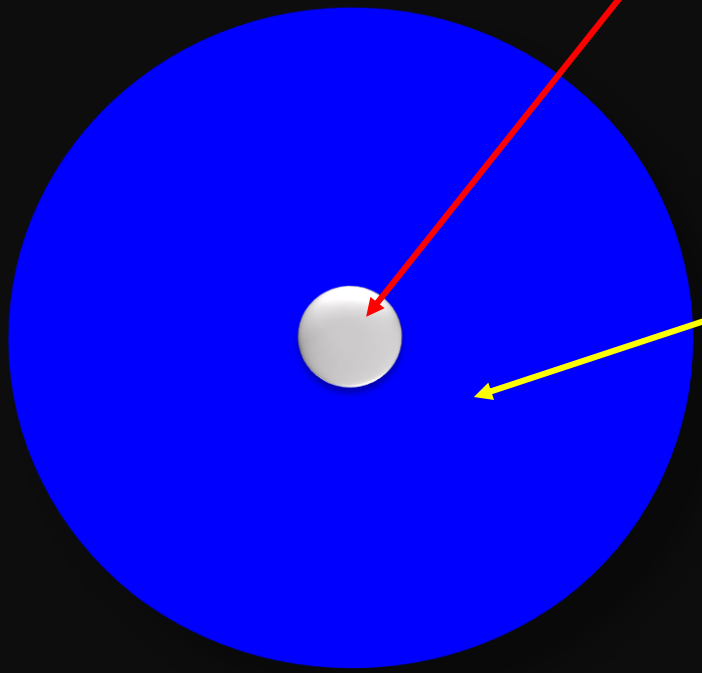
Advanced Food Microbiology

“Engineering is the art of modeling we do not wholly understand, into shapes we cannot precisely analyze, so as withstand forces we cannot properly asses, in such a way that the public at large has not reason to support the extent of our ignorance”



Laboratory Module A:
Experiment no.1: How to handle the instruments in microbiology lab.
Experiment no. 2: Different microbial culture media.
Experiment no. 3: Catalase , Oxidase and other common tests.

Laboratory Module B:
Experiment no. 4: Quantification of microbes using different selective media.
Experiment no. 5: Study on serial dilution in microbiology laboratory.
Experiment no. 6: Study on microbial colony morphology.
Experiment no. 7: Study on how to calculate colony forming unit(CFU).

Laboratory Module C:
Experiment no. 8: Quantification of Lactobacillus species in commercial yogurt.
Experiment no. 9: Quantification of Coliform bacteria in commercial fruit juice.
Experiment no. 10: Quantification of Salmonella species in commercial yogurt and fruit juice.

Laboratory Module D:
Experiment no. 11: Study on bacteria preservation and how to revive bacterial culture from glycerol stocks.
Experiment no. 12: Study on citrate utilization test using simmon citrate agar.

Food Microbiologist

Contributes to the knowledge about the behavior of microorganisms in food and processing environments

- New products/Brand maintenance
  - Conducts tests to verify shelf-life of new food products
- Research
  - Develops new and/or rapid testing methods
  - Studies “good” (those responsible for fermentation) and “bad” (those responsible for food borne illness) bacteria

Responsibilities

- Experimental design
- Perform, analyze, and report experimental results
- Troubleshooting

Case Study: Juice

Food microbiologists validate the pasteurization of juice to ensure pathogens such as E.coli 0157:H7 will not survive and cause foodborne illness.

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A Brief History

Early Food Preservation

900 AD – “Food Poisoning” Recognized

1795-Appert Developed Canning

1854-1864-FOOD MICROBIOLOGY BECOMES A SCIENCE

Louis Pasteur

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Why Study Food Microbiology?

Provide Clean, Safe, Healthful Food to Consumer

- Food Permits Growth
- Control of Microbial Growth

Prevent Food Spoilage

Prevent Food-borne Illnesses

Food Preservation and Production

Food-Borne Illness

ERS Estimates

- $6.9 Billion/Year Cost of FBI

CDC Estimates

- 76 Million Cases of FBI Annually
- 325,000 Hospitalizations
- 5,000 Deaths

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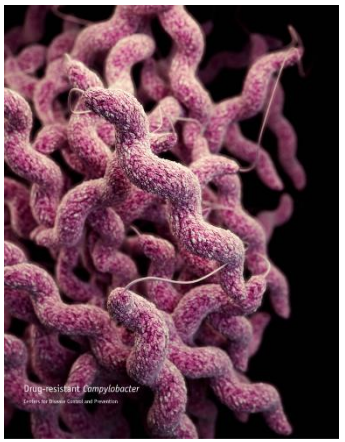
What Organism Causes the Most Cases of Food-Borne Illness Annually?

