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محمد رسول الله



Microbiology of Water

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Water:

- Water is transparent fluid which forms the worlds streams , lakes ,oceans and rain. It is major constituent of fluid of living organisms . It covers the 71% of earth surface

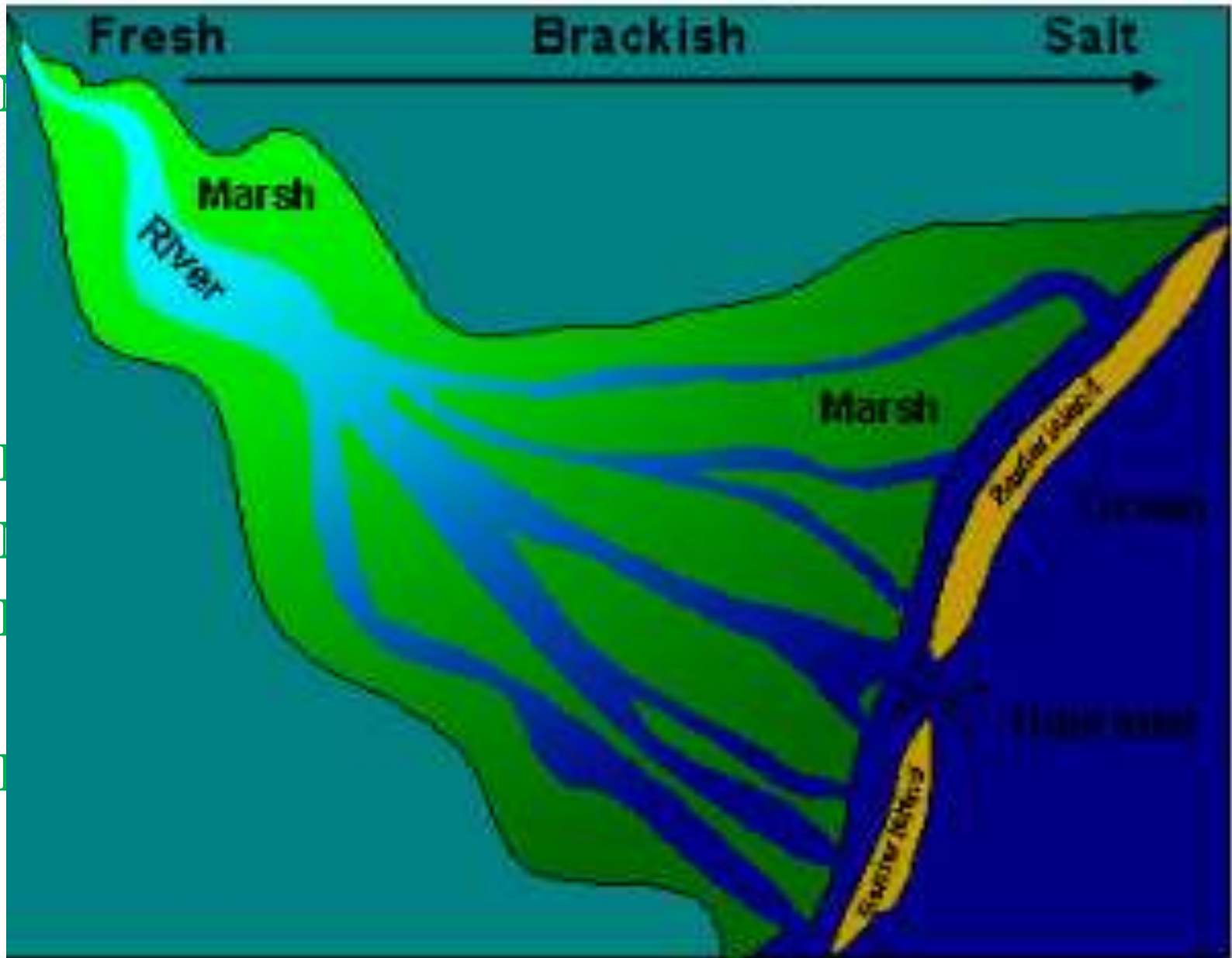


Importance of water

- Water is the medium of life.
- All organisms are composed primarily of water.
- Liquid water is the medium in which all cellular chemical reactions occur.
- Essential for survival and growth of organism.



The Estuary



Water Microbiology

- **Definition:**
- The study of microorganisms- (viruses, bacteria, algae , protozoa and microscopic fungi)- and their activities in fresh , estuarine and marine waters including springs , lakes, rivers and seas which inhabits these natural waters.



The aquatic environment:

The microbial population in a body of natural water is to a large extent determined by physical and chemical conditions. These conditions vary over a wide extremes when one compares streams, estuaries and open seas.

Some conditions are following:-

Temperature:

- Temperature of surface water varies from near 0 °C in polar region to 30-40 °C in equatorial region.
- More than 90% marine environment temp. is below 5 °C (*psychrophilic* microorganisms)
- Microorganisms do occur in hot springs ranges from 75-80 °C. i.e. *thermus aquaticus* (optimum temp. 70-72 °C)
- Some microorganisms associated with geothermal vents in Pacific Ocean are capable of growing at 250 °C.

• **Hydrostatic Pressure:**

- **Hydrostatic pressure** is the **pressure** that is exerted by a fluid at equilibrium at a given point within the fluid, due to the force of gravity.
- There are diff. in the hydrostatic pressure of surface water and water in oceanic depth
- Hydrostatic pressure affect chemical equilibrium → lowering pH of sea water. → change in solubility of nutrients i.e. HCO_3^- .
- Hydrostatic pressure increase B.P of water thereby maintaining the water in its liquid state at high temp. and pressure.

“Hydrostatic pressure increase with depth at the rate of 1atm per 10 m”

Example:

Barophilic microorganisms are isolated from depth of 1000-10000 m where pressure is greater than 100atm.

- **Light:**

- Most forms of aquatic life depend directly or indirectly upon the metabolic products of photosynthetic organisms . In most aquatic habitats these primary producers are algae and their growth is restricted to the upper layer of water through which the light can penetrate.

- **Salinity:**

- The degree of salinity in natural water ranges from zero in fresh water to saturation in the salt lakes.
- Concentration of salt varies between 33 and 37 g/kg. The major salts of sea waters are Chlorides, Sulphates and Carbonates of Na, K, Ca and Mg.
- Most marine microorganisms are **halophilic** . They grow best at salt conc. Of 2.5-4% whereas those from lakes and rivers are salt sensitive and don't grow at salt conc. Of more than 1%.

● **Turbidity:**

- There is a mark variation in the clarity of surface waters
- The Adriatic sea is clear at great depth whereas some near shore rivers are often turbid.
- The suspended material responsible for turbidity includes:-
 - i) Particles of mineral materials which originate from land.
 - ii) Detritus predominantly particulates organic materials such as cellulose, hemicellulose and chitin fragments.
 - iii) Suspended microorganisms.

Turbidity of water influences the penetration of light which in turn affects the photosynthetic zone.

- **Hydrogen ion concentration (pH):**

- Aquatic microorganisms in general can be grown at pH at 6.5-8.5.
- The pH of sea is 7.5-8.5.
- Optimum growth of most Marine species is obtain on the media at just to pH 7.2-7.6.
- Lakes and rivers may show a wider range in pH depending upon local conditions



Aquatic microorganisms:

The microbiology of fresh waters constitute a part of science of limnology (Study of Flora and conditions for life in Lake ,pond and streams)

□ Lakes and ponds:

❖ Lakes and ponds have characteristics zones:

- i)Littoral zone
- ii) Limnetic zone
- iii)Profundal zone
- Iv)Benthic zone

❖ Littoral zone:

- Littoral zone is situated along the shore which has considerable rooted vegetation and include the regions where light penetrates to the bottom

❖ Limnetic zone:

- Limnetic zone is situated in open area after littoral zone, which is determined by light compensation level (depth of effective light penetration).

