Lecture (11-12)

Air Microbiology
Water Microbiology

Air Microbiology

The air is a layer of gases surrounding the planet that is retained by Earth's gravity.

The atmosphere protects life on Earth by absorbing ultraviolet solar radiation, warming the surface through heat retention (greenhouse effect), and reducing temperature extremes between day and night (the diurnal temperature variation).

The common name given to the atmospheric gases used in breathing and photosynthesis is **air**.

AIR ENIRONMENT; WHY TO STUDY?

Air is a special important and microbes and their activities are of great importance in many ways since;

- Of all environments, air is the simplest one and it occurs in a single phase gas.
- Air environment is instrumental in the chain of biochemical reactions.
- Air is the environment that provides oxygen necessary for the life of living organisms. Without air, life is not possible.

COMPOSITION OF AIR

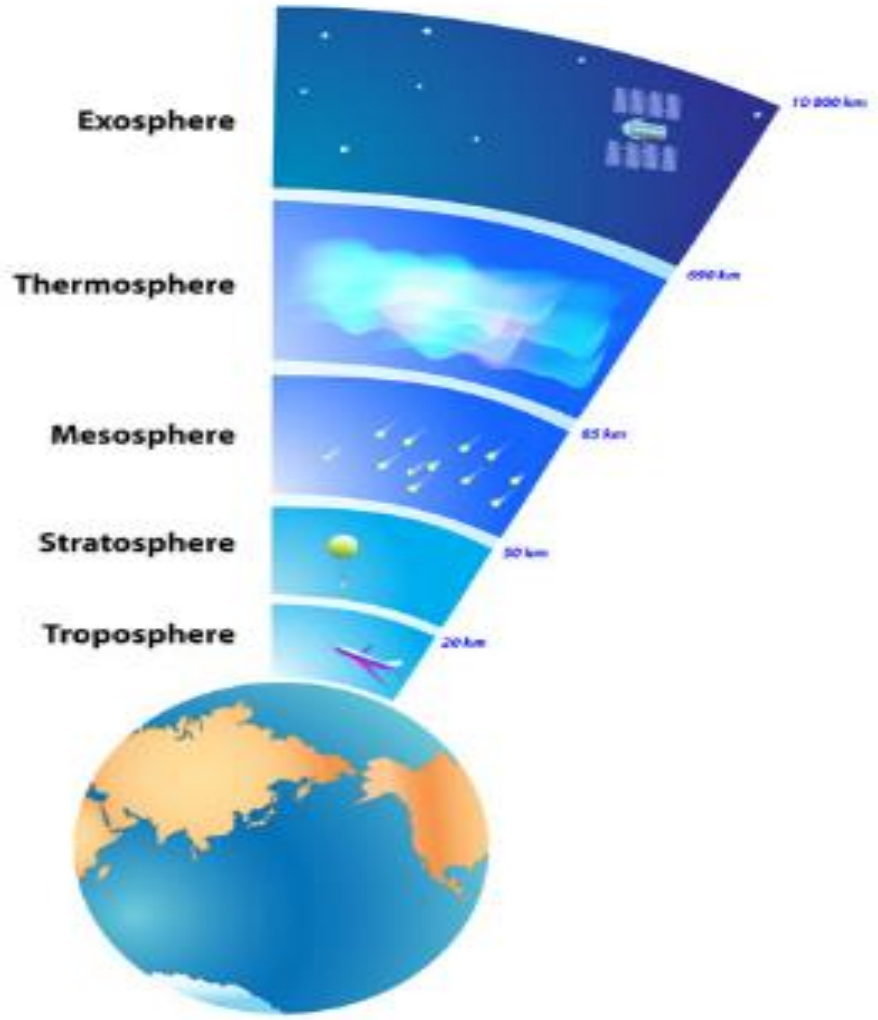
Air is a gaseous environment.

The relative quantities of various gases in air, by volume percentage are;

- Nitrogen 78%
- Oxygen 21%
- Argon 0.9%
- Carbon dioxide 0.03%
- Hydrogen 0.01%
- Other gases in trace amounts

In addition to various gases, dust and condensed vapor may also be found in air.

LAYERS OF AIR

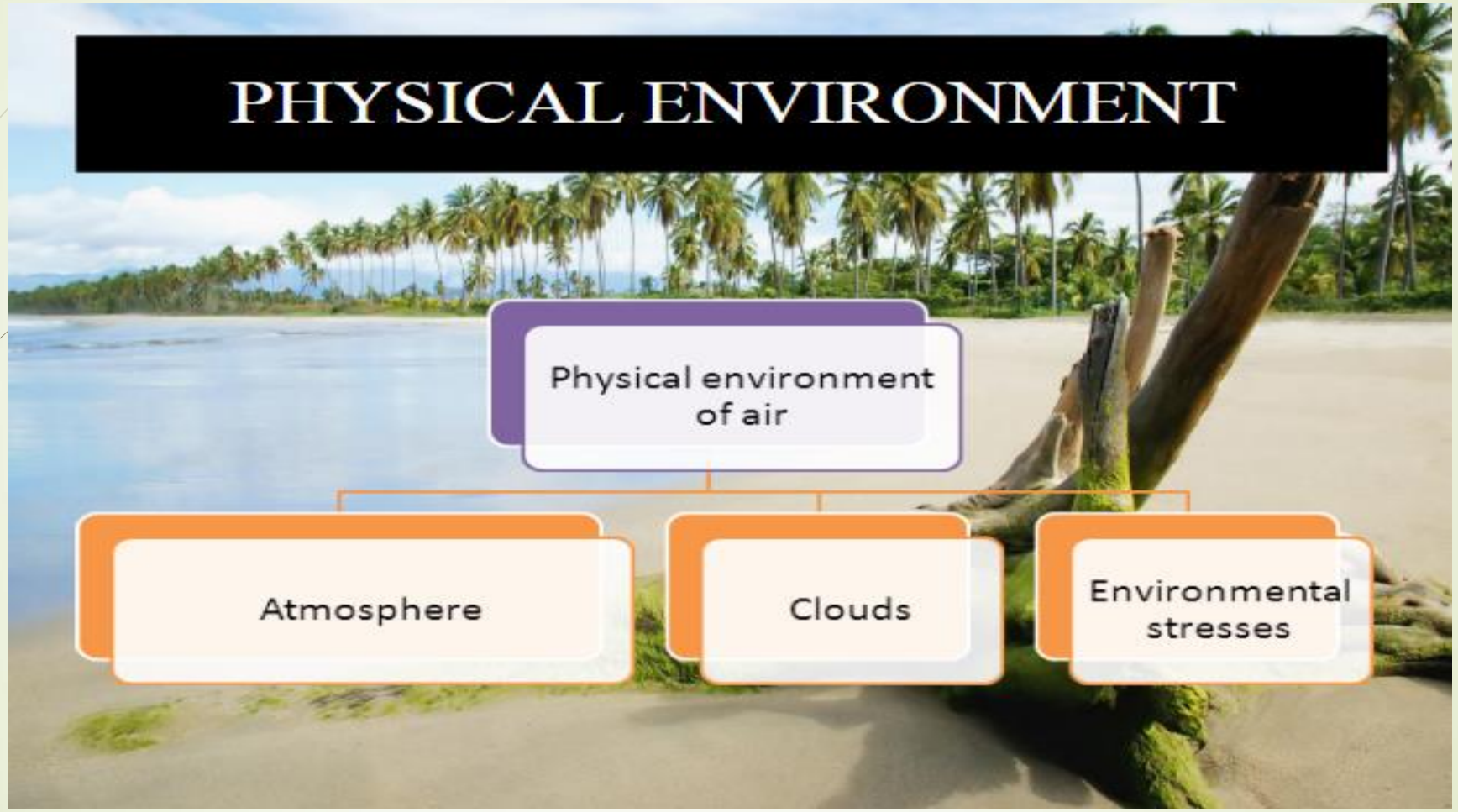


Various layers can be recognized in the atmosphere up to a height of about 1000km.

Although air content and atmospheric pressure vary at different layers, air suitable for the survival of terrestrial plants and terrestrial animals currently is only known to be found in Earth's troposphere and artificial atmospheres.

This layer is nearest to the earth. In temperate regions, troposphere extends up to about 11 km whereas in tropics up to about 16km. This layer is characterized by a heavy load of microorganisms.

Above the troposphere, the temperature starts to increase and the atmosphere become unsuitable for microbial growth and population.


PHYSICAL ENVIRONMENT
Physical environment of air
Atmosphere
Clouds
Environmental stresses

1) Atmosphere

In addition to water droplets, dust particles and other matter, air also contains microbes. These microbes follow a particular pathway in which they are suspended into the atmosphere which includes.

1- Launching into the air (source of the launching of airborne microbes stems from humans, animals and vegetation)

2- Transported (by various methods including winds, machinery and people)

3- Deposited in new atmosphere/environment

The atmosphere can have a variety of physical characteristics, and can be very extreme in terms of the relative humidity, temperature and radiation. These factors play a huge role in what kinds of microbes will survive in the atmosphere and how long they will stay alive.

2) Clouds

This is a second area in the air environment where bio aerosols can exist.

Cloud water is a mixture of organic and inorganic compounds suspended within moisture (contribution of microbial activity to clouds).

The conditions in clouds are not conducive to much life as;

1- Microbes present there must withstand freezing temperatures, the threat of desiccation, and extreme UV rays

2- Clouds have acidic environment, with a pH ranging from 3 to 7.

Nevertheless, there are extremophile microbes which can withstand all of these environmental pressures. Clouds serve as a transport for these microbes, dispersing them over long distances.

3) Environmental stresses

These stresses pose a variety of problems for survival of microbes and include;

Desiccation (it limits the amount of time that they can survive while suspended in the air)

Humidity (it affects the survival of organisms in air)

Temperature (too hot of temperatures can denature proteins, and too cold of temperatures can cause ice crystal formation)

Radiation (it can damage DNA within the cells)

In dry whether the microbial load of air is high while in wet weather the rain washes the microorganisms from the air.

WHAT IS AIR MICROBIOLOGY?

Aerobiology is defined as the study of life present in the air.

Or

Aero microbiology is related to the study of environmentally relevant microorganisms (bio aerosols).

The atmosphere as a habitat is characterized by high light intensities, extreme temperature variations, low amount of organic matter and a scarcity of available water making it a non hospitable environment for microorganisms and generally unsuitable habitat for their growth.

Nevertheless, substantial numbers of microbes exist within 300-1000 feet of earth's surface that have become attached to fragments of dried leaves, straw or dust particles light enough to be blown by wind.