Campylobacter

Campylobacter is a bacterium found in the intestines of many types of animals and is the most common bacterial cause of diarrhoeal illness.



What Organism Causes the Most Deaths Due to Food-Borne Illness?

Salmonella

Salmonella infection (**salmonellosis**) is a common bacterial disease that affects the intestinal tract. **Salmonella** bacteria typically live in animal and human intestines and are shed through feces. Humans become infected most frequently through contaminated water or food.

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Review of Microbiology

Mostly Single Celled

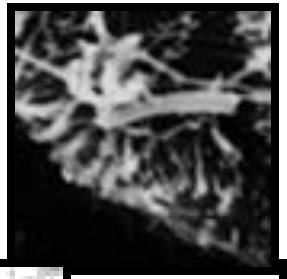
Groups

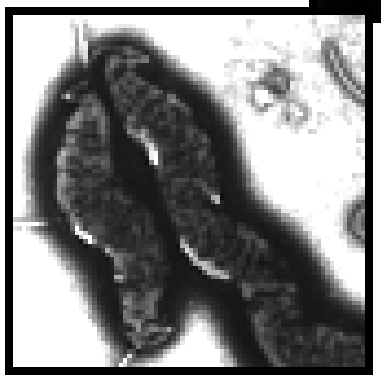
Morphologies

Gram Reactions

Size

Growth Rate





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The good, the bad and the ugly