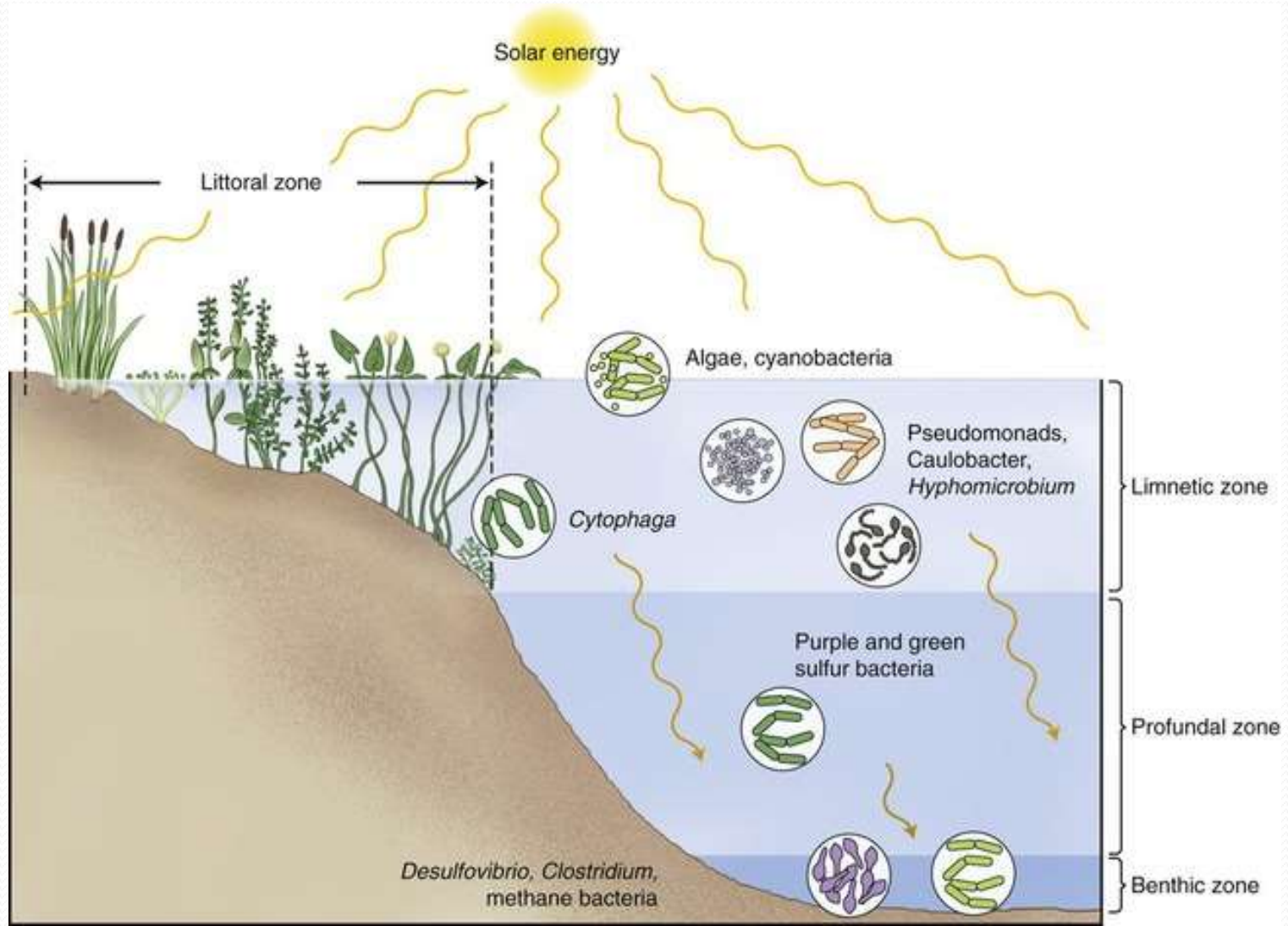
❖ Profundal zone:

- Profundal zone present beneath the limnetic zone in the deeper region of the open waters where the photosynthesis decrease progressively due poor penetration of light

❖ Benthic zone:

- The benthic zone is the deepest zone composed of soft

mud at bottom of ponds and lakes.



□ Streams:

- Streams obtain majority of nutrients from the flow of inorganic and organic material from the surrounding terrestrial system or lakes or ponds.
- To major extent the microbial flora reflects the immediate terrestrial conditions including the effects of agriculture and industrial practices.



□ The sea:

- Microorganisms are at all depth and at all latitudes in the sea water .
- They occur as plankton and in sediments of ocean.
- The great volume of open sea provides an environment with less variations in conditions than other aquatic environment.

➤ Marine Plankton

- The phytoplankton population comprises numerous species of Diatoms(algae),cyanobacteria(blue green-algae) ,dinoflagellates(flagellate protist) sillicoflagellates(unicellular algae) etc.
- This group of microorganism is chiefly responsible for the conversion of radiant energy to the chemical energy.

- The magnitude of this accomplishment is revealed by a calculation that suggest a requirement of 50 billion metric tons of phytoplankton to support the growth of potential world fish catch estimated at 50 million metric tons.
- Protozoa are present in large number in the region inhabited by phytoplankton . The zoo plankton feed upon phytoplankton organisms , bacteria or detritus . Observation indicates many zooplanktons avoid light, exhibiting diurnal migration .At night animals graze on phytoplankton at the surface and the day time sinked below the photic zone.

➤ Benthic population:

- Offshore sediments are inhabited by protozoa and bacteria.
- Large number are present at mud-water interface, the bacterial population may range from a few hundreds to the millions per mm.
- The count in sediments are as high as 10^8 bacteria per gram.
- Many bacteria are facultative or strict anaerobes.



Water born diseases

- H₂O can act as a vector for the transmission of bacterial, viral and protozoan agents which cause a variety of diseases (mainly intestinal)
- It can also be linked to viral/protozoan diseases transmitted by insects (aquatic hosts or insect breeding in H₂O - indirect)
- Water is responsible for, by some estimates, approximately **80%** of all infectious disease not just waterborne diseases, but any disease where water plays a role.

- **Water contains a variety of microbes**

including:

Viruses

Bacteria

Protozoa

Fungi



VIRUS

- Virus is microscopic entity consisting of a single nucleic acid surrounded by a protein coat and capable of replication only within the living cells of bacteria, animals or plants.
- Viruses that infect bacteria is called **Bacteriophage**.

