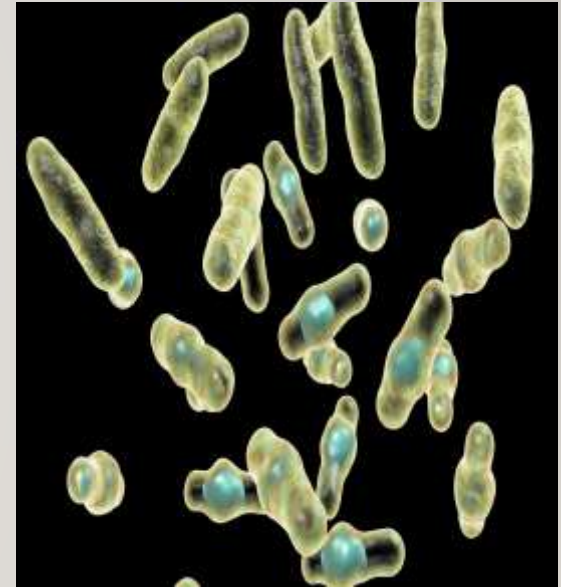
FAECAL STREPTOCOCCI

- do not multiply in water
- are more resistant to stress/disinfection
- last longer in the environment
- used as indicators of enteric viruses, and gastroenteritis for swimmers
- Members of the lactic acid bacteria
- Gram positive, non-motile, non-spore-forming, aerotolerant anaerobic bacteria that ferment sugars to lactic acid
- FC/FS ratio - ratio of fecal coliform counts to fecal strep counts
- $FC/FS > 4$: fecal contamination of human origin
- $FC/FS < 0.7$: fecal contamination of animal origin
- This relationship is only valid for recent fecal contamination (within the last 24 hours)