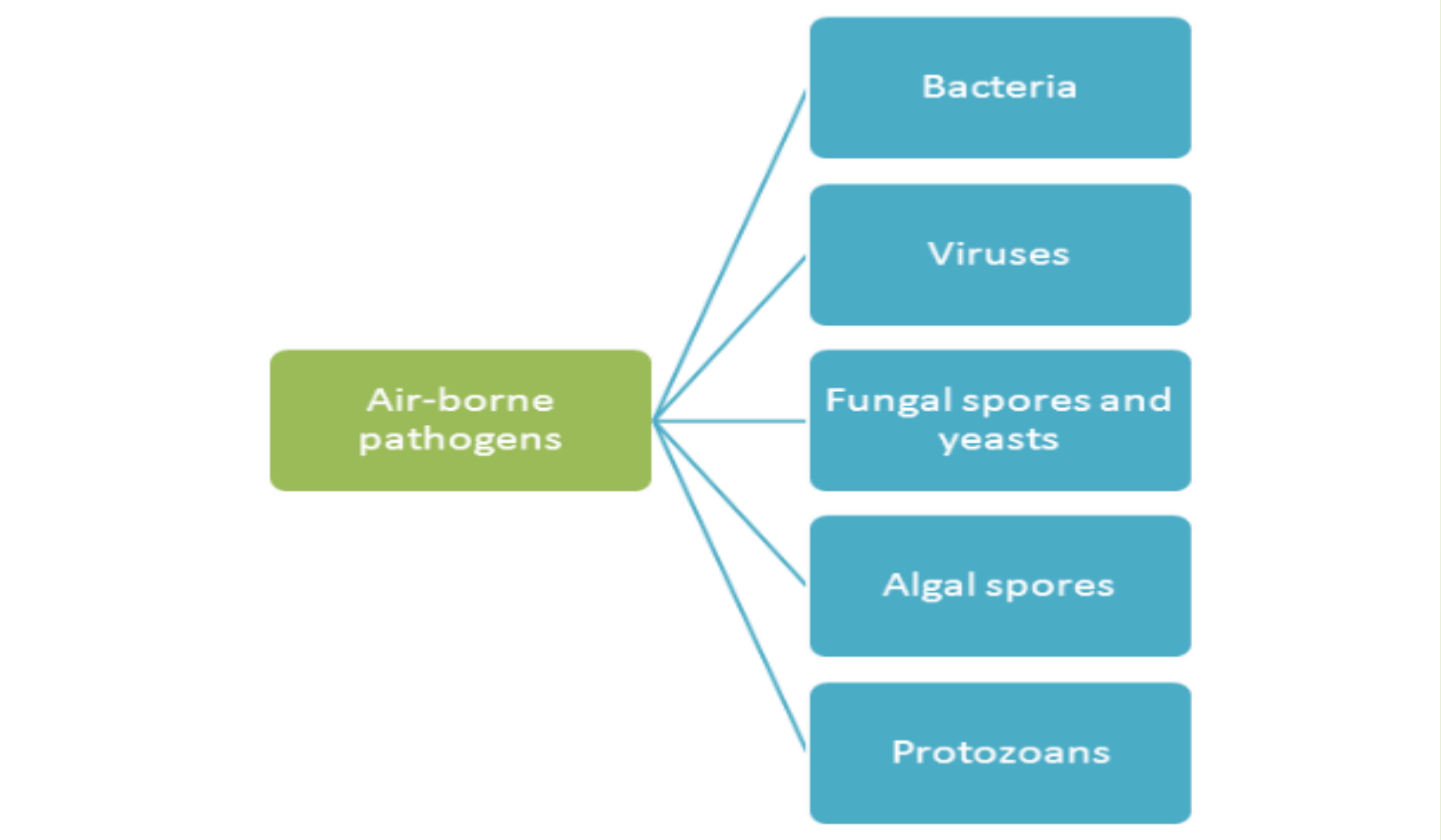
AIR MICROFLORA

Many different microorganisms can be in aerosol form in the atmosphere.

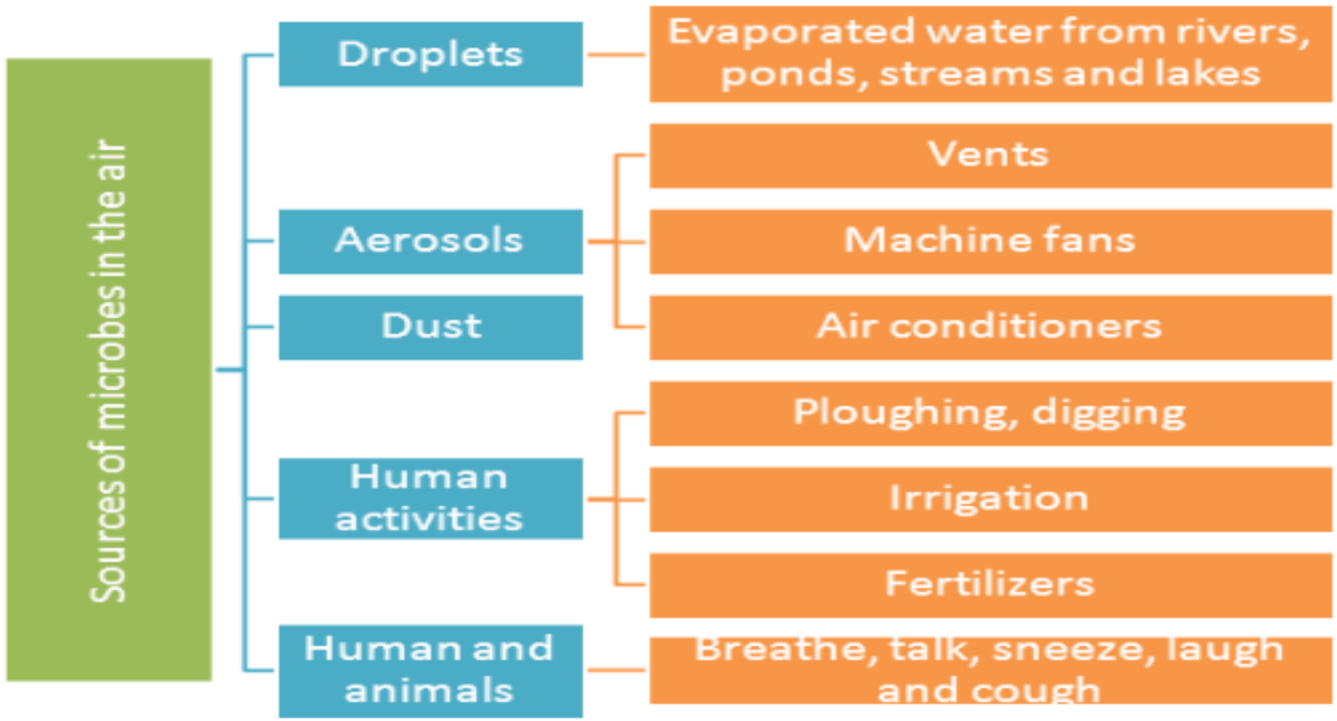
In order to survive in the atmosphere, it is important that these microbes adapt to environmental stresses.

Many of the microbes that are capable of surviving harsh conditions can readily form endospores, which can withstand extreme conditions.

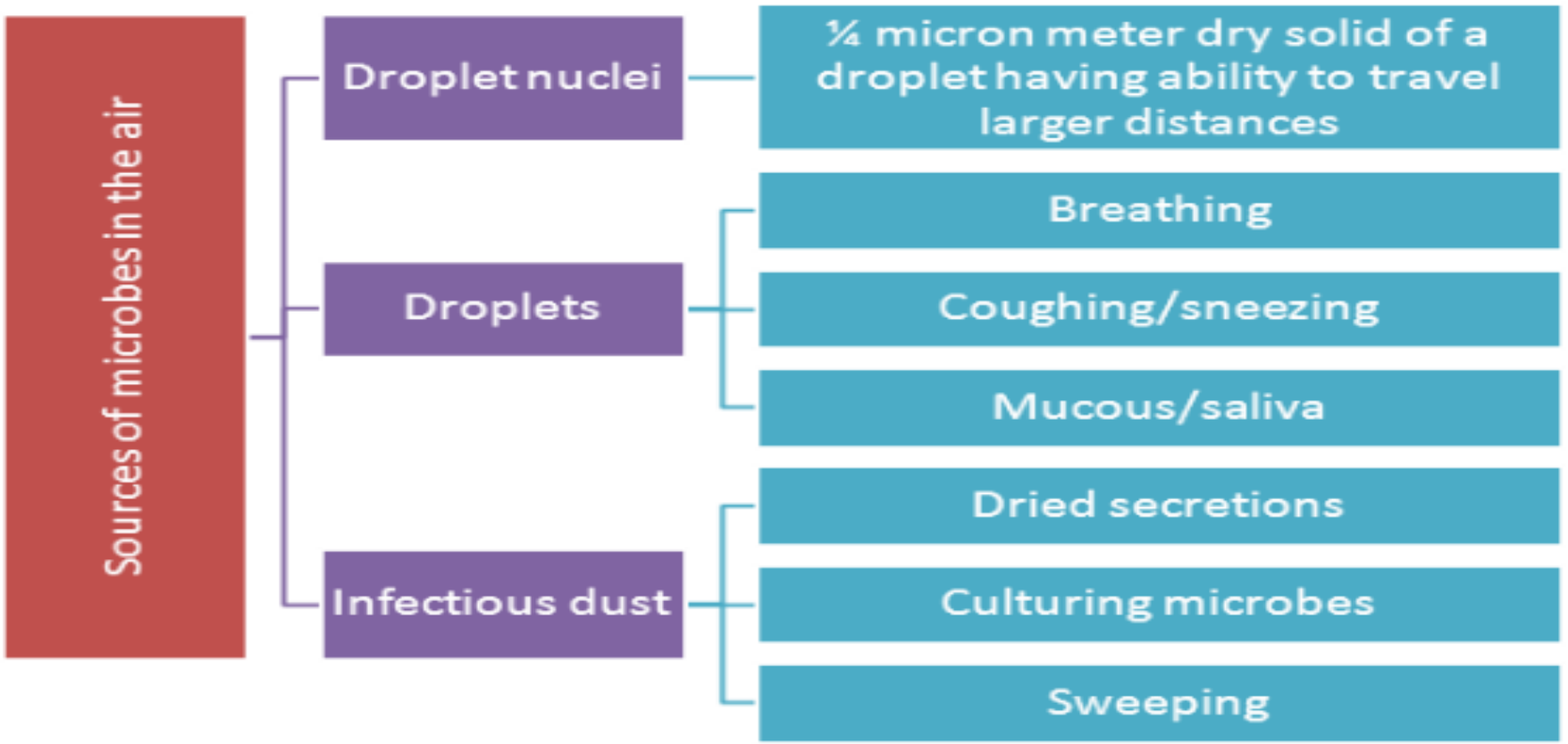
Many of these microorganisms can be associated with specific and commonly known diseases.


Air-borne pathogens
Bacteria
Viruses
Fungal spores and yeasts
Algal spores
Protozoans

SOURCES OF MICROBES IN THE AIR



FORMS OF DISCHARGE



AIR-BORNE DISEASES (Bacterial)

Bacteria	Disease
Streptococcus pyogenes	Sore throat
Corynebacterium diphtheriae	Diphtheria
Mycobacterium tuberculosis	Tuberculosis
Streptococcus pneumoniae	Pneumococcal pneumonia
Klebsiella pneumoniae	atypical pneumonia
Neisseria meningitidis	Meningococcal meningitis
Yersinia pestis	Bubonic plaque
Bordetella pertussis	Whooping cough
Haemophilus influenzae	Influenza
Nocardia asteroids	Nocardiosis

AIR-BORNE DISEASES (viral)

Virus	Disease
Influenza virus	Influenza
Hantavirus	Pulmonary syndrome
Hepatitis virus	Hepatitis
Herpes virus	Chicken pox
Picorna virus	Common cold
Flavivirus	Dengue fever
Rubella virus	Rubella
Measles virus	Measles
Influenza virus	Influenza
Hantavirus	Pulmonary syndrome

AIR-BORNE DISEASES (fungal)

Fungi	Disease
Aspergillus fumigatus	Aspergillosis
Blastomyces dermatiridi	Blastomycosis
Coccidioides immitis	Coccidioidomyosis
Cryptococcus neoformans	Cryptococcosis
Histoplasma capsulatum	Histoplasmosis
Pneumocystis carinii	Pneumocystitis

FOOD MICROBIOLOGY

Introduction

- Food production occurs at specific areas and at certain periods of the year due to variation in weather conditions.
- Food therefore has to be collected and stored for use during periods of low or no food production.
- However, storage is complicated by the fact that food begin to deteriorate shortly after harvest, gather or slaughter.

Food spoilage

- Food spoilage is defined as damage or injury to food rendering in unsuitable for human consumption.
- Food must be considered spoiled if it is contaminated with pathogenic microorganisms or various poisonous agents, such as pesticides, heavy metals etc.

Storage life of some foods

Food product	Storage life (days) at 21°C
Raw beef and mutton	1-2
Raw fish	1-2
Raw poultry	1-2
Dried salted or smoked meat and fish	360 or more
Fresh fruits	1-7
Dried fruits	360 or more
Leafy vegetables	1-2
Root crops	1-20
Dried seeds	360 or more

Food spoilage cont….

- In most cases there does not need to be an evident sign of spoilage, the food might look normal and only after eating it or by careful bacteriological and toxicological investigation, one is able to realize the defect.
- Food decay or decomposition is implied when the term spoiled is used.

Causes of food spoilage

- **(a). Growth and activity of microorganisms** Bacteria, yeasts and molds are microorganisms that cause food spoilage. They produce various enzymes that decompose the various constituents of food.
- (b). Enzyme activity: Action of enzymes found inherently in plant or animal tissues start the decomposition of various food components after death of plant or animal.
- (c). Chemical reactions: These are reactions that are not catalysed by enzymes.,e.g. oxidation of fat

Causes of food spoilage cont…

- (d). Vermin. Vermin includes weevils, ants, rats, cocroaches, mice, birds, larval stages of some insects. Vermin are important due to:

  (i). Aesthetic aspect of their presence,

  (ii) Possible transmision of pathogenic agents, (iii). Consumption of food.