Viral diseases and their pathogen:

➤ Diseases:

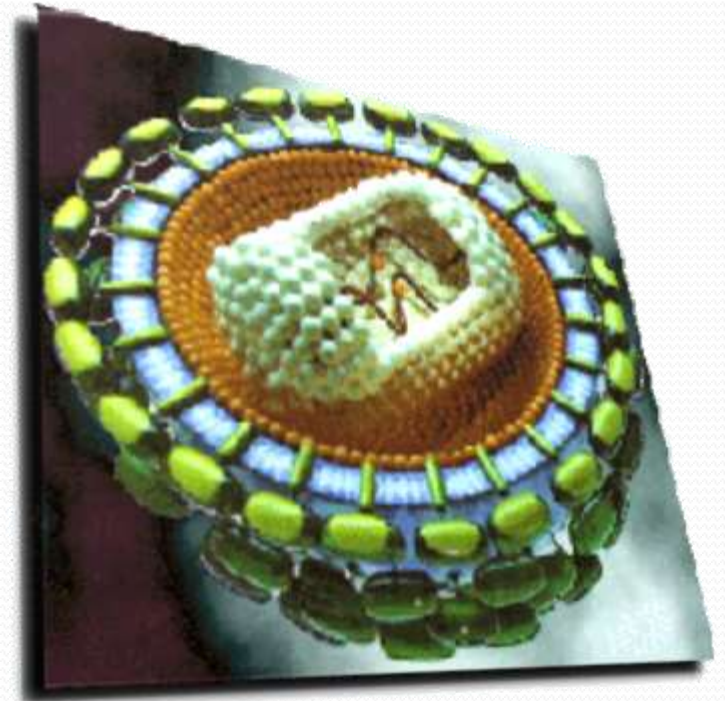
Enteritis, diarrhea, and dysentery

➤ Causative agent:

- Rotavirus

Symptoms:

severe vomiting



Diseases:

Hepatitis A

Causative agent: HAV

Hepatitis E

Causative agent: HEV

Symptoms:

Inflammation of liver ,vomiting ,Jaundice ,Nausea etc.

Treatment: Liver transplantation

Polio:

Causative agent: Polio virus

Symptoms:

Fever ,Sore throat ,Neck stiffness ,Pain in legs & arms etc.

Treatment: Polio vaccine.

BACTERIA

Bacteria vary in their shapes like bacteria have spherical(cocci), rod (bacillus) and spiral (spirillum) shaped arrangement and are organized in specific cellular shape.

Bacterial diseases & their pathogen:

Diseases:

❑ Enteritis & diarrhea

- *Causative agents:*

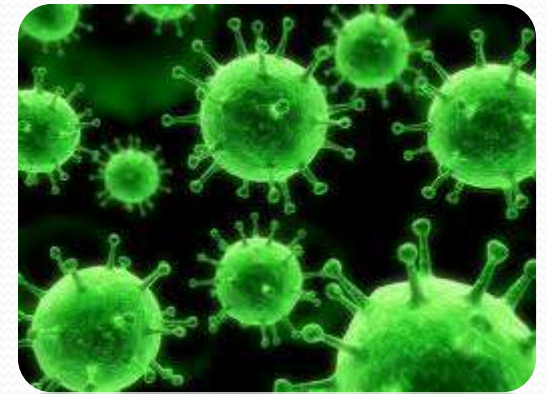
- E.coli

Symptoms: Vomiting , Fever ,Nausea.

❑ Enteric fever

- *Causative agent* : Salmonella Typhi, Salmonella Paratyphi

- *Symptoms:* Poor appetite ,Headache etc



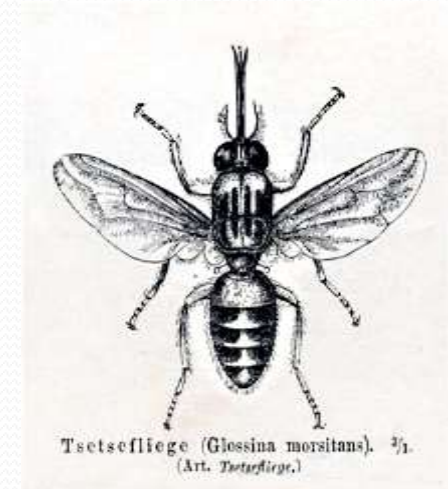
Protozoa

- These are unicellular organisms with diameters in the range of 2-100 μm .

Protozoan infection & their pathogen :

Diseases:

- ❑ *Malaria*: Plasmodium
- Symptoms : Fever ,Fatigue ,vomiting etc.
- ❑ *African sleeping sickness*: Tsetse fly
- Symptoms : Headache ,fever , Joint's pain.



FUNGI

Fungi are serve as decomposers, absorbing nutrients from dead leaves or other organic matter in soil and water

Fungi are found in very less quantity/numbers in water. This is due to their **competition with other heterotrophic** organisms like some bacteria and archae for food intake. their low abundance is an indicator for their loss during competition.

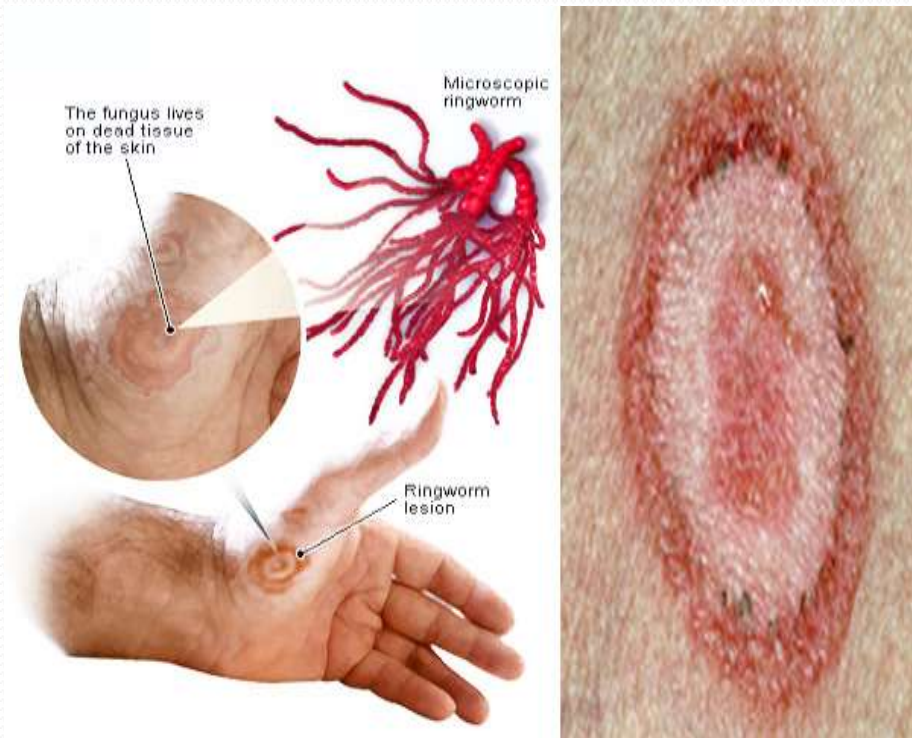
•Fungal disease

•Ringworm:

The fungi that cause ringworm can live on surfaces, particularly in damp areas like public showers.

Causative agent:

Trichophyton (fungi)



Algae

- Algae may produce toxins in sea food which can cause illness to human beings by using contaminated seafood.

Algal disease:

- Amnesic Shellfish Poisoning (ASP):

Causative agent:

Pseudo-nitzschia sp.(Diatom)

Symptoms:

Short terms memory loss , Brain damage etc.