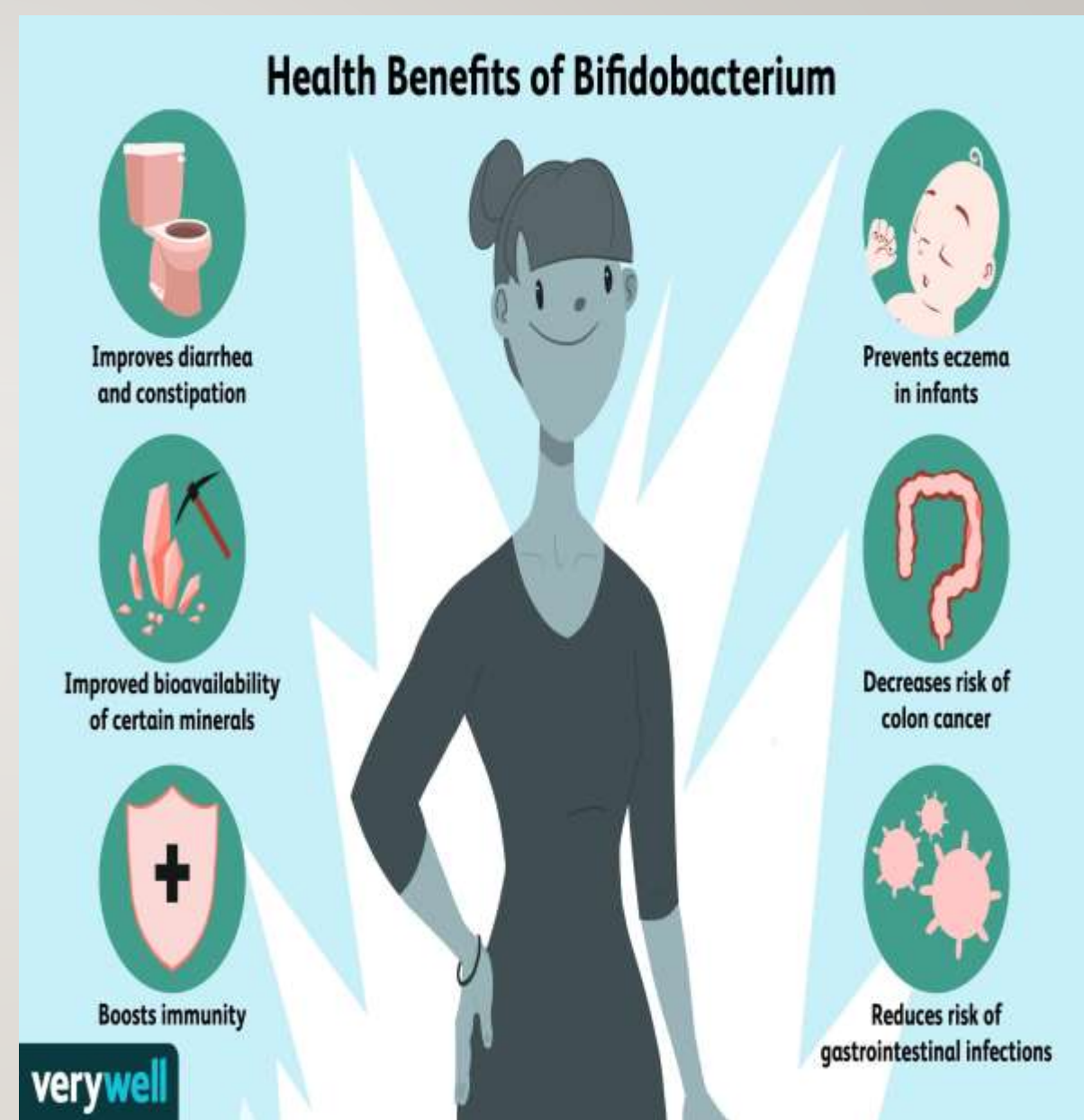


ANAEROBIC BACTERIA

- *Clostridium perfringens* is a Gram-positive, rod-shaped, anaerobic, spore-forming pathogenic bacterium of the genus Clostridium.
- They are sulfite reducing bacterium found in colon and represents approximately 0.5% of faecal microflora.
- The bacteria can form endospores, capable of surviving adverse conditions for long periods of time. When favorable conditions are encountered, the endospores