- (e). Physical changes. These include those changes caused by freezing, burning, drying, pressure, etc.

Microbial spoilage of food

- Bacteria, yeasts and molds are the major causes of food spoilage.
- They produce various enzymes that decompose the various constituents of food.
- Molds are the major causes of spoilage of foods with reduced water activity e.g dry cereals and cereal product
- Bacteria spoil foods with relatively high water activity such as milk and products.

Sources of microorganisms in food

The primary sources of microorganisms in food include:

1. Soil and water
2. Plant and plant products
3. Food utensils
4. Intestinal tract of man and animals
5. Food handlers
6. Animal hides and skins
7. Air and dust

Factors affecting microbial growth in food

(a)Intrinsic factors:

These are inherent in the food. They include:

- Hydrogen ion concentration (pH),
- moisture content,
- nutrient content of the food,
- antimicrobial substances ad
- biological structures.

1. Hydrogen ion concentration (PH)

- Most bacteria grow best at neutral or weakly alkaline pH usually between 6.8 and 7.5.
- Some bacteria can grow within a narrow pH range of 4.5 and 9.0, e.g. salmonella
- Other microorganisms especially yeasts and molds and some bacteria grow within a wide pH range, e.g. molds grow between 1.5 to 11.0, while yeasts grow between 1.5 and 8.5.

pH values of some food products

Food type	Range of pH values
Beef	5.1 - 6.2
Chicken	6.2 – 6.4
Milk	6.3 – 6.8
Cheese	4.9 - 5.9
Fish	6.6 - 6.8
Oyester	4.8 - 6.3
Fruits	< 4.5 (most < 3.5)
Vegetables	3.0 – 6.1

- Microorganisms that are able to grow in acid environment are called acidophilic microorganisms.
- These microorganisms are able to grow at pH of around 2.0.
- Yeasts and molds grow under acid conditions.
- Other microorganisms such as *vibrio cholerae* are sensitive to acids and prefer alkaline conditions.
- Most bacteria are killed in strong acid or strong alkaline environment except Mycobacteria.

Minimum and maximum pH for growth of some specific microorganism

Microorganism	Minimum	Maximum
Escherihia coli	4.4	9.0
Salmonella typhi	4.5	8.8
All bacteria	4.0	9.0
Molds	1.5	11.0
Yeast	1.5	8.5

2. Moisture content

- The effect of moisture is in terms of water activity: - the amount of free water in a food medium.
- The amount of free water is important for growth of microorganisms.
- If there is lack of this free water microorganisms will not grow.
- Water activity is defined as the vapour pressure of a food substance to that of water at the same temperature. (Aw = VPFood/VPWater)

Moisture content

- The water activity is therefore equal to 1.0.
- Food products have a water activity of less than 1.0.
- A saturated salt solution has a water activity of 0.75.
- Salting and drying reduces the water activity of a food product.

Water activity of some food products.

Food Product	Water activity
Raw meat and milk	0.99- 1.0
Luncheon meat	0.95
Boiled ham, sliced bacon	0.90
Dried grains	0.80

Water activity levels

- Growth of microorganisms is greatly affected by the level of water activity(Aw) in the food.
- Inhibition of growth occurs if the water activity for food is lowered beyond an organism's minimum level of water activity that is necessary for growth.
- Microorganisms have varied minimum water activity requirements that supports their growth in food.

Minimum water activity that supports growth of some microorganisms

Microorganism	Water activity
Clostridium botulinum, Bacillus cereus, Pseudmonas aeroginosa, Salmonella spp.	0.95 0.95 0.95 0.95
Staphylococcus aureus (anaerobic), Candida spp., Saccharomyces	0.90
Staphylococcus aureus (aerobic)	0.86
Penicillium spp.	0.82
Most spoilage yeast	0.88
Most spoilage molds	0.80
Osmotic yeast	0.70

Nutrients content of the food

- Microorganisms require proteins, carbohydrates, lipids, water, energy, nitrogen, sulphur, phosphorus, vitamins, and minerals for growth.
- Various foods have specific nutrients that help in microbial growth.
- Foods such as milk, meat and eggs contain a number of nutrients that are required by microorganisms.
- These foods are hence susceptible to microbial spoilage.

Antimicrobial substances

- Antimicrobial substances in food inhibit microbial growth.
- Various foods have inherent antimicrobial substances that prevent (inhibit) microbial attack.
- Such inhibitors are like lactinin and anti-coliform factors in milk and lysozyme in eggs.

Biological structures

- Some foods have biological structures that prevent microbial entry.
- For example, meat has fascia, skin and other membranes that prevent microbial entry.
- Eggs have shell and inner membranes that prevent yolk and egg white from infection.

(b). Extrinsic factors

- Are factors external to the food that affect microbial growth. They include:
  1. Temperature of storage,
  2. Presence and concentration of gases in the environment
  3. Relative humidity of food storage environment.

1. Temperature

- The growth of microorganisms is affected by the envirnmental temperatures.
- Various microorganisms are able to grow at certain temperatures and not others.
- Bacteria can therefore be divided into the following groups depending upon their optimum tmperature of growth.

(i). Pyshrophilic microorganisms

- These grow best at about 20°C but also down to -10°C in unfrozen media.
- Psychrophilic bacteria can cause food spoilage at low temperatures.
- Several of the microorganisms found in the soil and water belong to this group.

(ii). Mesophilic bacteria

- These organisms grow between 25°C and 40°C, with an optimum growth temperature close to 37°C
- Some such as *Pseudomonas aeroginosa* may grow at even lower temperatures between 5-43°C
- None of the mesophilic bacteria are able to grow below 5°C or above 45°C.
- Most pathogenic bacteria belong to this group.

(ii). Thermophilic bacteria.

- These grow at temperatures above 45°C. Often their optimum growth temperatures is between 50°C and 70°C.
- Growth of some bacteria occur at 80°C.
- Bacteria in this group are mainly spore formers and are of importance in the food industry especially in processed foods.

Note that:

- The effect of temperature on microbial growth also depends upon other environmental conditions such as:
  - Growth factors in the nutrient medium,
  - pH of the food, and
  - Water activity.

2. Concentration of gases in the environment

- This relates to the presence and concentration of gases in the food environment.
- Various microorganisms require for growth, either high oxygen tension (aerobic), low oxygen tension(microaerobic) or absence of oxygen (anaerobic).
- Some microorganisms may grow either in high oxygen tension, or in the absence of oxygen (facultative anaerobes).

Foods affected by various groups

- Anaerobic or facultatively anaerobic sporeformers are most likely to grow in canned foods .
- Microaerophilic bacteria are most likely to grow in vacuum packed foods since they have low oxygen tension, while
- Aerobic bacteria are likely to grow on the surface of raw meat.
- Aerobic molds will grow in insufficiently dried or salted products

3. Relative humidity

- Relative humidiy is the amount of moisture in the atmosphere or food environment.
- Foods with low water activity placed at high humidity environment take up water, increase their water activity and get spoiled easily.
- For example, dry grains stored in a environment with high humidity will take up water and undergo mold spoilage.

Food preservation

- Food preservation is a process through which physical and /or chemical agents are used to prevent microbial spoilage of food.
- Food preservation aims at treating food in a manner to prolong its storage life
- In food preservation, efforts are made to destroy organisms in the food,or
- Increase the period taken by microorganism to adapt to the food environment before they start to spoil the food.

Food preservation principles

- Two general principles are employed in food preservation.
- (1). Inhibition priciple
- (2). Killing principle

(1). Inhibition principle

- In this principle, food preservation is achieved by inhibition of growth and multiplication of microorganisms.
- The inhibition principle can be achieved by any of the following methods:
- (a). Reduction of water activity e.g. By drying and salting
- (b). Reduction in pH e.g. by fermentation and addition of acids.
- (c). Use of preservatives, e.g. sodium benzoate
- (d). Use of low temperatures (chilling or freezing)
- (e). Smoking – which has a drying and preservative effect

Inhibition methods

- Preservation of food by inhibition methods does not necessarily imply the destruction of organisms,
- On removal of the inhibiting influence, the food will undergo spoilage as the microorganism present will grow and multiply to cause spoilage.

Food preservation by lowering pH

- Many food products can be preserved by lowering pH so that the growth of spoilage and pathogenic bacteria is prevented.
- The lowering of pH can be achieved by addition of acids and fermentation
- Fermentation is the breakdown of carbohydrates under anaerobic conditions into alcohol or lactic acid and carbon dioxide.

Food preservation by lowering water activity

Lowering of water activity can be achieved by:

- Addition of high content of salt: Sodium chloride and sometimes nitrats and nitrites
- Addition of high content of sugar
- Drying: sun/air drying; electrical drying or freeze drying.

The salting procedure

The salting procedure can be performed in four ways:

1. Dry cure in which the meat or fish is rubbed with salt
2. Pickling: The products are immersed in pickle of brine, usually containing about 15% salt.
3. The injection cure: concentrated salt injected to muscles
4. Direct addition method

Preservation of food by addition of high content of sugar

- Monosaccharides such as glucose(dextrose) and fructose are more effective in reducing the water activity than disaccharides like sucrose.
- Thermophiles are more susceptible to the action of sugar than than other bacteria.
- Osmophilic yeasts are able to tolerate very high concentrations of sugar and cause food spoilage.

Food preservation by use of low temperatures

- Two methods are employed to arrest microbial growth and multiplication.
- These are chilling (cold storage) and freezing.
- Chilling is keeping food at temperatures between 0-15°C. The commom chilling temperatures ranges between 4-5°C.
- Freezing is keeping food at temperatures between 0°C and -35°C.

Effect of low temperatures

- Low temperatures are used to retard chemical reactions and actions of food enzymes and to slow down or stop the growth and activity of microorganisms in the food.
- A low enough temperature will prevent growth of any microorganisms.
- Spores are not usually injured at all by freezing. However, most parasites are killed by freezing.

(2). Killing principle

- In this principle, spoilage microorganisms are destroyed (Killed) in the food, and the food protected against subsequent contamination by being enclosed in an air tight container.

Methods employed to achieve the killing principle

1. **Heat treatment:** through **pasteurization** or **sterilization**
2. **Irradiation** with either ionizing or electromagnetic radiation e.g gamma rays, cobalt 60 radioactive particles. Radiations kill microorganisms by destruction of DNA and by creating toxic reactive compounds in a medium and in microbial cells
3. **Use of gases:** by use of ethylene oxide or ozone. The gases destroy both vegetative cells and spores.

Pasteurization

- Is the process of heat treatment at specific temperatures and times.
- Pasteurization is aimed at destroying all pathogenic microorganisms without affection the nutritive value of the food.

Three methods of pasteurization

a. Low temperature long time (63°C for 30 min)
b. High Temperature short time (72°C for 15 seconds)
c. Flash method (80°C for 1-2 seconds)
d. UHT (120°C for 1-2 seconds)

Sterilization

- Is the use of physical or chemical means to destroy all microorganisms that are present in the food.
- Sterilization can be achieved by:
  a. Heating at high temperatures, e.g. 100-140°C
  b. Irradiation:Irradiation kills bacteria, spores, and insects as well as inactivates enzymes.

Applications

- In pracice, often a combination of inhibition and killing principles and the various methods are used depending on the food type. e.g.
  - use of pasteurization and chilling of milk,
  - lowering of water activity and low temperature storage,
  - use of preservatives and low temperature etc.

Important terminologies on use of heat in food preservation

- D- value
- Z- value
- F-value

Decimal reduction Time (D-Value)

- Is the time required at any temperature to destroy 90% of the spores or vegetative cells of a given organism.
- The higher the temperature, the faster is the rate of destruction and the shorter it takes to kill 90% of the cells.
- For example, D-value for *Clostridium sporogenes* in a given food at 120°C is 1 minutes, at 115°C is 4 minutes, at 110°C is 10 minutes.

D-Value cont..

- The larger the initial number of vegetative cells or spores, the longer it will take to destroy 90 % of the cells at a given temperature.
- D- value is numerically equal to the number of minutes required for the survivor curve to trasverse one log cycle.
- If the intial number is one million per ml, one log cycle will reduce this number to 100 per ml.

Z-value

- **The Z value:** Is the number of degrees the temperature has to be increased in order to reduce the thermal death time tenfold.
- The z value is relatively constant and depends very little upon the environment.
- For spores of bacteria, the z - value used is 10°C.