SPREAD AND SYMPTOMS OF DISEASE

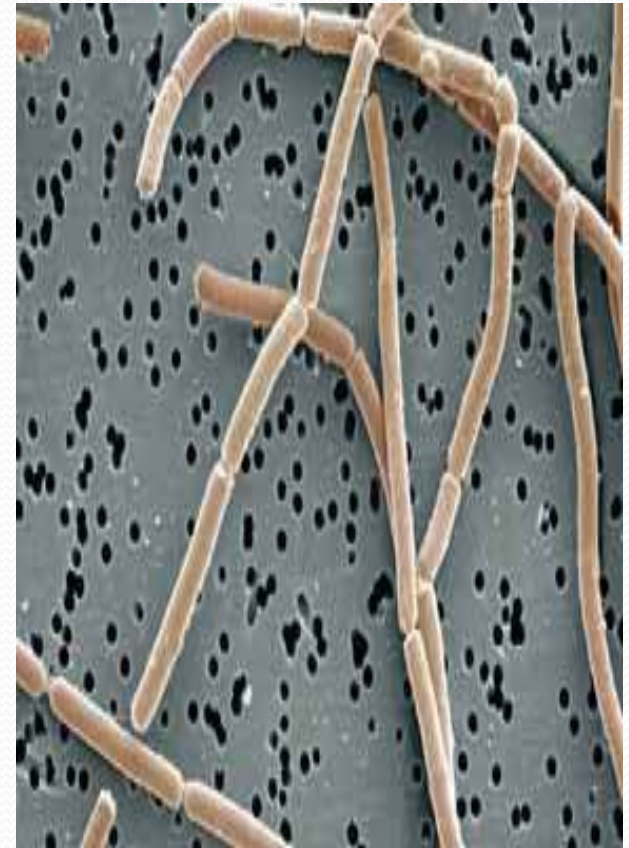
- A person may be infected by direct drinking contaminated water.
- By coming in contact with contaminated water.

Common symptoms

- Abdominal pain
- Fever
- Diarrhea
- Condition are more severe in peoples with weakened immune system.

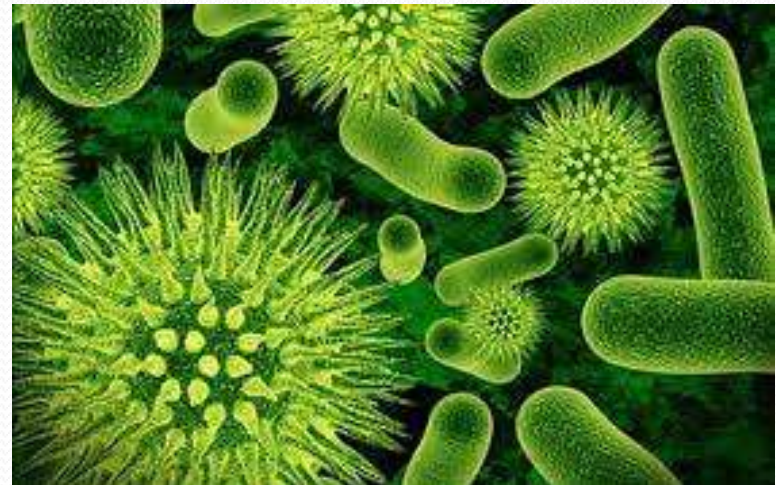
Microbiological Examination of Water

- Individual pathogen numbers may be too low to detect in a reasonable sized water sample. Isolation and detection of some pathogens can take several days, weeks, or months.



Indicator Microorganisms:

- Indicator microorganisms are used to indicate an increased risk of pathogen contamination due to fecal contact. They are not dangerous to human health but are used to indicate the presence of a health risk.



Characteristics of a Useful Indicator:

- Always present when pathogens are present
- Not present in the absence of the pathogen
- Correlated with degree of pollution
- More easily detectable than a pathogen
- Non-pathogenic in nature.

Bacterial-Indicator Organisms

Common Groups:

❖ Coli forms

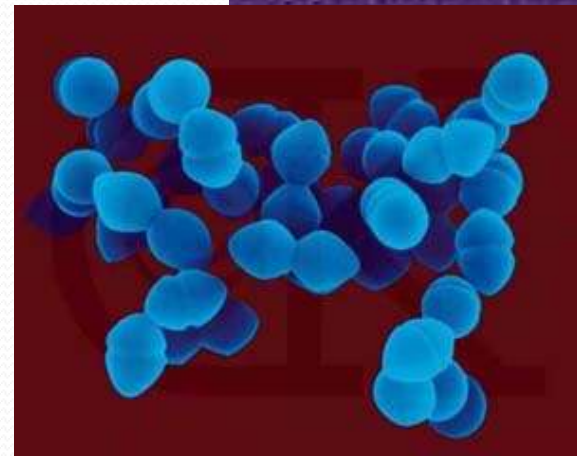
- Total coli forms
- Fecal coli forms

❖ Streptococci

- fecal streptococci
- enterococci

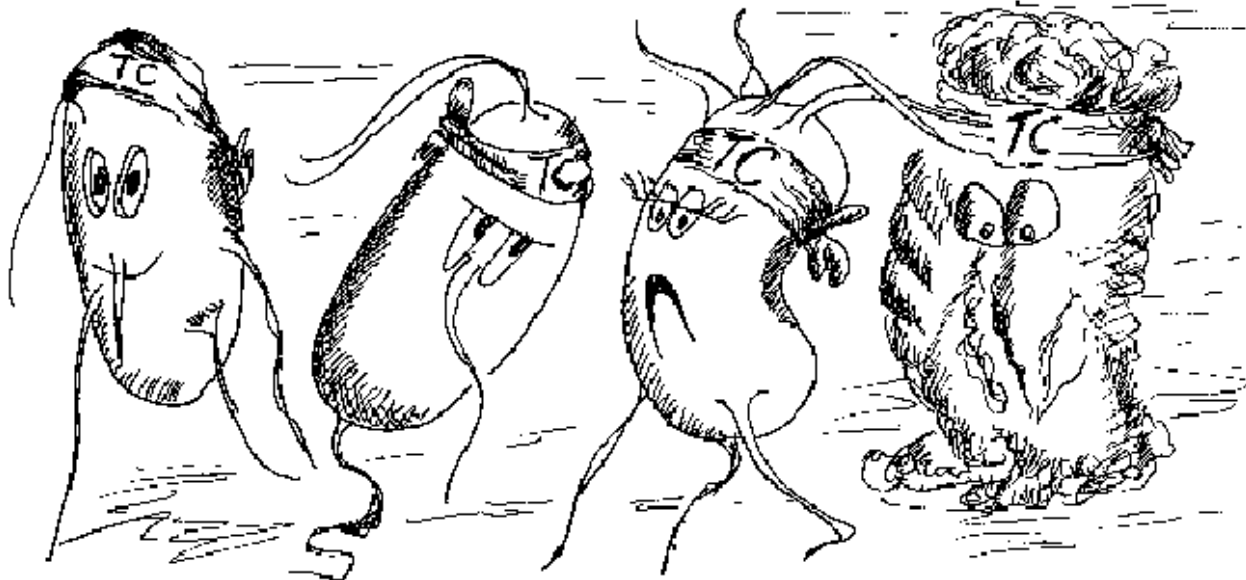
❖ Spore Formers

- *Clostridium perfringens*




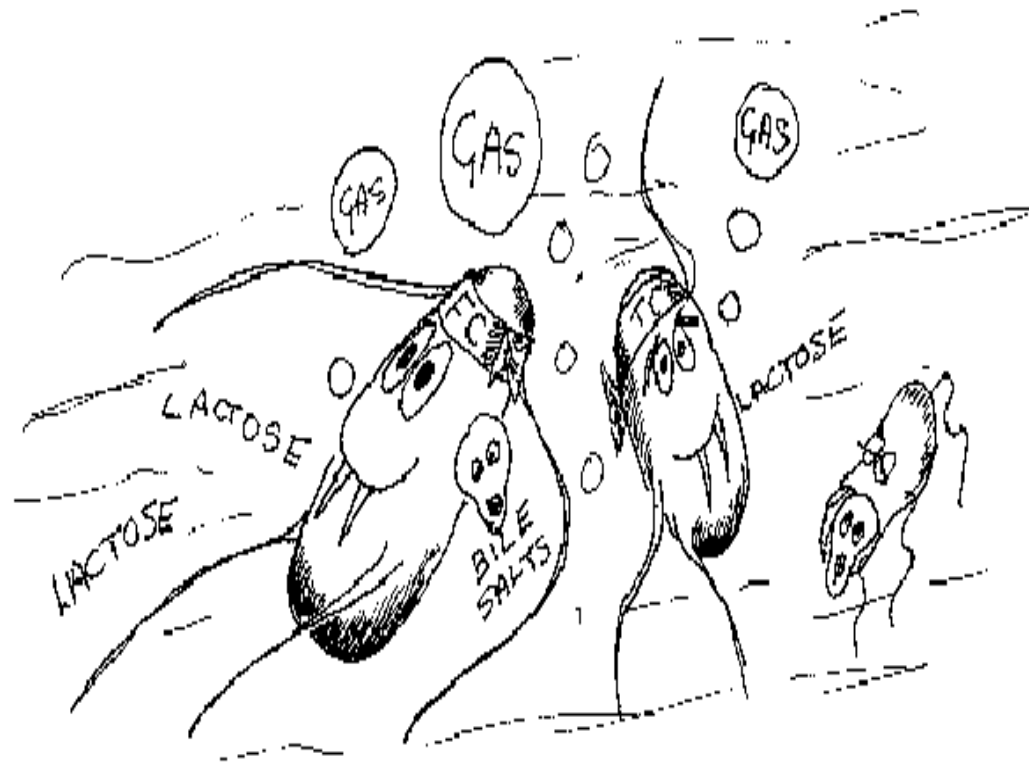
Coliform Bacteria:

- Coliform bacteria (*E. coli*-like) are the most often used indicator bacteria for water quality assessment in the U.S.



Characteristics of coliforms:

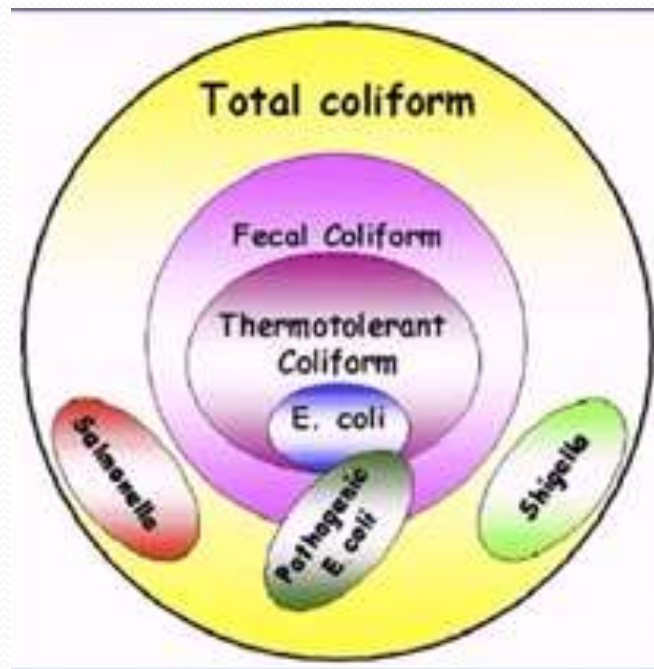
- Aerobic or facultative,
- Gram-negative,
- Non-spore forming,
- Bacilli, 
- Which ferments Lactose to form acid and/or gas with in 24 hours at 35°C



Coliform groups

Total coliforms – indicate water in contact with plant or animal life (universally present)

Fecal coliforms – mammal or bird feces in water



Factors Affecting Ratio of Indicator Organisms to Pathogens:

- All treatment methods and environmental conditions affect pathogens and indicators differently.
- Chlorinated water may have zero indicators and pathogens, but loaded with viruses.
- The ratio of indicators to actual pathogens is not fixed

Water Quality

- Feces from human populations with higher infection rates are of greater concern.
- Microbes are filtered from water that percolates into groundwater.
- Some pathogens are transmitted to human in drinking and recreational water.
- It involves variety of steps which depend upon the type of impurities in the raw water source.




Most Probable Number & Membrane Filter methods

Most Probable Number OR

Multiple tubes method:

- The **Most Probable Number** method is used to check **potability** (if water is safe enough to be drinking water) of water.
- The MPN method looks for the presence of potential pathogenic bacteria that may be in the water due to **contamination** of the water supply.
- Water supplies are generally derived from ground sources and have to be checked for safety levels of bacterial-contamination.

- 
- **MPN** method enumerates the enteric bacteria called coliforms, specifically **fecal coliforms** (*E. coli*)
 - Coliforms are **Gram negative bacilli that have the ability to ferment lactose with the production of acid and gas.**

➤ **MPN test includes 3 levels of testing:**

Presumptive
Test

Confirmatory
Test

Completed
Test

1- Presumptive Test:

- The **presumptive** test looks for presence of coliforms in the water sample by inoculating lactose broths with the water sample.

Composition of lactose broth:

Ingredients per liter of demineralized water:	
Peptone 190 (Pancreatic Digest of Gelatin)	5.0 g
Beef Extract	3.0 g
Lactose	5.0 g
pH 6.7 ± 0.2 at 25C	

Procedure:

- Took three sets of test tubes (with Durham tube) containing **lactose broths(2ml)** are inoculated with varying dilution of the sample:-

- **first set** of 5 tubes inoculated with 10ml of sample;
- **second set** of 5 tubes inoculated with 1ml of sample;
- **third set** of 5 tubes inoculated with 0.1ml of sample

- Then all test tubes incubate at 35 °C for 24 hours.

- Those tubes that show presence of **acid and gas** are marked as **+ve** and those with **no acid/gas** as **-ve**.
- Then count the +ve marked tubes from all sets.
- The combination of positives in the 3 sets is used to figure out the MPN /100ml of water using the table provided.

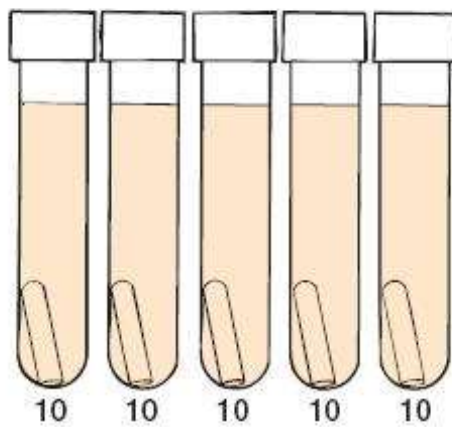


Water sample

Inoculate 15 tubes: 5 with 10 ml of sample, 5 with 1.0 ml of sample, and 5 with 0.1 ml of sample.