germinate rapidly; *C. Perfringens* has the shortest generation time, 6.3 minutes, reported for any organism.



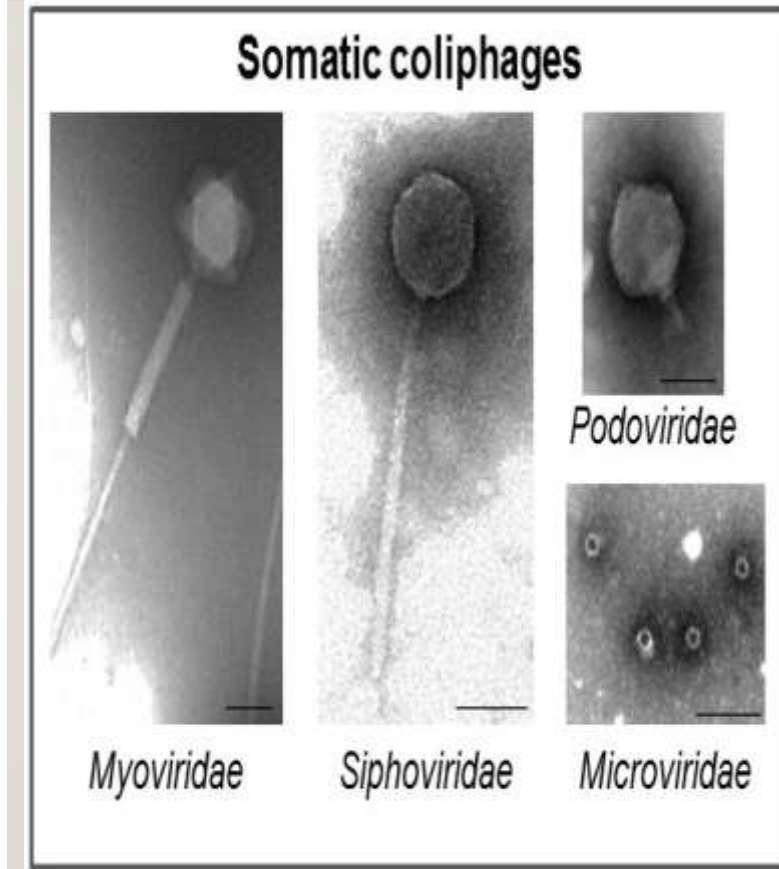
- *Bifidobacterium* is a genus of gram-positive, nonmotile, often branched anaerobic bacteria. They are ubiquitous inhabitants of the gastrointestinal tract though strains have been isolated from the vagina and mouth (*B. Dentium*) of mammals, including humans.
- Some species are *B. faecale*, *B. bifidum*, etc.