Z- value

- The spore killing effect of a heat treatment can be expressed as a function of temperature and the time the material has been exposed to that heat.
- For example, when it takes 1 min to kill 90% of the remaining spores at 120°C, it will take 10 min to obtain the same effect at 110°C, and it will take 100°C.

F-value

- **F-value**. The F-value express the time taken to expose food to the same amount of heat required to destroy spores and vegetative cells of a particular organism using different temperatures.
- For example, food heated at 121.1°C for 2 minutes will give a value F=2. To get the same F-value of 2 using 111.1°C, one needs to heat the food for 20 min.

F-value

- Heating such a food at 111.1°C for 2 minutes will give F value of 2/10 = 0.2.
- This means that one can obtain the same killing effect of spores and /or vegetative cells at a lower temperature, provided the time of exposure is longer.
- Thus, F-value shows the heat treatment given to a food product to destroy bacteria.

F-value

- As far as spore killing is concerned, F=1 is equal to 1 min at 121°C (or 10 min at 111.1°C or 100 min at 101.1°C.)

Water Microbiology

Introduction

Water

- very essential factor needed by man (used for cooking, drinking, etc.)

-open and widely accessible, making it susceptible to contamination by chemicals and bacterial pathogens

-once contaminated, it would be harmful for human consumption.

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
associated diseases

1 There are waterborne diseases, such as cholera, typhoid, bacillary dysentery, infectious hepatitis;

2 **Water-washed diseases, such as trachoma, scabies, dysentery, louse-borne fever;**

3 **Water-based diseases, such as schistosomiasis, and Guinea worm;**

4 **And water-related diseases (involving an insect vector) such as malaria, sleeping sickness, or onchocerciasis.**

40% of annual worldwide deaths attributed to these diseases

H_2O 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 worm invasions and viral/protozoan diseases transmitted by insects (aquatic hosts or insect breeding in H_2O - indirect)

Water-associated diseases can be classified under 4 different categories: -

1. Water-borne diseases

Mainly enteric diseases resulting from the ingestion of faecally-contaminated H_2O (man, animal and bird excreta)

In developed countries, classical H_2O -borne diseases are mostly low infective dose infections - cholera and typhoid fever (rare), leptospirosis (rare); viral infections; *Campylobacter* (bacterium) and *Giardia* and *Cryptosporidium* (protozoa) infections - becoming more common in Ireland

- In developing countries (or as a result of the breakdown of sanitary services in developed countries - earthquakes, war etc.), a variety of other, high-infective dose diseases can be transmitted via H_2O - infectious hepatitis, *Vibrio* (bacterial) infections; bacillary dysentery; other viral infections etc. (human and/or animal origin

- All water borne diseases can also be transmitted by other routes that permit ingestion of faecal matter - e.g. contaminated food

2. Water-washed diseases

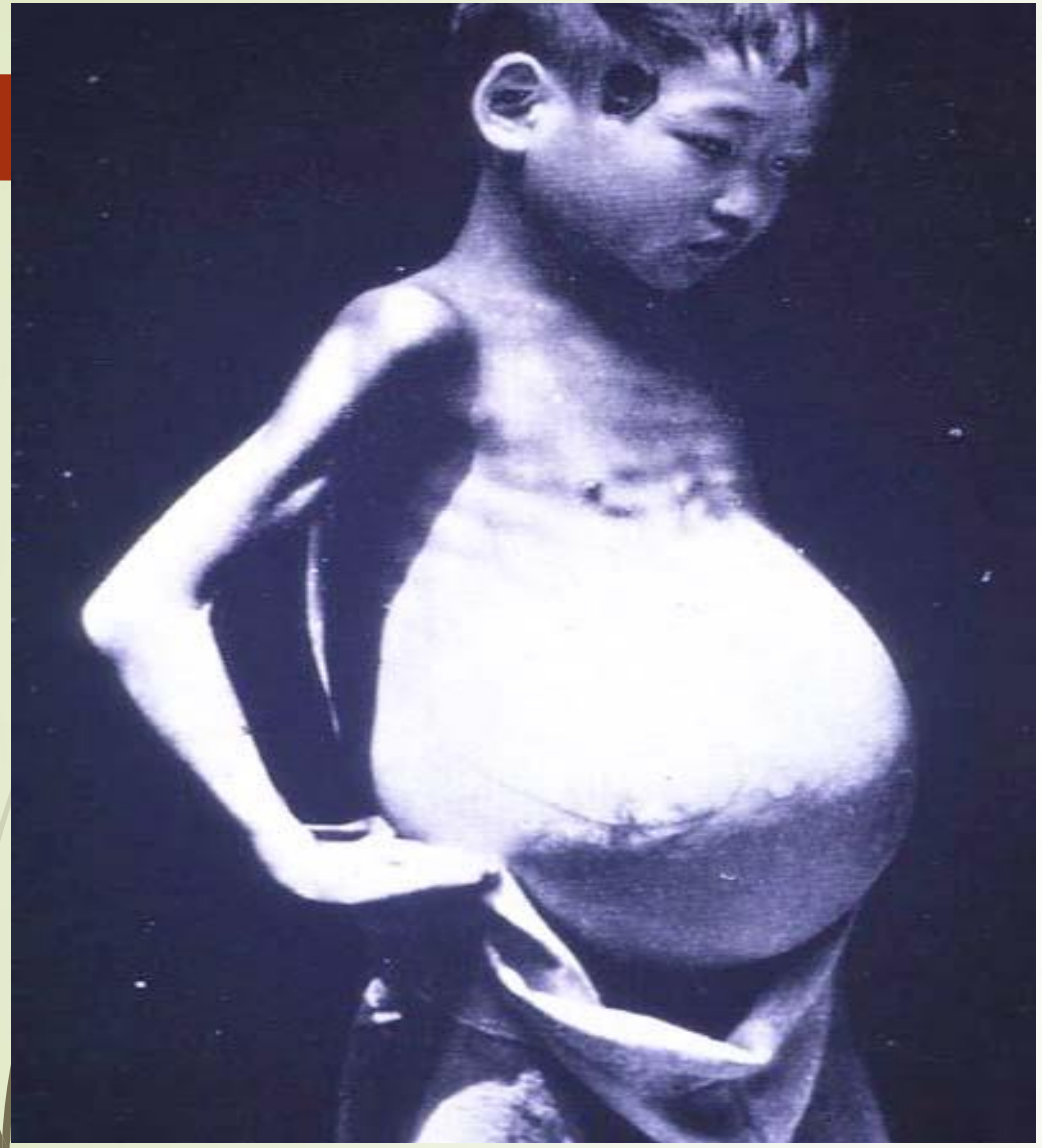
- **Diseases linked to H_20 resultant poor personal hygiene**
- **Obviously more common in tropical, 3rd world countries where H_2O supplies may be scarce**
- **Intestinal and non-intestinal infections**
- **Intestinal: *Shigella* (dysentery); typhoid; cholera; *Campylobacter*; Giardia; Cryptosporidium; viruses**

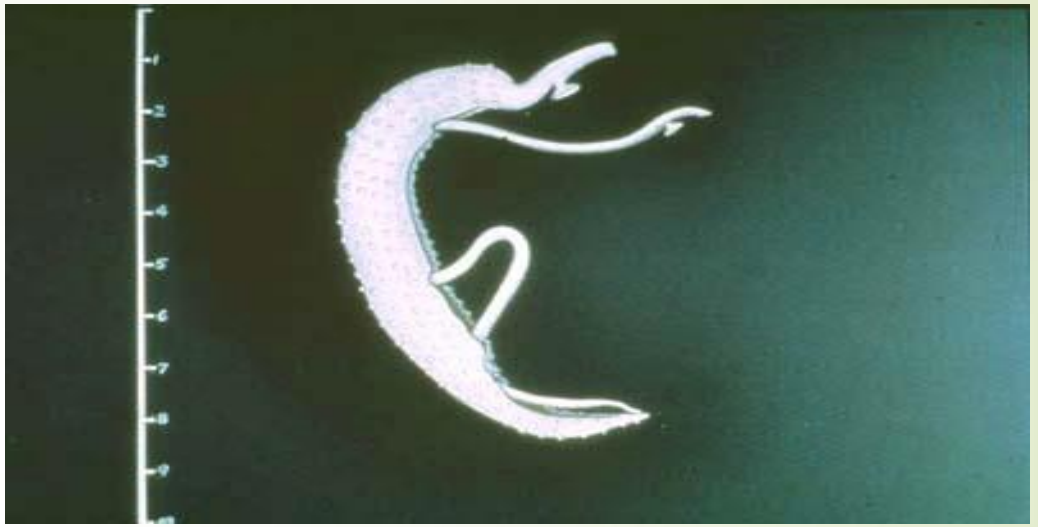
- **Non-intestinal: Infections of the skin and mucous membranes - bacterial skin sepsis; scabies; fungal infections such as ring-worm; fungal mouth ulcers**

3. Water-based diseases

- **Diseases caused by pathogens that have a complex life-cycle which involves an intermediate aquatic host**

- **All of these diseases are caused by worms, e.g. Schistosomiasis caused by the Schistosoma worm which uses aquatic snails as an intermediate host, also the Guinea worm (*Dracunculus medimensis*) which uses a small crustacean as an intermediate host**





<u>Schistosomiasis</u> affects 200 million people worldwide per annum

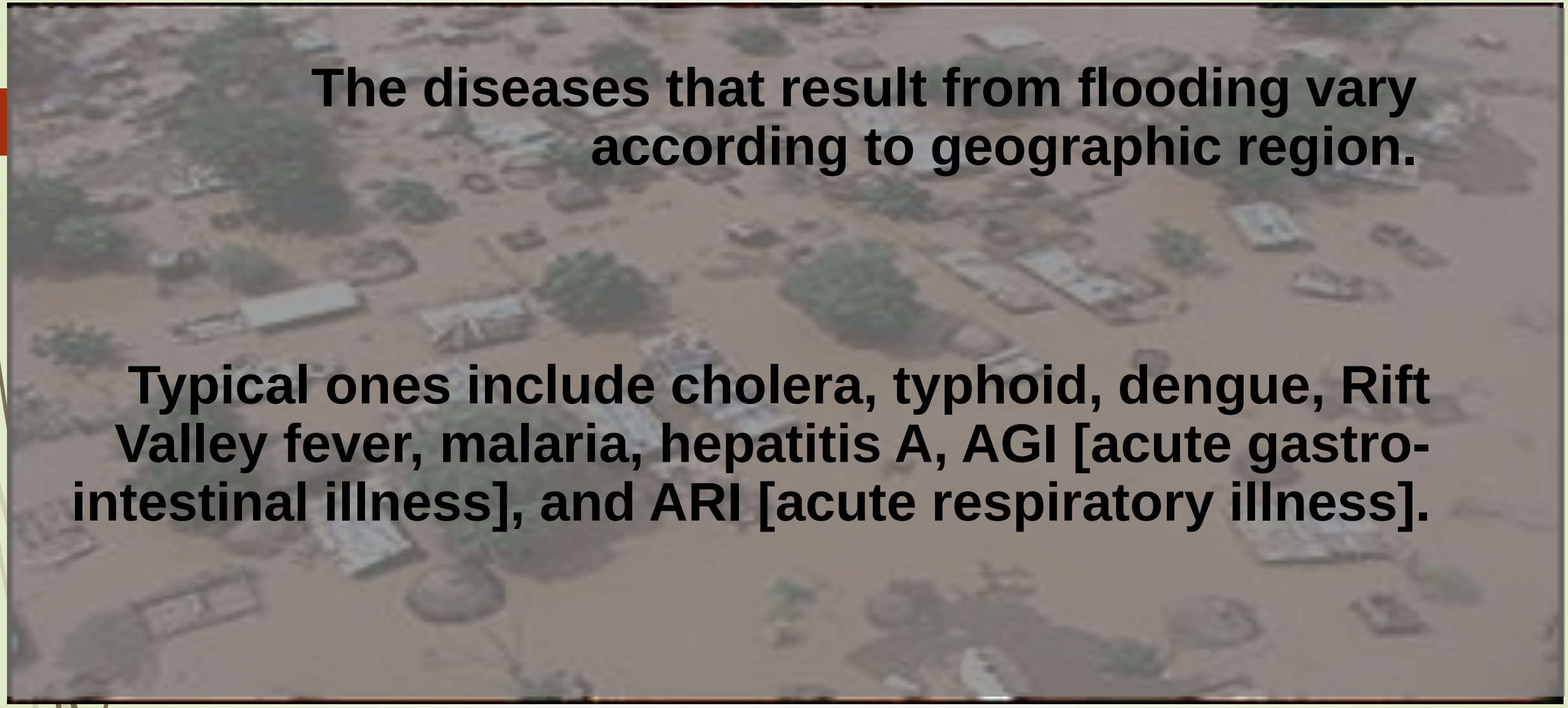
4. Water-related diseases

Diseases caused by pathogens carried by insects that live near H_2O and act as mechanical vectors

Very difficult to control and diseases are very severe

Examples:

- **Yellow fever (viral disease) is transmitted by the mosquito *Aedes* spp.;**
- **Dengue (viral) carried by the mosquito *Aedes aegypti* (breeds in water);**
- **Malaria is caused by a protozoan (*Plasmodium* spp.) and is also spread by a mosquito (*Anopheles* spp.);**
- **Trypanosomiasis (Gambian sleeping sickness) is also caused by a protozoan transmitted by the riverine Tetse fly (*Glossina* spp.)**



The diseases that result from flooding vary according to geographic region.