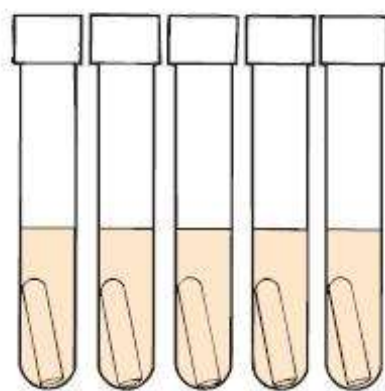
Double-strength broth

Single-strength broth



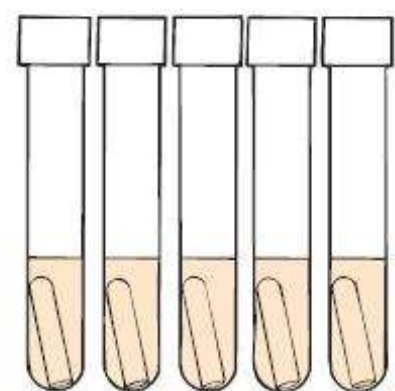
10 10 10 10 10

(ml)



1.0 1.0 1.0 1.0 1.0

(ml)



0.1 0.1 0.1 0.1 0.1

(ml)

Presumptive

Lactose or lauryl tryptose broth

Negative presumptive. The absence of gas in broth tubes indicates coliforms are absent. Incubate an additional 24 hours to be sure.



Negative

← 24 ± 2 hours 35°C →



Positive

After 24 hours of incubation, the tubes of lactose broth are examined for gas production.

**Table 1. Table of Most Probable Numbers (MPN) Per 100 ML
of Sample using Three Tubes of Each Dilution**

Number of positive tubes in dilutions			MPN per 100 ml	Number of positive tubes in dilutions			MPN per 100 ml
10 ml	1 ml	0.1 ml		10 ml	1 ml	0.1 ml	
0	0	0		2	0	0	9.1
0	1	0	3	2	0	1	14
0							20
0							26
0							15
0							20
0							27
0							34
0	2	0	6.2	2	2	0	21
0	2	1	9.3	2	2	1	28
0	2	2	12	2	2	2	35
0	2	3	16	2	2	3	42
0	3	0	9.4	2	3	0	29
0	3	1	13	2	3	1	36
0	3	2	16	2	3	2	44
0	3	3	19	2	3	3	53
1	0	0	3.6	3	0	0	23
1	0	1	7.2	3	0	1	39
1	0	2	11	3	0	2	64
1	0	3	15	3	0	3	95
1	1	0	7.3	3	1	0	43
1	1	1	11	3	1	1	75
1	1	2	15	3	1	2	120
1	1	3	19	3	1	3	160
1	2	0	11	3	2	0	93
1	2	1	15	3	2	1	150
1	2	2	20	3	2	2	210
1	2	3	24	3	2	3	290
1	3	0	16	3	3	0	240
1	3	1	20	3	3	1	460
1	3	2	24	3	3	2	1100
1	3	3	29				

Standard :

If MPN/100 ml is 3 or less than 3 than water is suitable for drinking

Confirmed test:

- Some spore forming bacteria give false positive test in presumptive test.
- Confirmed test is done to determine that the coliforms are of fecal origin or not. And they are *E. coli* or not.
- **Procedure:**
- Transfer the lactose broth with gas into two following media:-
 - Brilliant green lactose bile broth (BGLB):** this medium inhibit growth of lactose fermenter other than coliforms; thus gas formation in BGLB medium confirmed that coliforms are present.
 - Eosin Methylene Blue (EMB) agar:** Indicator produce characteristic colonies
 - E.coli** : small colonies ,dark ,almost black centers with greenish metallic sheen
 - Enterobacter** : large pinkish colonies ,dark centers ,rarely show metallic sheen

Com

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- i. Lacto
- ii. Agar s colour exam: prese:

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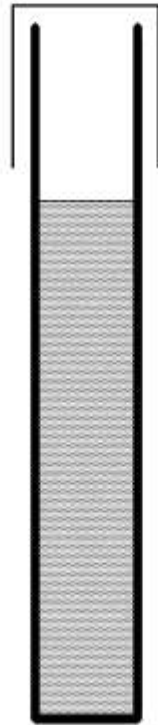
Result:

containin

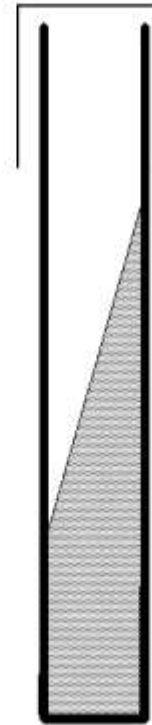
Agar plate



Agar deep



Agar slant



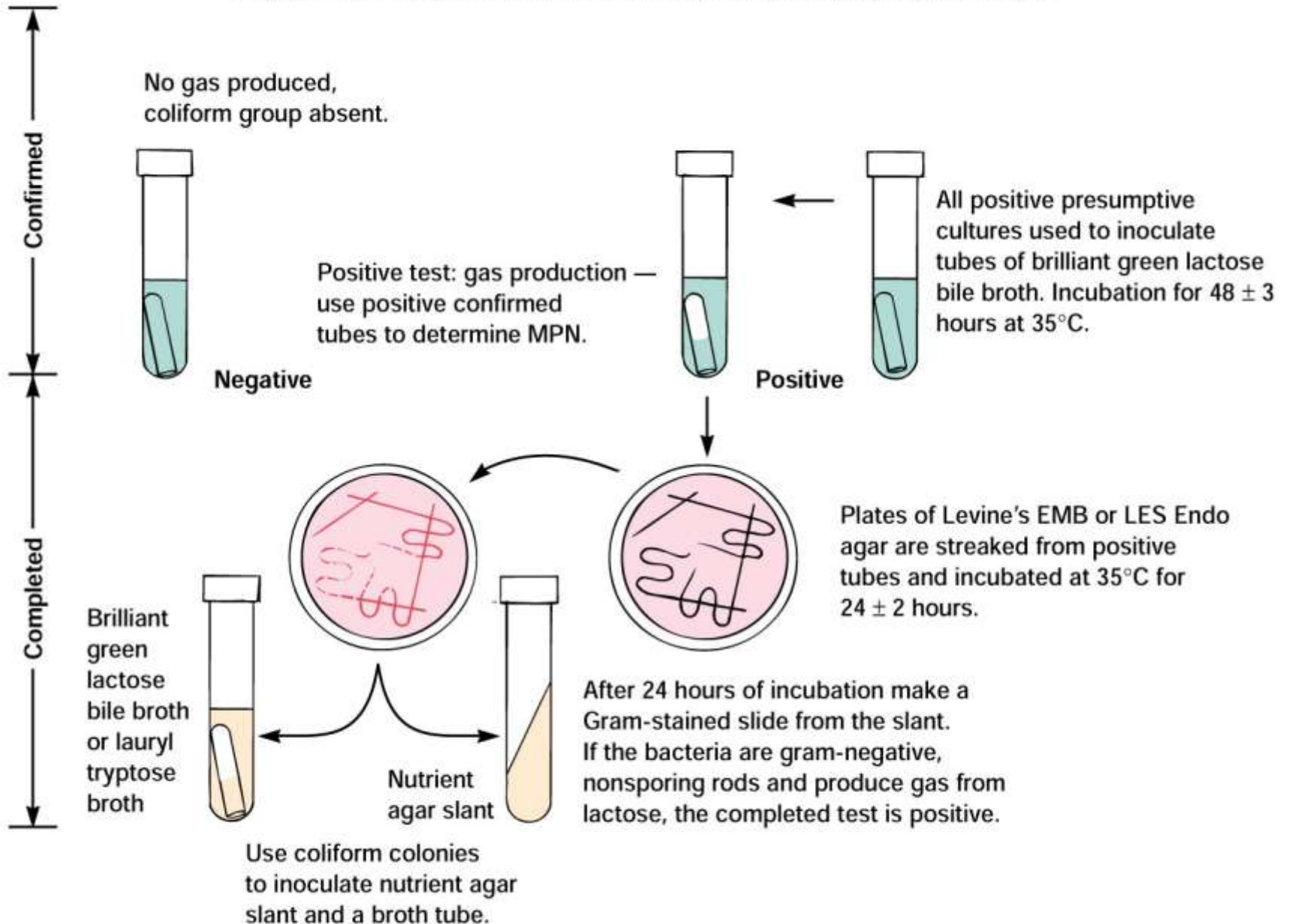
EMB plate

→ red
microscopic
eria

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Flow chart of Confirmed and Completed MPN