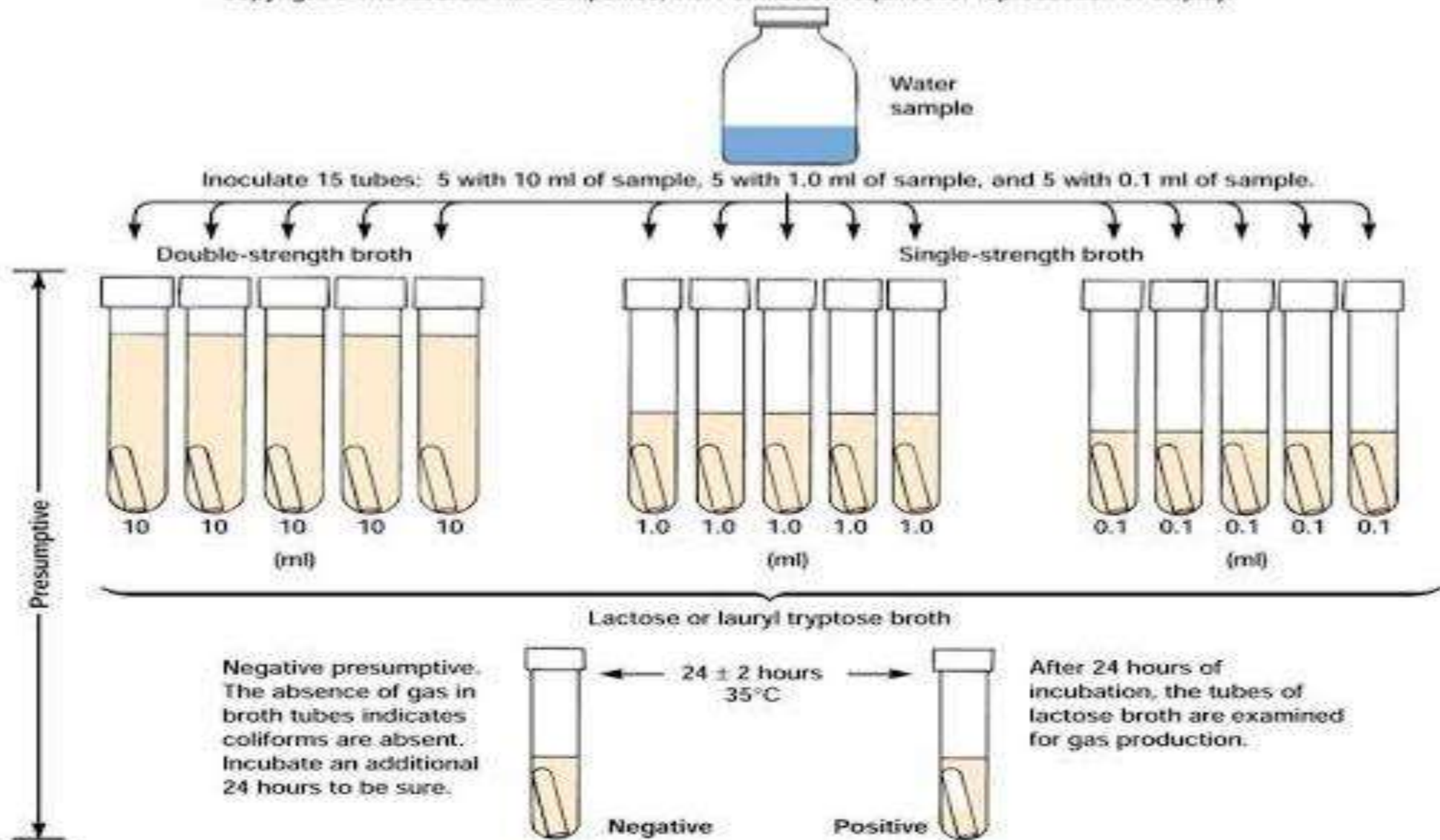
BACTERIOPHAGE

Coliphage – one example

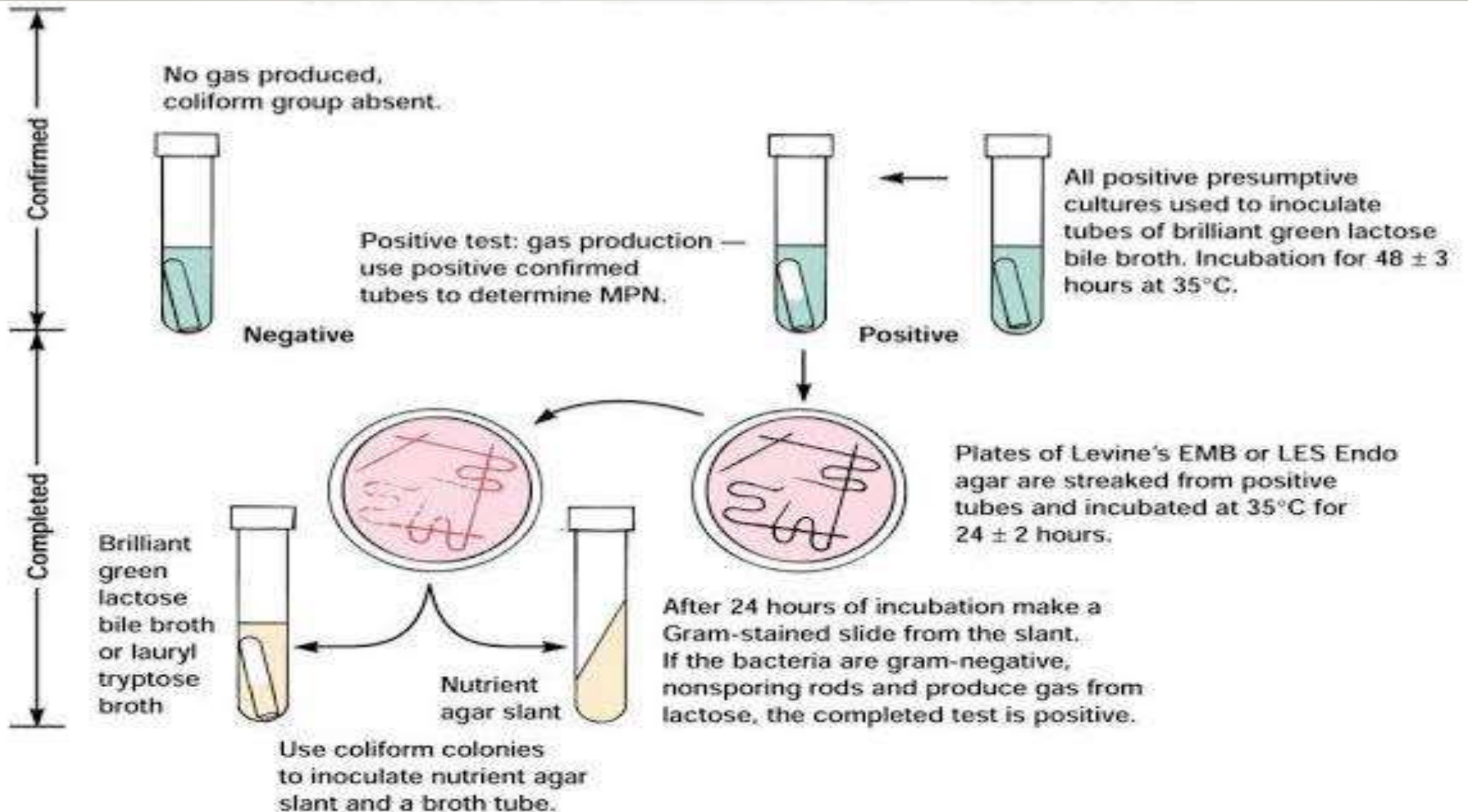
- bacteriophage that infect coliforms, particularly *E. coli*
- similar to enteric viruses in size, morphology, and performance in environment
- found in higher numbers than enteric viruses in wastewater and other waters
- rapid and easy detection methods available
- survive for 7 days in shellfish without increasing in numbers
- routinely used as indicator microorganisms to determine the effectiveness of wastewater treatment processes
- resistant to disinfection