Typical ones include cholera, typhoid, dengue, Rift Valley fever, malaria, hepatitis A, AGI [acute gastro-intestinal illness], and ARI [acute respiratory illness].

Flooding

Problems involved in getting clean, safe water to people in the developing world

- **Water supplies in communities highly susceptible to municipal, agricultural, and industrial contamination.**

Standing water is a major problem in malaria and other vector-borne diseases.

Cholera, giardiasis, hepatitis, shigellosis, typhoid, and AGI

Absence of Sanitary Infrastructure

Often in the developing world gastroenteritis and other infections cause unnecessary mortality

HOWEVER, NOT JUST A PROBLEM OF THE DEVELOPING WORLD

WBD's in a developed world context

- Growing problem in Ireland primarily due to deterioration of ground and surface water quality
- Massive volume of wastes produced in intensive agriculture can contaminate a water supply if not managed correctly

General causes of WBDOs include:

1) No treatment

2) Breakthrough at treatment plant

3) No disinfectant residual

4) Direct sewage contamination through pipe leakage, breakage, back-siphoning, and cross-connections

Where the problems arise

Corrosion of pipe networks allowing contamination during distribution

Biofilm ***formation leads to:***

- **biofouling;**
- **foul odour, smell, colour, and the general impression of "dirty water";**
- **biocorrosion;**
- **disinfection survival and proliferation of pathogens;**
- **resistance;**
- **transfer of antibiotic and virulence factors**

Problems with Microbiological Monitoring

Current indicator organisms may not be adequate for the following reasons:

The presence of coliforms in water only reflects sewage contamination - not potential pathogens like *Legionella*

Coliform behaviour and die-off is not comparable to the behaviour of viruses and protozoa

Die-off rates of faecal coliforms have been demonstrated to vary enormously

Problems with Microbiological Methods (cont'd)

Techniques used to identify indicators rely on growth and culture - many organisms can be viable in the environment but unculturable using current methods ('Plate-count anomaly')

Molecular methods based on DNA probes and PCR still not adequate

No detection methods for these organisms at the moment

Use of conventional indicators meaningless

How many of the 350,000 cases of food/water borne illnesses in Ireland last year caused by these organisms??

Detection of m.o. in water

- Indicator and index m.o.
  - Coliform
  - faecal coliforms
  - *E. coli*
- Detection techniques
  - Multiple tube fermentation or MPN method
  - Membrane filtration method

Bacteria found in water

- Natural aquatic bacteria
  - mostly are Gram-negative bacteria e.g. *Pseudomonas*, *Acinetobacterium*, etc.
- Soil-dwelling bacteria
  - Enterobacteriaceae (e.g. *Enterobacter*), *Streptomyces*, *Bacillus*
- Intestinal m.o.
  - Coliform, *E. coli*, Faecal Streptococci

Introduction

Bacterial pathogens

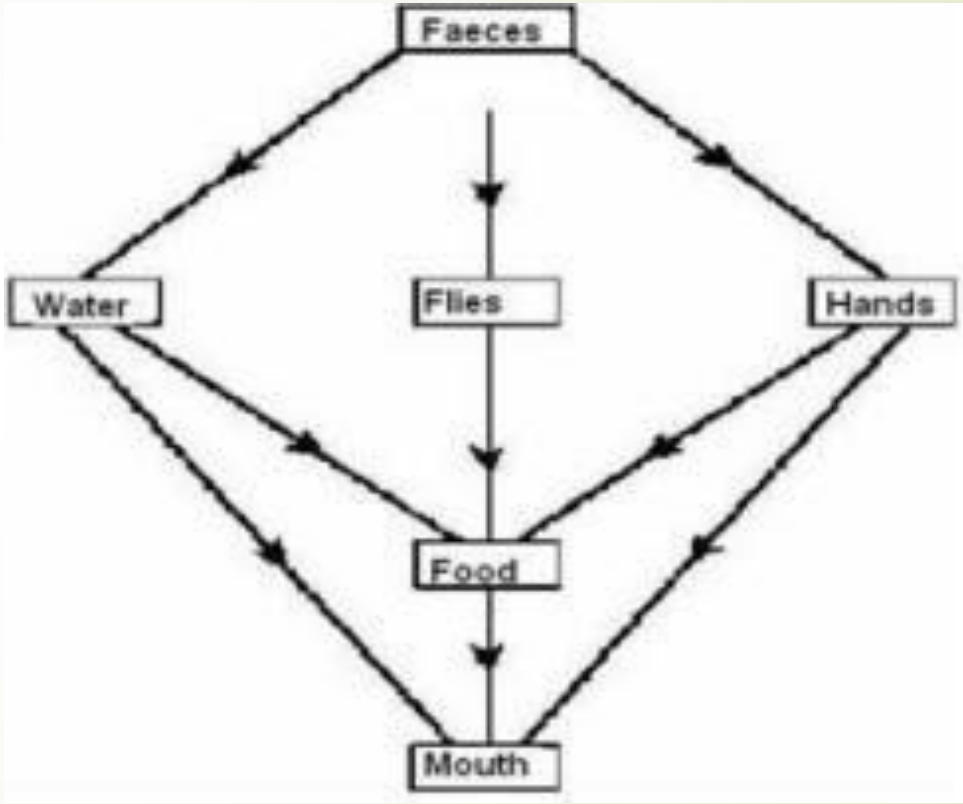
-may cause water-borne diseases such as Shigellosis, Malaria, Campylobacteriosis, Cholera, Giardiasis, etc.(bacteria ,parasite)

Prevalence:

In developing countries, 4/5 of all the illnesses are caused by these, with diarrhea being the leading cause of

Water Borne Diseases

- Water-borne diseases are any illness caused by drinking water contaminated by human or animal faeces, which contain pathogenic microorganisms.
- The germs in the faeces can cause the diseases by even slight contact and transfer.



Water Borne Diseases

- **Bacterial infections**
- Botulism - Clostridium botulinum bacteria - gastro-intestinal food/water borne
- Campylobacteriosis
- Cholera - Vibrio cholerae bacteria - gastro-intestinal often waterborne
- Chronic granulomatous disease - caused by the Mycobacterium marinum infection and localized in skin, frequently occurred with aquarium keepers.[3]
- Diarrheal disease due to *E. coli*.

Water Borne Diseases

- Dysentery - Shigella/Salmonella bacteria - gastro-intestinal food/water
- Legionellosis - cause Pontiac fever and Legionnaires' disease
- Leptospirosis
- Otitis externa- "Swimmer's Ear"
- Typhoid - Salmonella typhi bacteria - gastro-intestinal water/food borne. Salmonellosis - due to many Salmonella species. Water/food/direct contact borne.
- Vibrio illness caused by the bacteria of Vibrio vulnificus, Vibrio alginolyticus and Vibrio parahaemolyticus commonly found in seafood and recreational water.

Viral Sources of Waterborne Disease

- Hepatitis A: inflammation and necrosis of liver
- Norwalk-type virus: acute gastroenteritis
- Rotaviruses: acute gastroenteritis, especially in children
- Enteroviruses: many types affect intestines and upper respiratory tract
- Reoviruses: infects intestines and upper respiratory tract

Objectives

1. To perform the Most Probable Number (MPN) Technique for testing the potability of different water samples.
2. To interpret results of water analyses using the MPN table.
3. To be familiar with common water-borne diseases and their causative reagents.

Problems when testing water

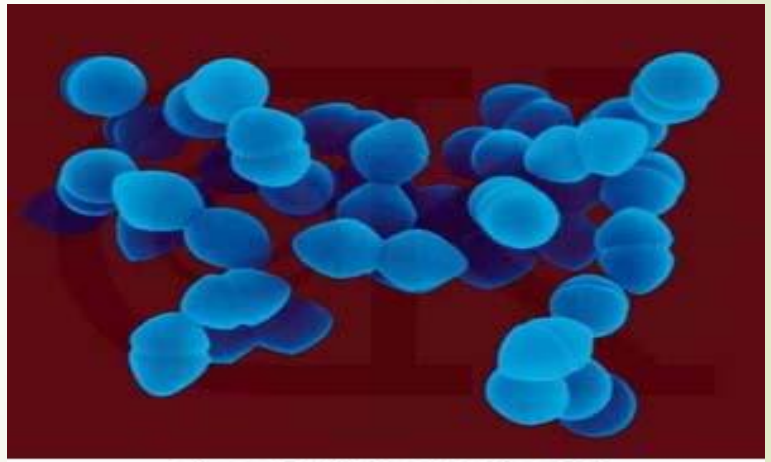
- Numerous water borne pathogens
- Individual pathogen numbers may be too low to detect in a reasonable sized water sample
- Isolation and detection of some pathogens can take several days, weeks, or months
- Absence of one particular pathogen does not rule out the presence of another.

Indicator Organism Concept

- Widely used in determination and estimation of water contamination correlated to the presence of pathogens
  .
- **Why used:**
- Population large enough to isolate in small water samples (100 mL)
- Rapid
- Inexpensive
- Safe, not culturing pathogens

Bacterial-Indicator Organisms Common Groups

- Coliforms(recent fecal contamination)
  - Total coliforms
  - Fecal coliforms
  - *Escherichia coli*
- Streptococci
  - *fecal streptococci*
  - *Enterococci*
- Spore Formers(indication of old fecal contamination)
  - *Clostridium perfringens*



Characteristics of a Useful Indicator

- Useful for all water types
- 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
- Survive longer than the pathogen
- Not dangerous to work with

Coliforms

- Coliforms- refers to the various genera of the family *Enterobacteriaceae* which are lactose fermentors and are commonly found contaminants in water.
- Organisms that are under the genus *Escherichia, Enterobacter*, *Klebsiella*, *Serratia*, *Citrobacter*.
- Collectively, this group of Gram-negative bacilli are referred to as "coliforms" because they share similar morphological and biochemical characteristics.

Coliform Group (total coliform)

- *Enterobacteriaceae*
  - Facultative anaerobe
  - Gram negative
  - Non-spore forming
  - Rod shaped
  - Ferment glucose
  - Produce gas and acid within 48 h at 35 C

- Coliforms genera
  - *Enterobacter*
  - *Klebsiella*
  - *Citrobacter*
  - *Escherichia*

- In addition to Ferment lactose

Coliforms

- Most of these organisms are members of the normal flora of humans and/or animals and are considered opportunistic pathogens.
- Most are found in the colon. Most of these organisms possess fimbriae that is used as appendages for adhesion purposes.

E.coli

- *E. coli* are found in intestine, their ability to survive for brief periods outside the body makes them an ideal indicator organism to test environmental samples for fecal contamination. Indicator organisms indicate that water received contamination of intestinal origin.

- *E. coli* are Gram negative bacterium that is commonly found in the lower intestine of warm-blooded animals,

  while other coliforms(*Enterobacter, Klebsiella)* can be found on plants and in soil.