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Disadvantages:

- ❖ **labor intensive ,Large amount of glassware is required**
- ❖ **Its lack of precision, large errors**
- ❖ **still requires survival and culture of organisms in lab**

Membrane Filtration



Membrane Filtration

Categorized into four processes based on membrane pore size;

- * A. Microfiltration
- * B. Ultrafiltration
- * C. Nano filtration
- * D. Reverse Osmosis

Microfiltration

- * Size: 0.1-10 microns
- * Removes Sand, Silt, Clays, *Giardia-lambia*(flagellated protozoan parasite), Algae, some bacterial species and oil Emulsions.
- * It does not remove viruses.



... microfiltration

- * Used in :
 - * purification of juices and beverages.
 - * cold sterilization of pharmaceuticals.
 - * separation of oil and water emulsions.
 - * pre treatment of nano filtration or reverse osmosis



Ultra Filtration

- * 0.003 to 0.1 microns
- * Removes: Soluble organic materials, protozoa, bacteria, most viruses



... ultrafiltration

- * Used in :
 - * dairy (milk and cheese)
 - * food, metal and textile industry
 - * pre treatment of Nano filtration and Reverse Osmosis.



Nano Filtration

Size: 0.001 microns

- * Pressure driven filtration process.
- * 600 to 1000 kilopascal pressure required.



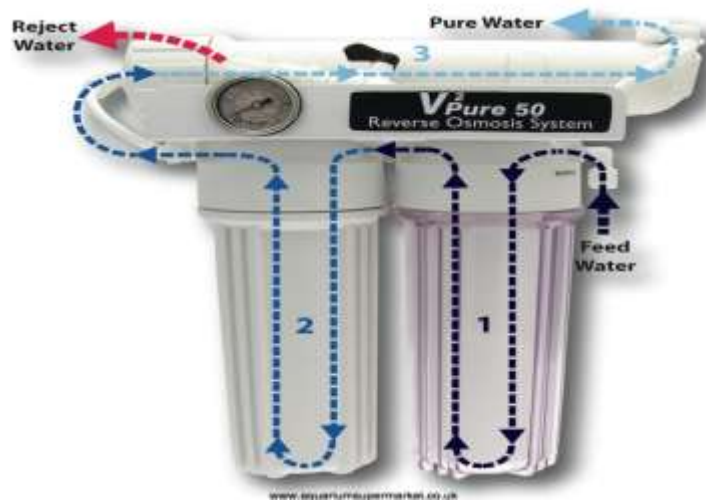
... nano Filtration

Removes

- * micro-molecular organic compounds,
- * microorganisms, hardness of water, demineralization, Colour removal
- * Used in desalination.

Reverse Osmosis

- * 0.0001 microns
- * Removes: Natural organic substances, pesticides, cysts, bacteria, viruses
- * Used in desalination

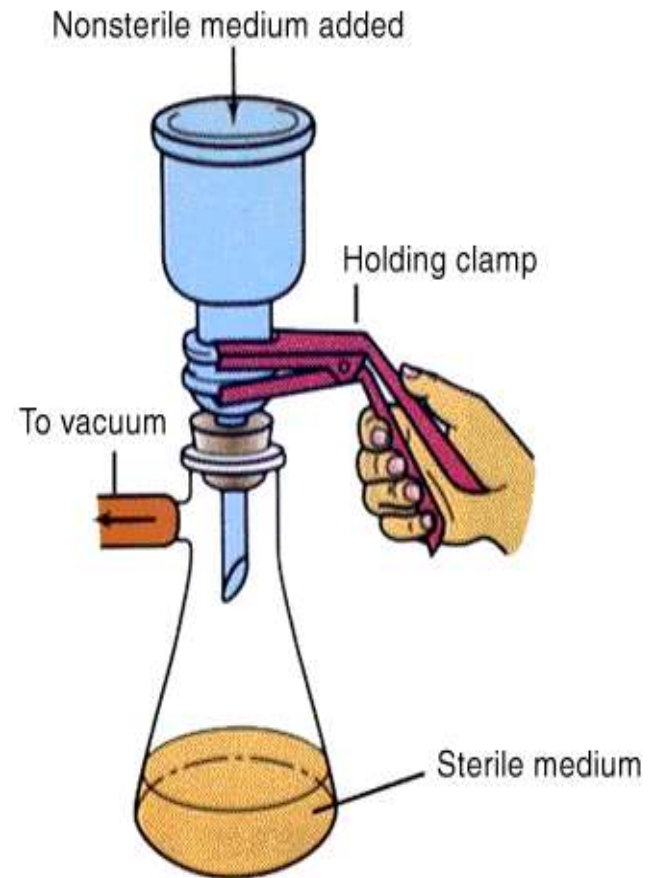
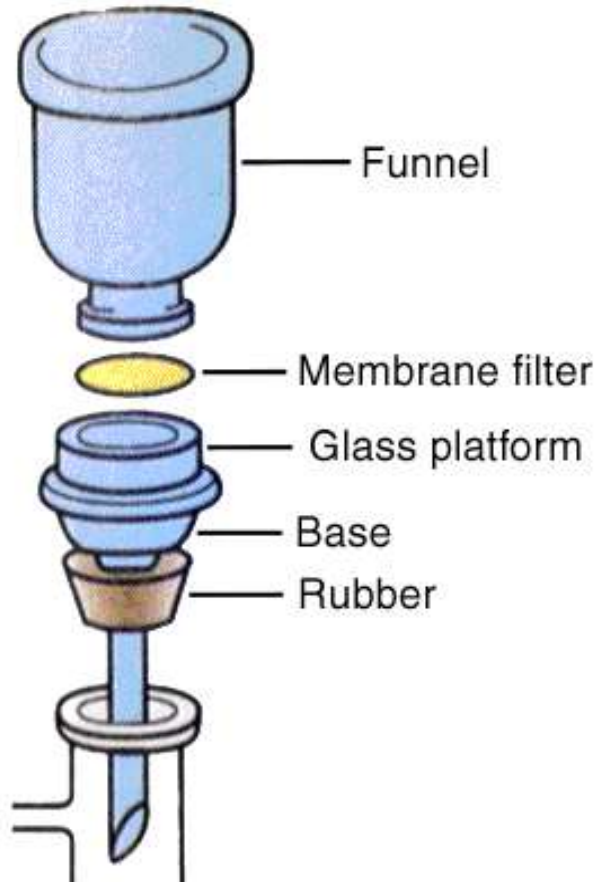