Good-bacteria are important in food production

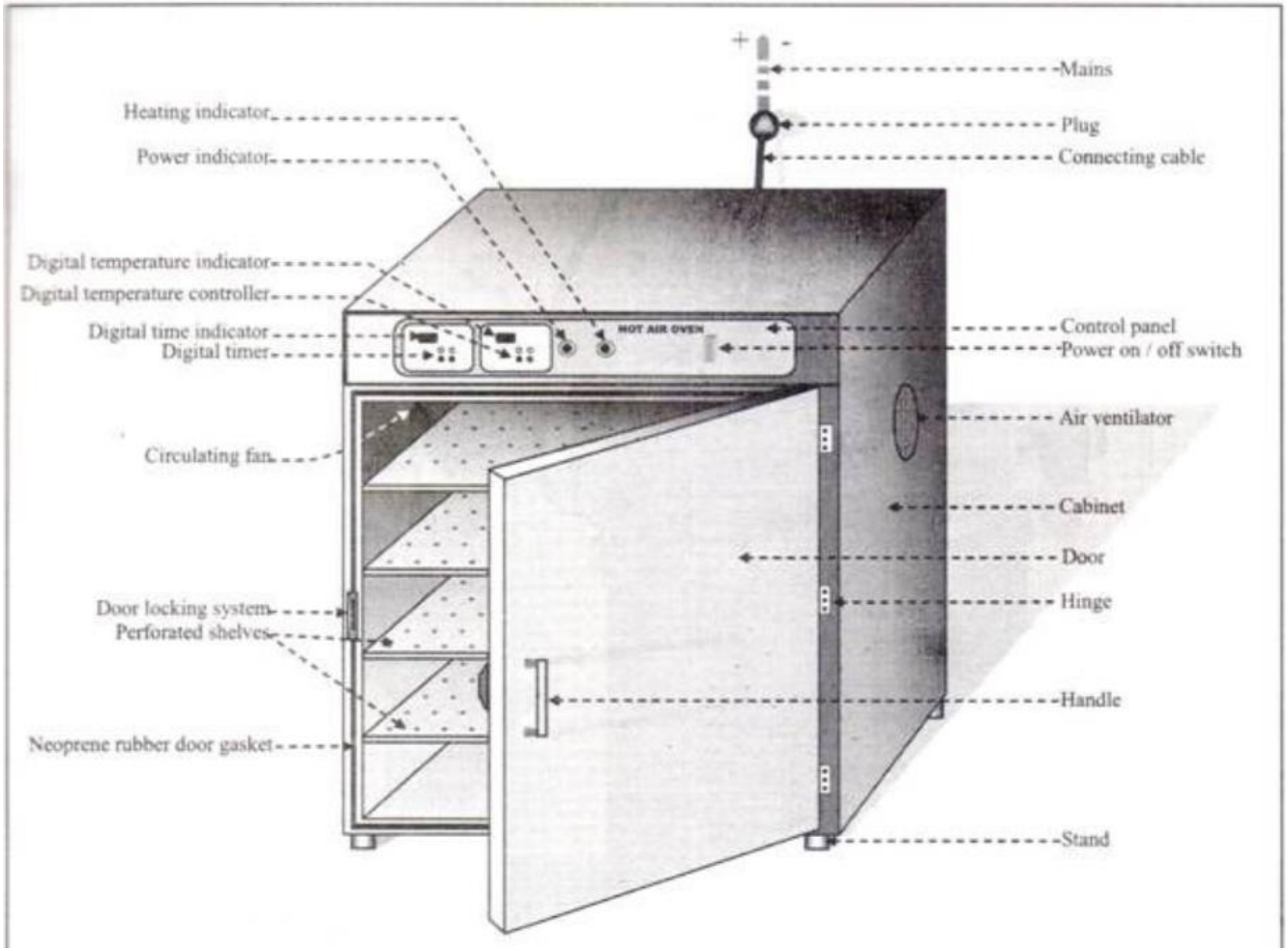
Bad-some bacteria cause food poisoning

Ugly-some bacteria cause food spoilage

Experiment no.1: How to handle the instruments in microbiology lab.
Experiment no. 2: Different microbial culture media.
Experiment no. 3: Catalase , Oxidase and other common tests.

1. Drying Oven:

For preparation of certain reagents, the glassware's, after proper cleaning and rinsing with distilled water, are required to be dried. They are dried inside the drying oven at 100°C till the glassware's dry up completely.



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Autoclaving is a sterilization method that uses high-pressure steam. The **autoclaving** process works by the concept that the boiling point of water (or steam) increases when it is under pressure.

An **autoclave** is a pressure chamber used to sterilize equipment and supplies by subjecting them to high pressure saturated steam at 121 °C for around 15–20 minutes depending on the size of the load and the contents.

Autoclave

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Is a device used to grow and maintain microbiological cultures.

The incubator maintains optimal temperature, humidity and other conditions such as the carbon dioxide (CO_2) and oxygen content of the atmosphere inside.

Incubator

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Centrifuge

- is an apparatus that rotates at high speed and separates substances of different densities.

Lab. refrigerator

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hot plate / stir plate

used to heat and stir substances.

Magnetic stirring bars

Balance

used to measure an object's mass to a very high degree of precision.

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Biological Safety Cabinets

is an enclosed, ventilated laboratory workspace for safely working with materials contaminated with pathogens.

Water bath

is a device that maintains water at a constant temperature.

It is used in the microbiological laboratory for incubations.

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Experiment no.1: How to handle the instruments in microbiology lab.
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Inoculating loops and needles

Inoculating loops are used to transfer microorganisms to growth media or for staining slides.

The wire forms a small loop with a diameter of about 5 mm.

The loop of wire at the tip may be made of platinum or nichrome.

Needles are straight wires (no loop) used to pick up bacteria from closely packed colonies or to inoculate in a very defined area.

needles commonly used to inoculate semi-soft media.

Bunsen burner

is a common piece of laboratory equipment that produces a single open gas flame, which is used for heating and sterilization.

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Anaerobic jar

is an instrument used in the production of an anaerobic environment.

This method of *anaerobiosis* is used to culture bacteria which die or fail to grow in presence of oxygen.

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Glassware

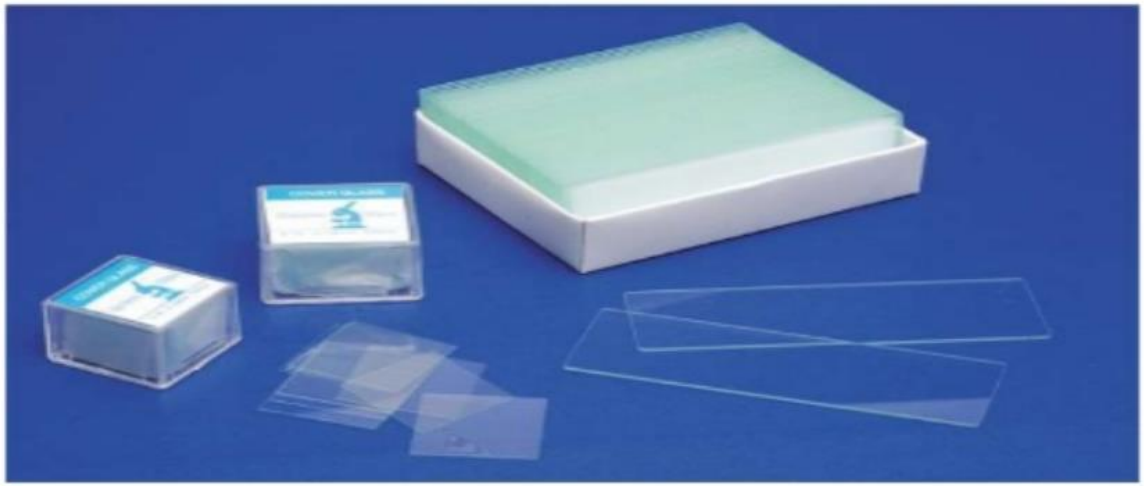
Glass slide and cover slip

- **Glass slide:**

used to place specimens on to observe under the microscope.