Multiple Tube Fermentation Technique to determine Most Probable number

- uses a specified number of test tubes to statistically predict the number of organisms present (based on the expected population of organisms in the sample)
- tubes may also contain an inverted inner vial (Durham tube) for gas collection
- ideal for wastewater samples and non-potable samples, because the analyst can accommodate highly turbid samples by diluting prior to analysis

Multiple Tube Fermentation Methods

- Specific dilution is made
- Inoculate multiple tubes (3 or 5) of media with water sample
- Incubate
  - 35 C or
  - 44.5 C
- Count positive growth tubes
- Use Most-Probable-Number (MPN) table to estimate density

Methodology

Sampling for Tap Water Samples

1. • Clean the tap. Let water flow for 1-2 minutes. Sterilize tap with flame and collect sample
2. • Analyze water samples not more than 6hours after sampling or 24hours if chilled.
3. • Multiple Tube Fermentation Technique

Multiple Tube Fermentation Technique

Presumptive Test

Confirmatory Test

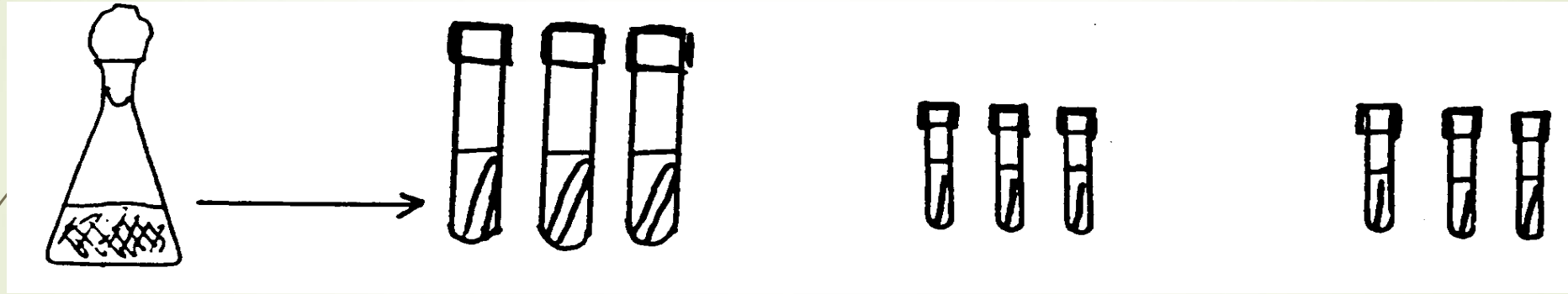
Completed Test

Presumptive Test

1. **Prepare and sterilize 3 Ds Mac broth(10ml) with Durham tubes, 6 Ss Mac broth (5ml) with Durham tubes**
2. **Inoculation: first 3 tubes with 10ml of the original sample, next 3 with 1ml of the of the original sample, next 3with 0.1ml of the original sample.**
3. **Incubation for 35-37 ˚C for 48 hours. Observe for gas production, turbidity, and change in color to yellow. If positive proceed to Confirmatory Test.**

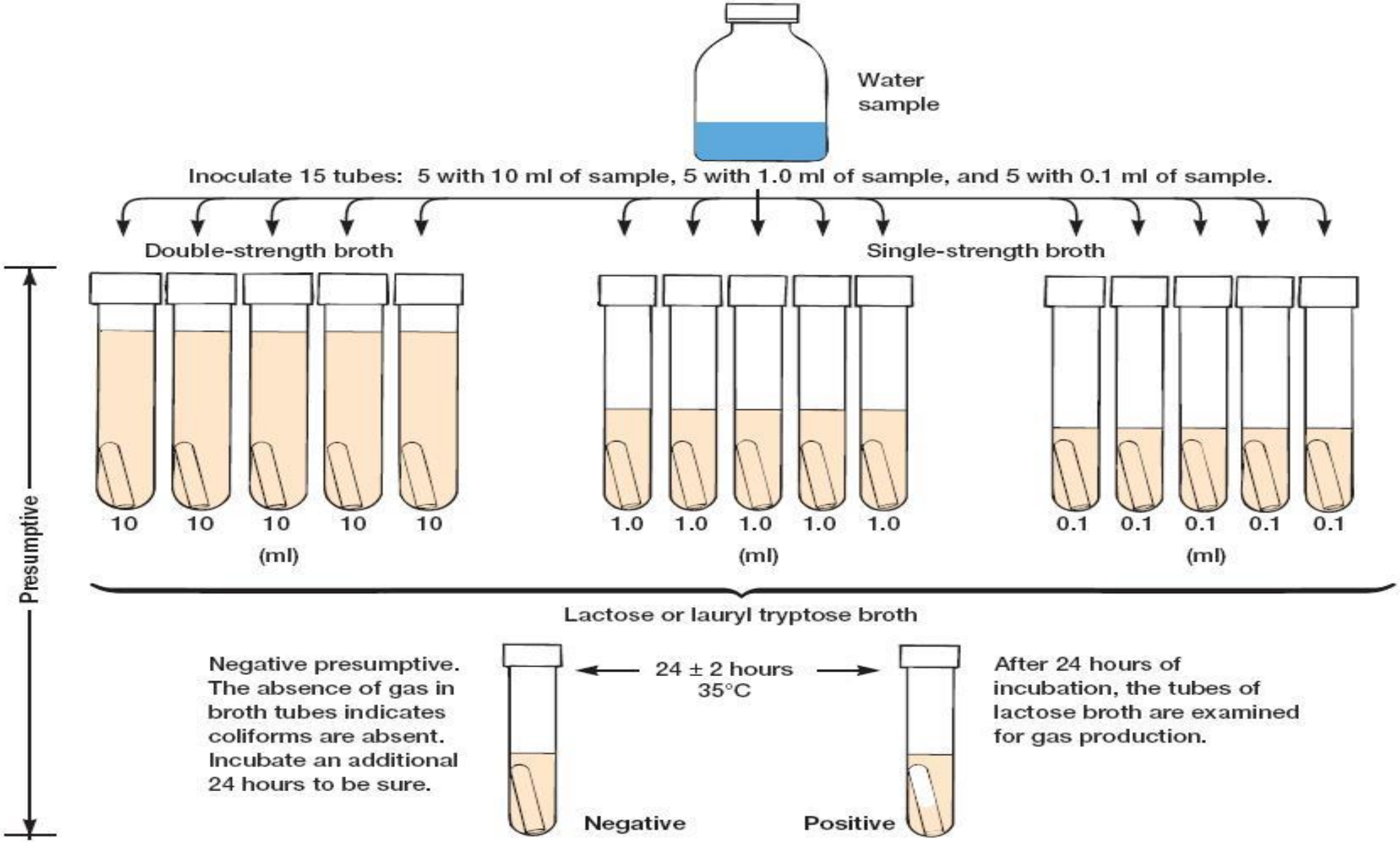
Bacteriological analysis of water:
Most Probable Number (MPN) technique

coliform: acid and gas from lactose <24 hours/37^{0}C
indicator organism: *E. coli*

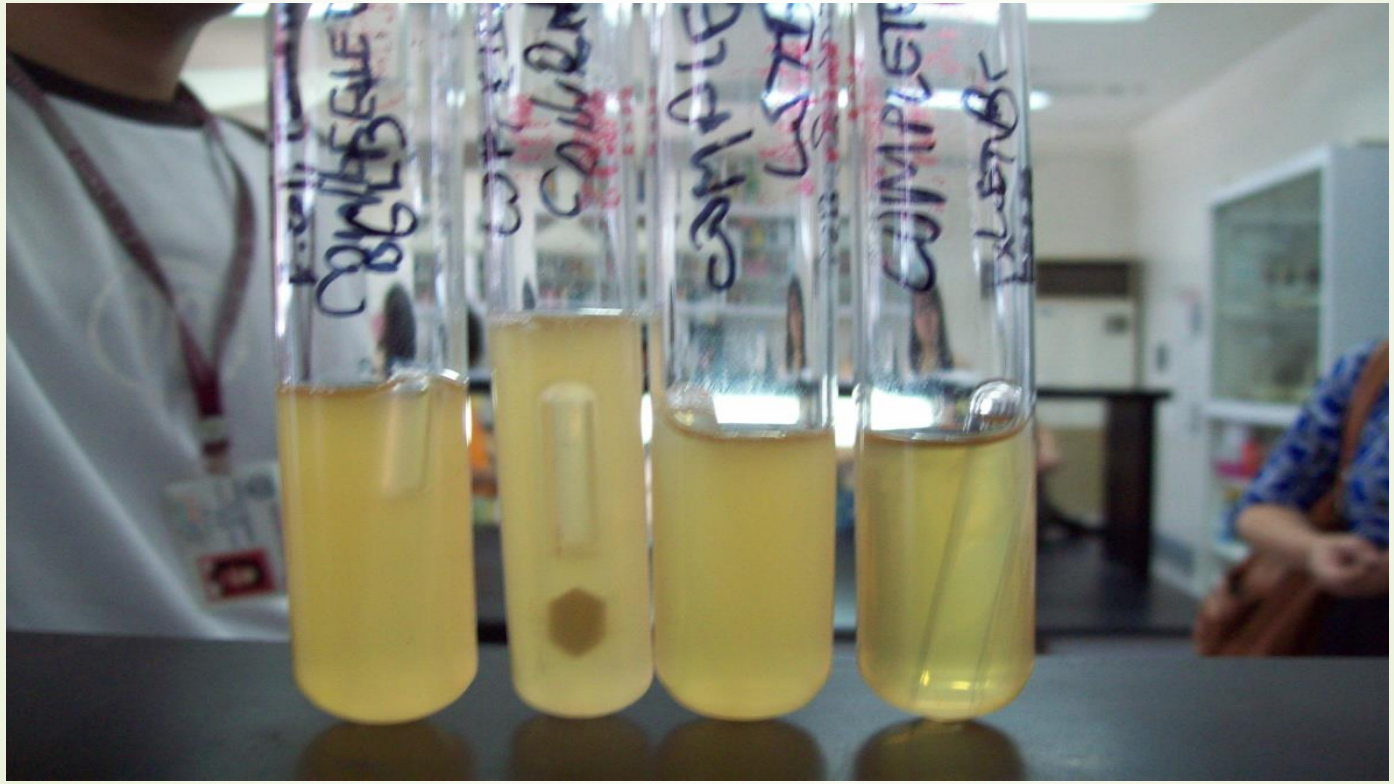


Water sample	double strength	single strength	single strength
	10 ml sample	1.0 ml sample	0.1 ml sample

*Normally 5 Durham tubes are inoculated but this exercise is modified to three tubes in the interest of economy.


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.
24 ± 2 hours
35°C
Negative
Positive
After 24 hours of incubation, the tubes of lactose broth are examined for gas production.

Presumptive test



Confirmatory Test

1

- BGLB tubes (10ml) with Durham tubes were prepared and sterilized.
- Loopful of suspension from the positive presumptive tubes was inoculated and incubated at 35°C for 48hours.

2

- EMB plate is inoculated and incubated at 35C
- Loopful of suspension was inoculated and incubated at 44°C for 24 hours.
- Sometimes an IMViC reaction is inoculated.

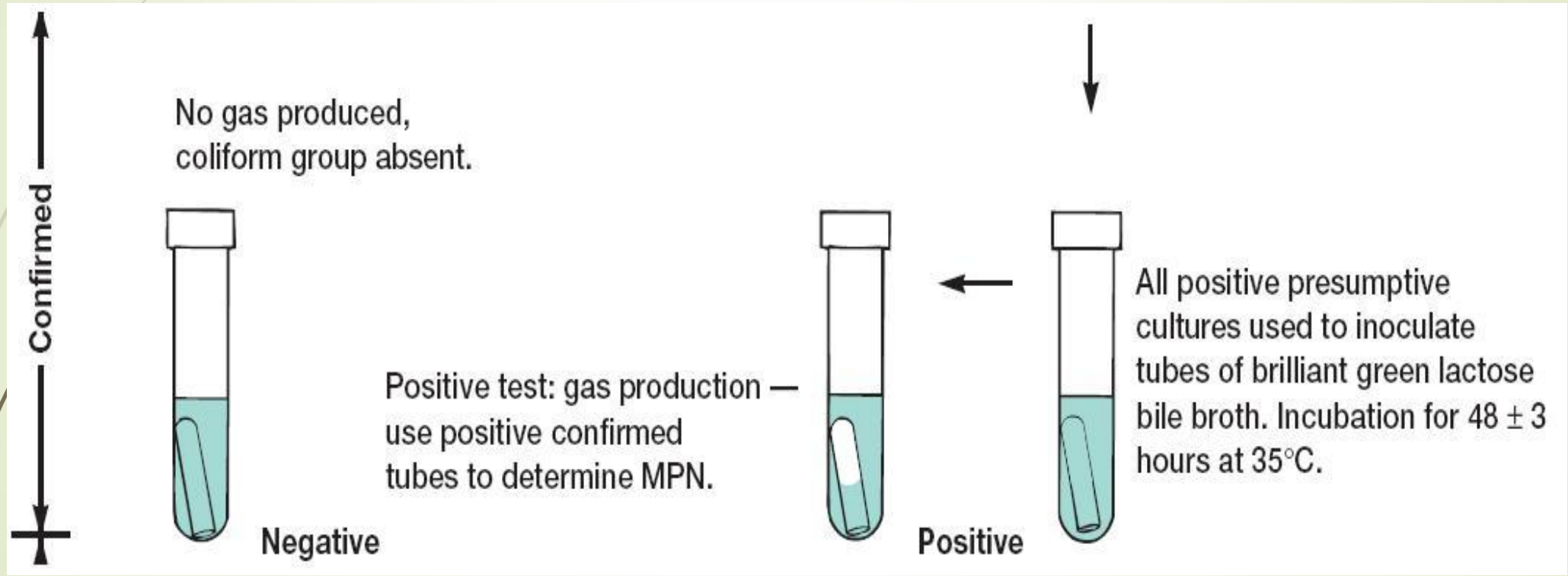
3

- BGBL : Turbidity and gas production is observed.
- EMB: Green metallic sheen growth is observed on the plate.
- IMViC reaction : ++--
- Those positive for the confirmatory test were subjected to the completed test.