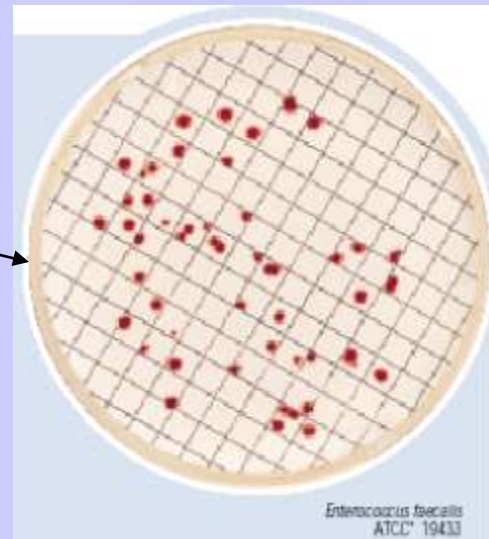
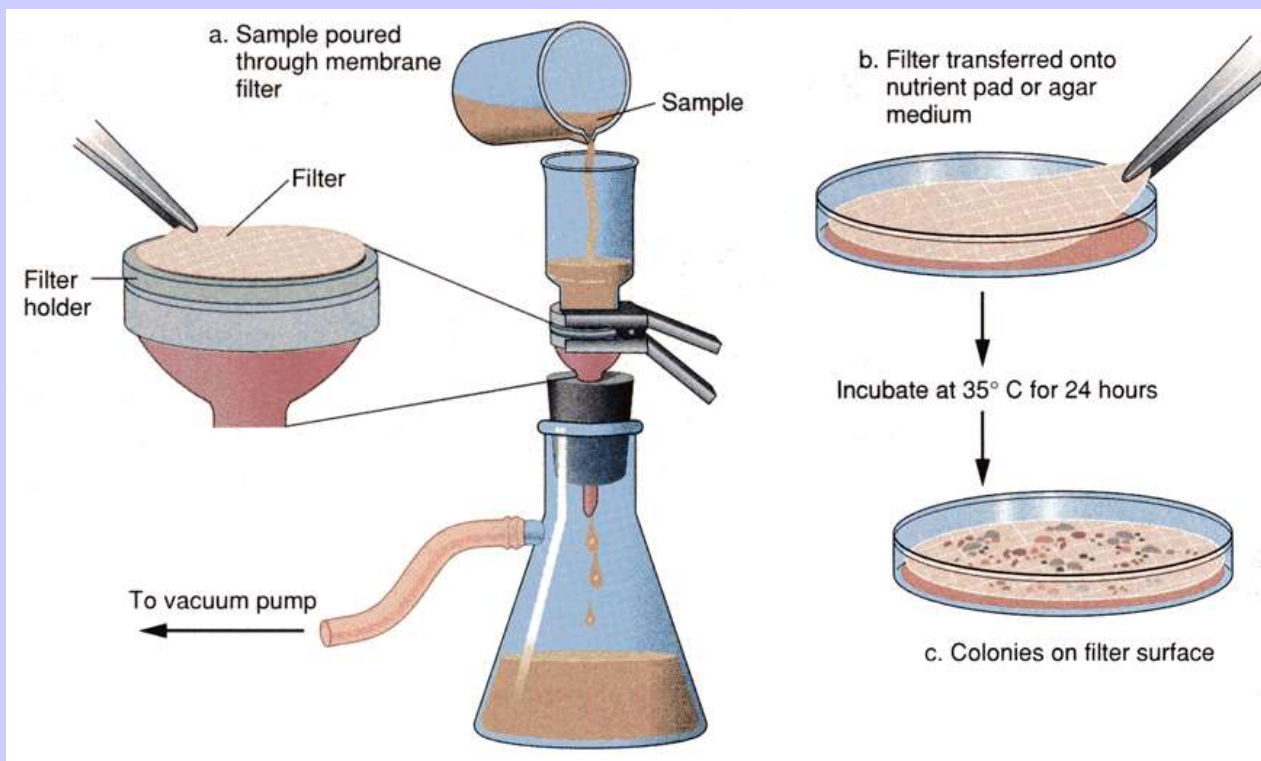
Membrane Filter Methods

- * Filter water through a membrane filter (Bacterial cells can not pass through)
- * Place membrane on selective media (EMB)
- * Incubate
 - * 35°C
- * Count colonies



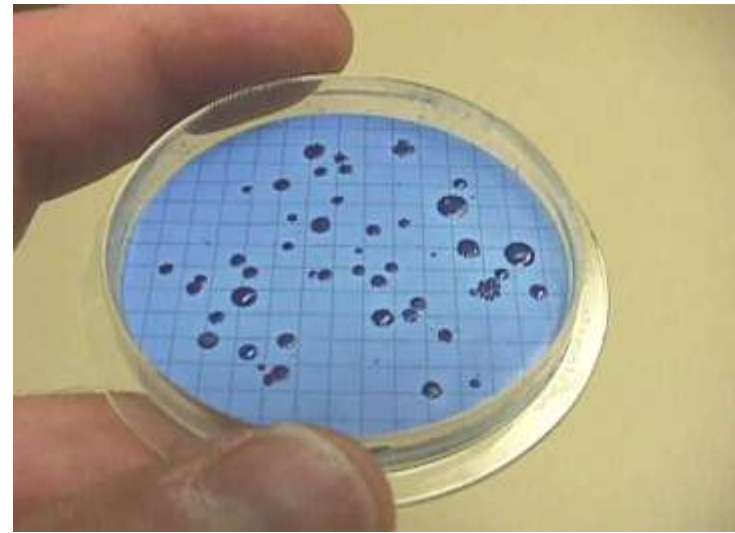
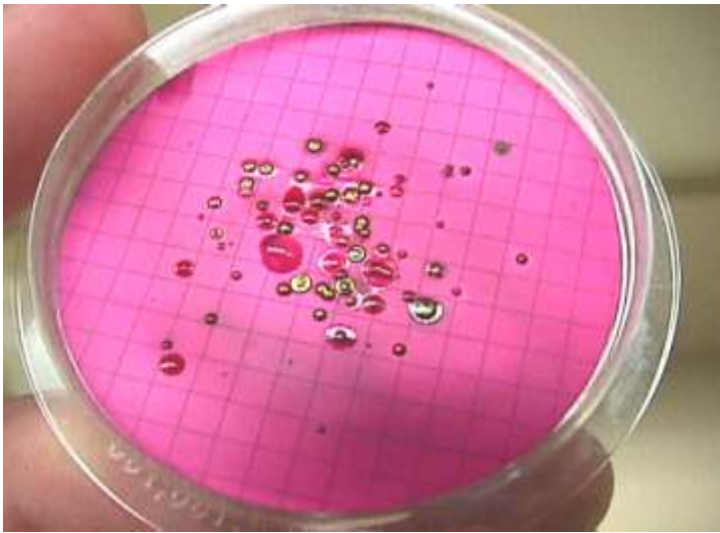
MF method





Membrane filtration method

Results:



Coliform bacteria produce colonies with a characteristic **"metallic green sheen"**

Membrane filtration method

Advantages

- ✓ More than 100ml samples can be tested
- ✓ Effective and acceptable technique. Used to monitor drinking water in government laboratories.
- ✓ Rapid
- ✓ Lower chance of contamination esp. on low scale
- ✓ More accurate

Disadvantage:

- ✓ Not suitable for turbid or waste water.

QUERRIES

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جَزَاءَكُمْ اللَّهُ خَيْرًا كَثِيرًا
وَجَزَاءَكُمْ اللَّهُ أَحْسَنَ الْجَزَاءِ

THANK YOU!!!