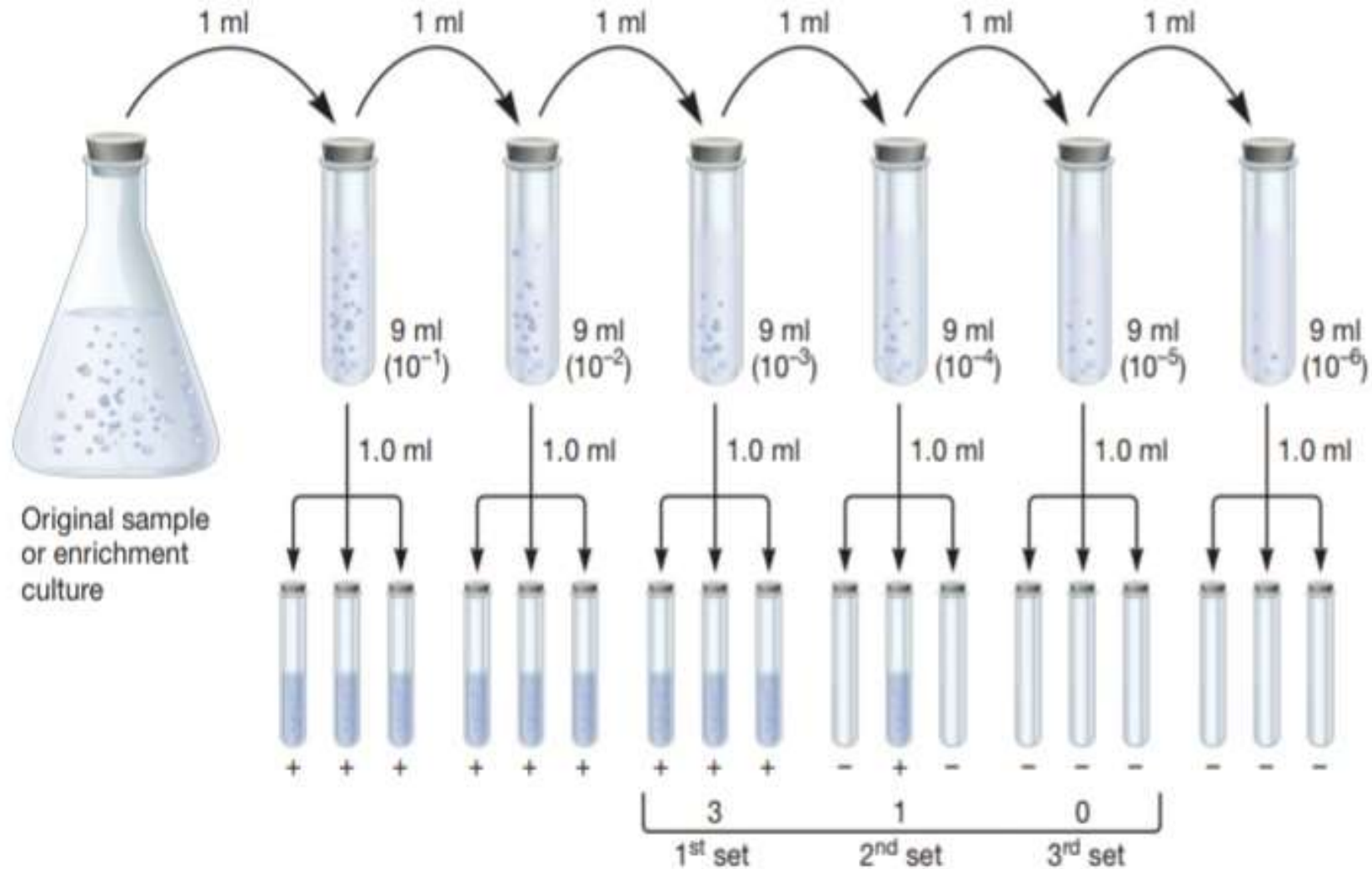
MULTIPLE TUBE DILUTION TECHNIQUE [FOR COLIFORM] (PRESUMPTIVE TEST)