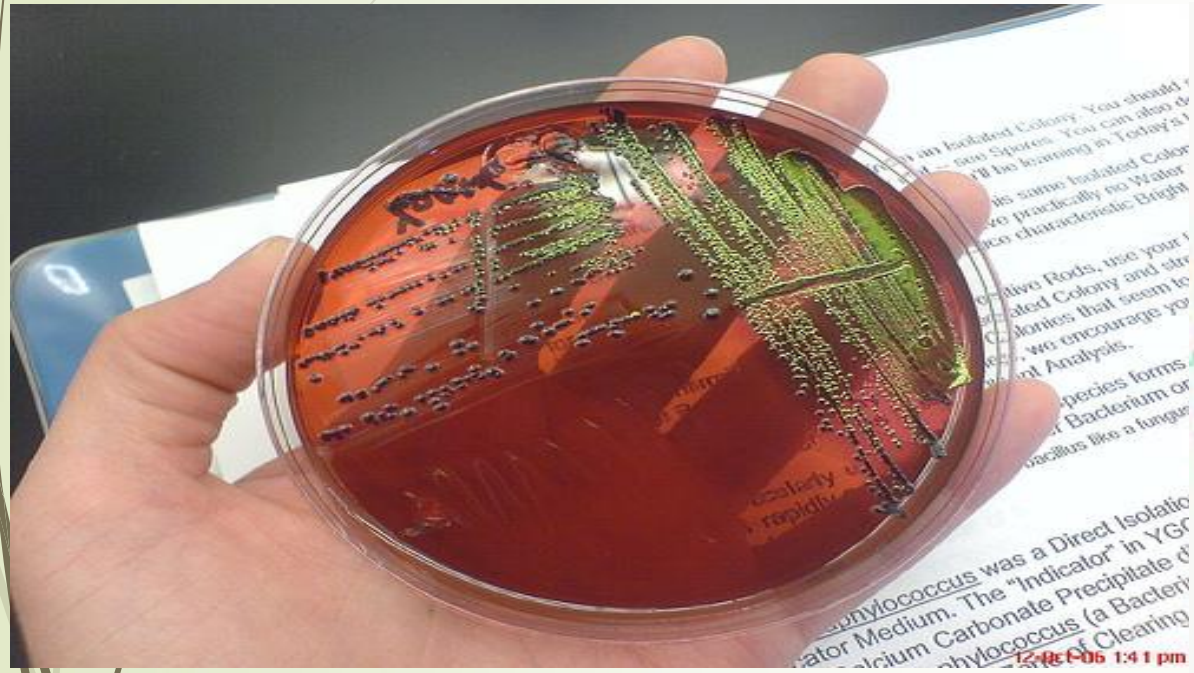
BGLB components

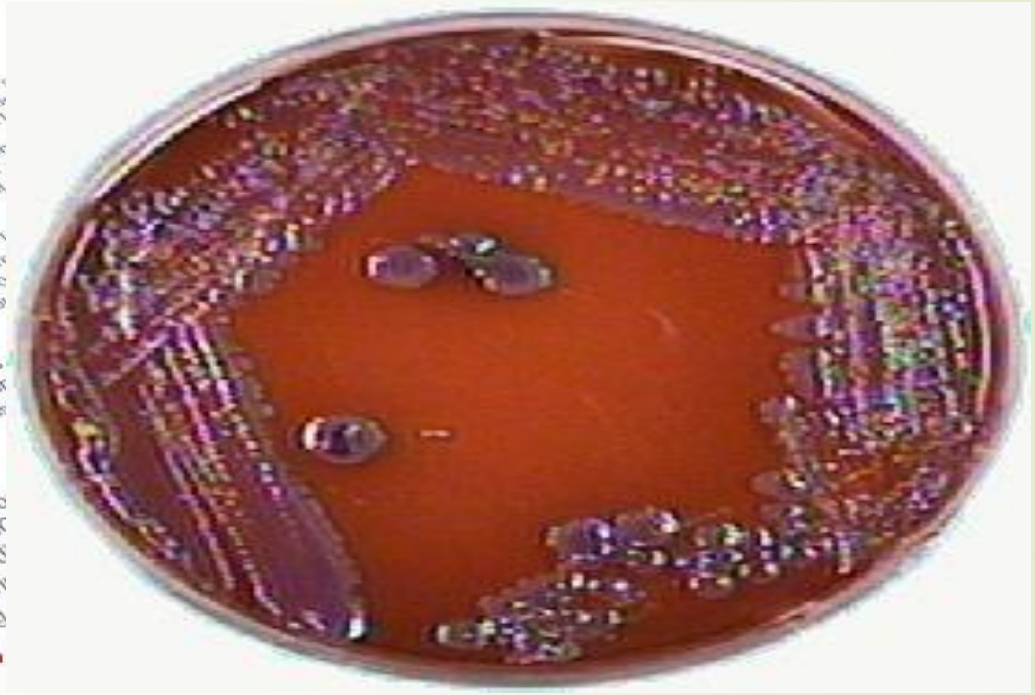
- Peptone: a source of nitrogen, vitamins and minerals.
- Lactose: fermentable carbon source
- Oxgall (bile) and brilliant green: inhibitor of gram-positive bacteria and most gram-negative bacteria except coliforms
- Basic fuchsin and erioglaucine: pH indicators
- Monopotassium phosphate: buffering agent.

BGBL medium


Confirmed
No gas produced,
coliform group absent.
Positive test: gas production —
use positive confirmed
tubes to determine MPN.
All positive presumptive
cultures used to inoculate
tubes of brilliant green lactose
bile broth. Incubation for 48 ± 3
hours at 35°C.
Negative
Positive

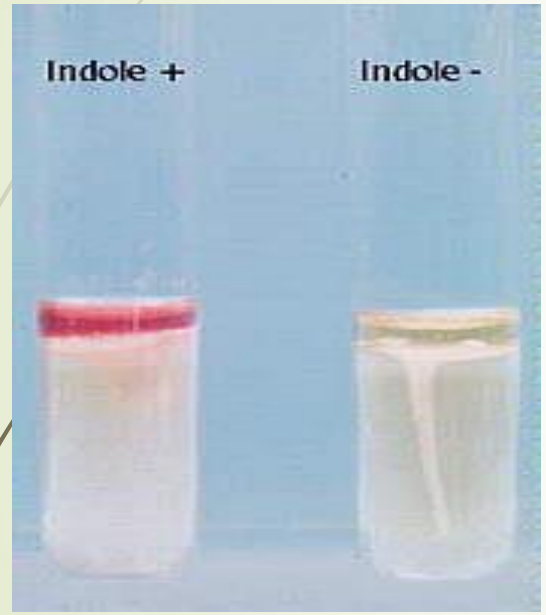
Confirmatory Test

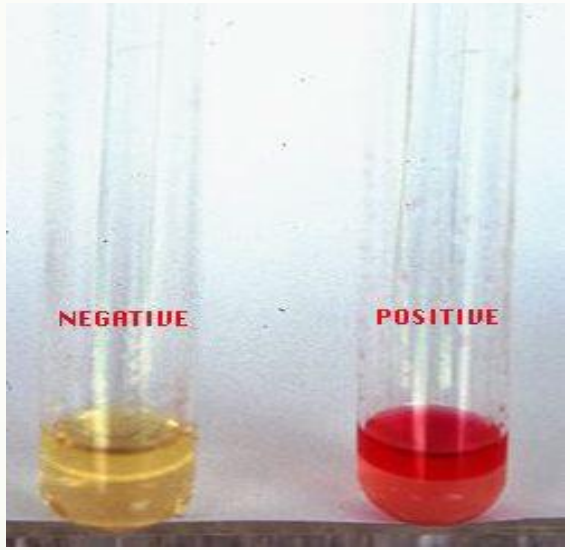


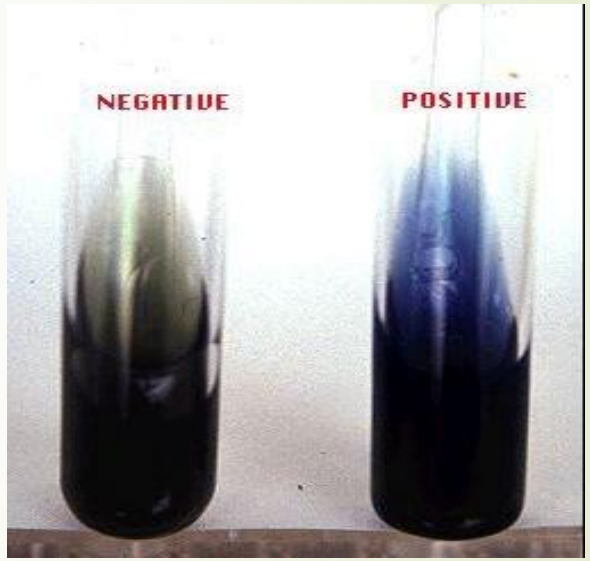


E. coli vs E. aerogenes

IMViC Test







Indole MR- VP Citrate

MPN Table

10mL tubes Positive	1-mL tubes positive	0.1 mL tubes positive	MPN/100 mL
0	0	0	0
0	0	1	2
0	0	2	4
0	1	0	2
0	1	1	4
0	1	2	6
0	2	0	4
0	2	1	6
0	3	0	6
1	0	0	2
1	0	1	4
1	0	2	6
1	0	3	8
1	1	0	4
1	1	1	6

Completed Test

1. • **Representative colonies were chosen,**
   • **inoculated onto Lactose broth , and incubated for 35°C for 24hours. (Look for gas production)**
2. • **Perform gram-staining**
3. • **Culture on NA slant**

Reading results

- Presumptive test:
- Any + ve tube is given a value of 1
- Any –ve tube is given a value of 0
- Add result of 3 tubes of 10ml Ds Mac broth ,then 3 tubes of 1ml Ss Mac broth, then 3 tubes of 0.1 ml Ss Mac broth
- Get value of 3 Numbers e.g. 1 0 3 look it in MPN table ,the index is 8 coliforms /100ml of water.
- If confirmed ,then it should be reported.

Directions for collection

- Bottle is pre-sterilized. Do not open or remove cap or touch inside of bottle.
- Do not rinse bottle .It contains sodium thiosulfate to neutralize the bactericidal effect of chlorine.
- When collecting tap or well water ,allow water to run ,or pumped out for several min.
- This is to provide a representative sample from source

Directions for collection

- Lake , river etc. select a good place to obtain sample , and extend bottle away from body.
- Best volume is 100ml , 50 ml is OK.
- The sample should be tested as soon as possible .If not possible it should be refrigerated until testing.

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	Number of positive tubes in dilutions			MPN per
10 ml	1 ml	0.1 ml	100 ml	10 ml	1 ml	0.1 ml	100 ml
0	0	0		2	0	0	9.1
0	1	0	3	2	0	1	14
0	0	2	6	2	0	2	20
0	0	3	9	2	0	3	26
0	1	0	3	2	1	0	15
0	1	1	6.1	2	1	1	20
0	1	2	9.2	2	1	2	27
0	1	3	12	2	1	3	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				

Typical Water Quality Standards

- Water standards when coliforms are used as pollution indicator
- Drinking Water and swimming pool water
  - No coliforms contamination acceptable less than 3 coliforms /100ml of sample
- Recreational water
  - 100- 200 fecal coliforms /100 ml
- Fish and wildlife habitat
  - 5000 fecal coliforms/100 ml

False +ve and –ve

- **False positive:**
- *Enterobacter aerogenes - a soil* organism; use the IMViC test for Distinction

- **False negative:**
- *A case of Salmonella typhi in* Riverside in 1965 in USA

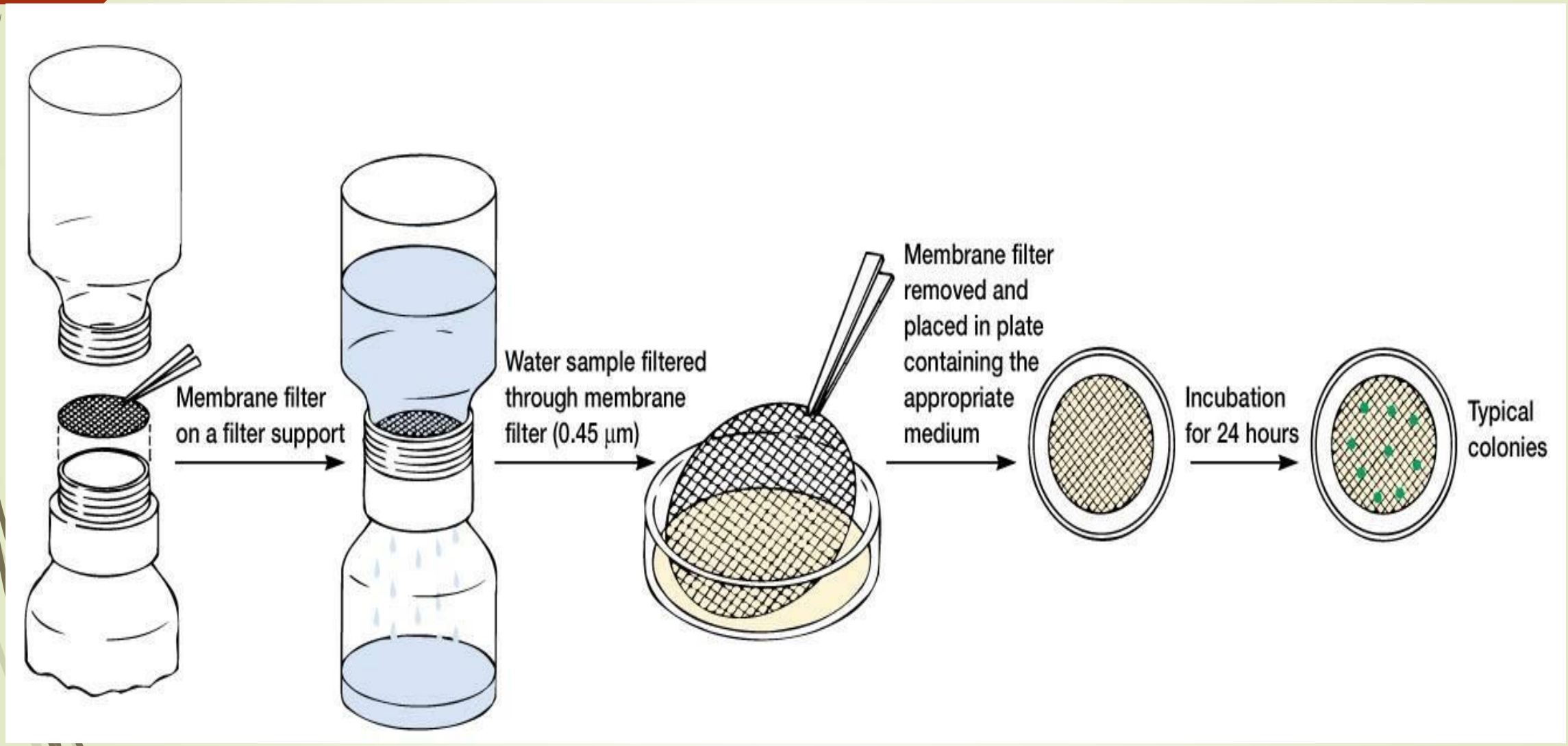
Water Microbiology II

Membrane filtration method

Membrane Filter Methods

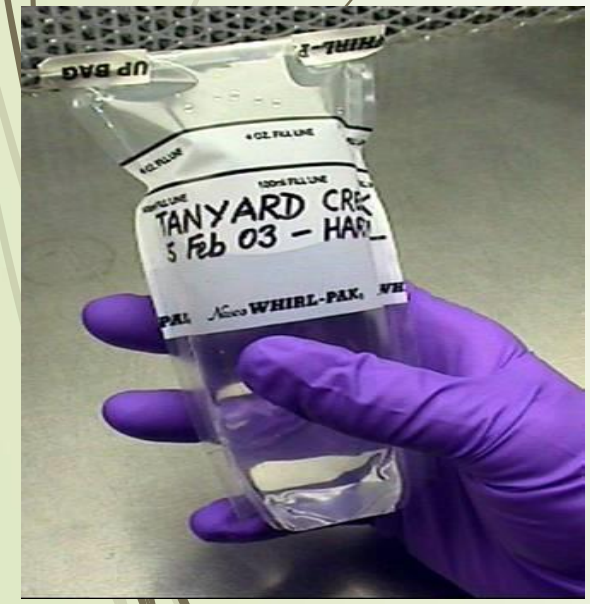
- Filter water through a 0.45 or 047 µm membrane filter(Bacterial cells can not pass through)
- Place membrane on selective media(EMB)
- Incubate
  - 35 C total Coliform
  - 44.5 C fecal Coliform
- Count colonies

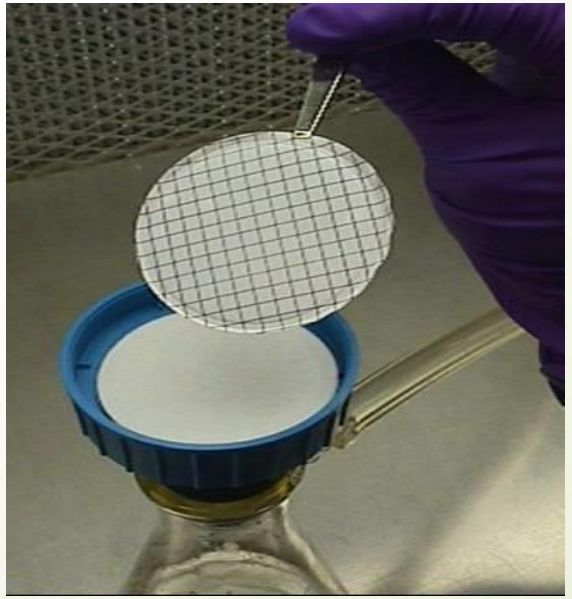
MF method

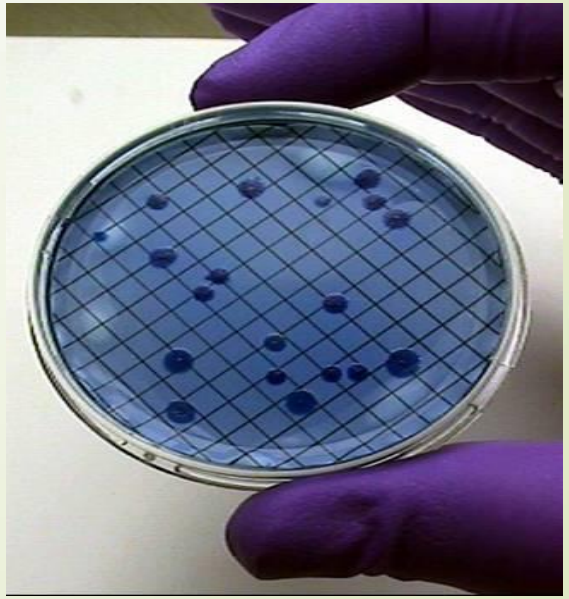

Membrane filter
on a filter support
Water sample filtered
through membrane
filter (0.45 μm)
Membrane filter
removed and
placed in plate
containing the
appropriate
medium
Incubation
for 24 hours
Typical
colonies

MF method

Filter water through a 0.45 µm membrane filter
Place membrane on selective media
Incubate
    35 C total Coliform
    44.5 C fecal Coliform
Count colonies

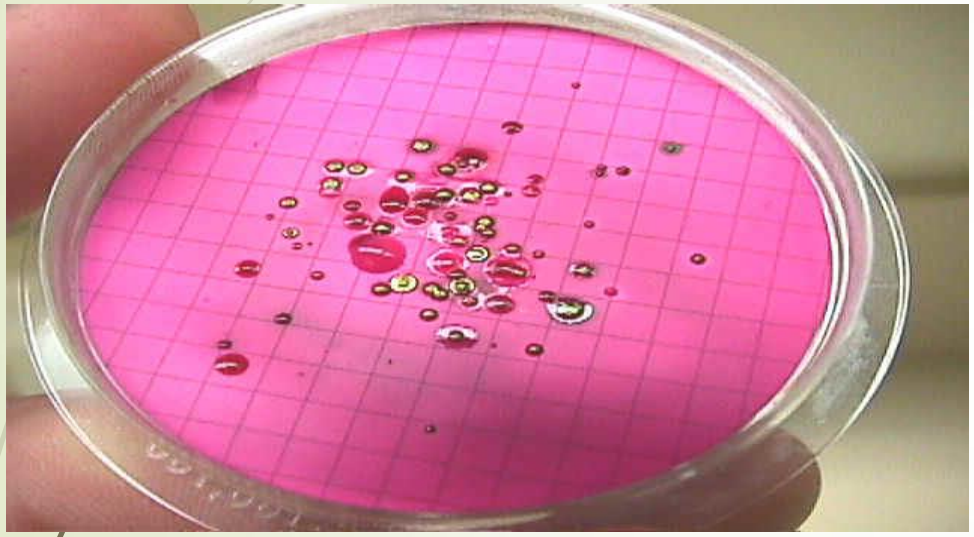


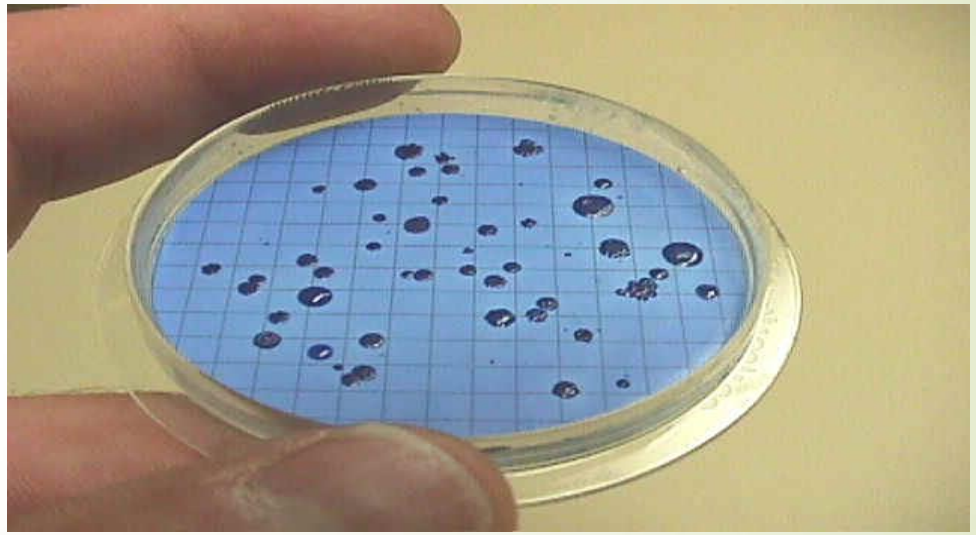




Membrane filtration method

Results:





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

RESULTS

- **Quality limit**: less than 1 Coliform /100ml of water , the water is potable.
- **Action limit**: 4 coliforms/100ml of water, it means that the water company must take immediate action to remedy the problems that are responsible for the presence of coliforms in water.

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

Disadvantages

MPN test

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

M F method

- **Not suitable for turbid or waste waters.**

MPN Advantages

- It is relatively simple and cheap.
- It is the method of choice for determining fecal coliforms densities and timing of contamination
- ideal for wastewater samples and non-potable samples, because the analyst can accommodate highly turbid samples by diluting prior to analysis