CONFIRMED TEST AND COMPLETED TEST



MULTIPLE TUBE DILUTION TECHNIQUE [FOR STREPTOCOCCI]



- 1 A dilution series of the sample is made. Usually ten-fold dilutions are used.
- 2 1.0 ml of each dilution is used to inoculate triplicate tubes of growth medium.
- 3 The tubes are evaluated for growth. The first set of dilutions that fails to show growth (or the set with the least number of tubes without growth) is used to bracket a set of three dilutions.
- 4 The **MPN** is determined by consulting a table that has been established using statistical analysis. (Only a small part of the **MPN** table is shown here.)

(a) Procedure for **MPN** analysis

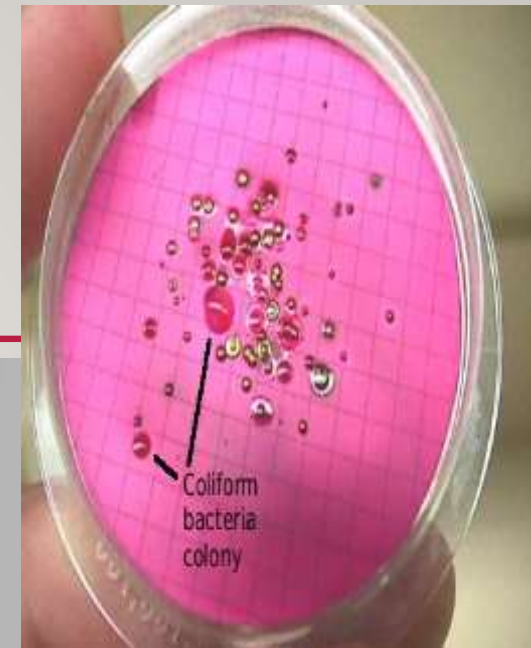
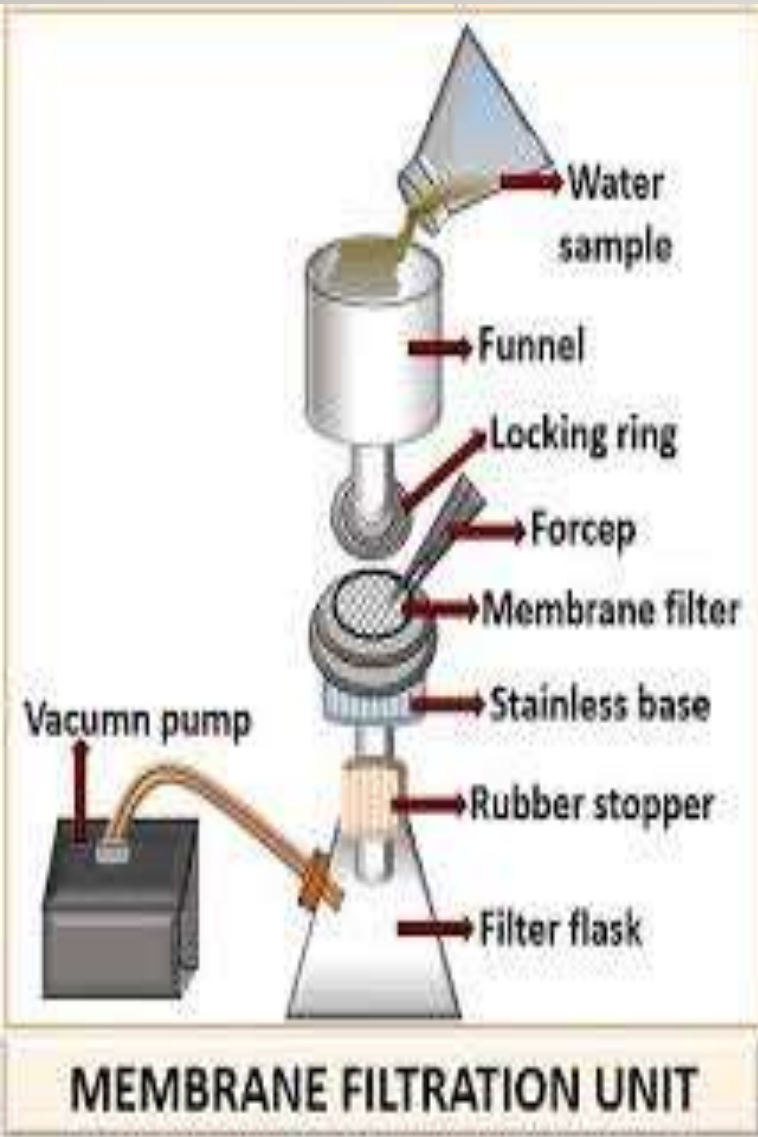
$$\text{MPN}/100\text{ml} = \frac{\text{Number of positive tubes} \times 100}{\sqrt{(\text{ml of sample in negative tubes}) \times (\text{ml of sample in all tubes})}}$$

$$\sqrt{(\text{ml of sample in negative tubes}) \times (\text{ml of sample in all tubes})}$$

Sample MPN Table

Number of Tubes Giving Positive Reaction			MPN Index per 100 ml	95 Percent Confidence Limits	
3 of 10 ml each	3 of 1 ml each	3 of 0.1 ml each		Lower	Upper
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120
3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	380
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1300
3	3	1	460	71	2400
3	3	2	1100	150	4800

MEMBRANE FILTER TEST



FAECAL COLIFORMS VERSUS NON FAECAL COLIFORMS

FAECAL COLIFORMS

Facultative anaerobes that originate from the intestine of warm-blooded animals

Live in the intestine of the warm-blooded animals

Indicate the presence of pathogenic microorganisms in food and water

Optimal temperature for lactose fermentation is 44 °C

Develop dark-blue colonies

Ex: *E.coli*

NON FAECAL COLIFORMS

Rod-shaped Gram-negative bacteria that belongs to the family Enterobacteriaceae

Live in soil, aquatic environment, and on vegetation

Ferment lactose, producing an acid and a gas

Optimal temperature for lactose fermentation is 37 °C

Form pink colonies

Ex: *Enterobacter, Klebsiella*

THANK YOU