- **Cover slip:**

used to cover specimens on a microscope slide.



slide and coverslip

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Glassware

Petri dishes

often used to make **agar plates** for microbiology studies.

The dish is partially filled with warm liquid containing agar and a mixture of specific ingredients that may include:

nutrients

blood

salts

Carbohydrates

dyes

indicators

amino acids

or

antibiotics

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Graduated Cylinders

Pipet

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Glassware

Beaker

Glass or plastic

Used to stir, heat(if glass) and measure liquid volume in ml.

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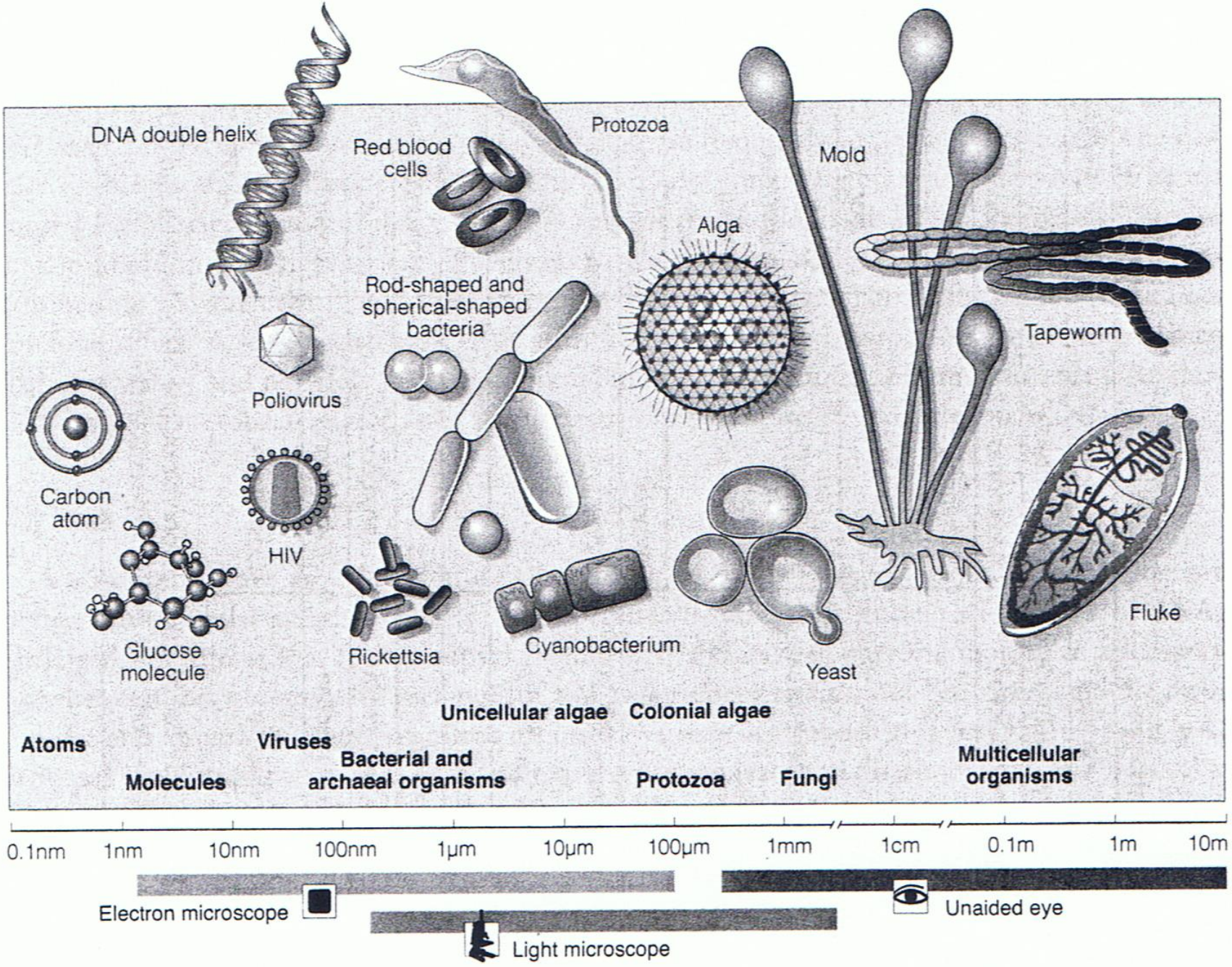
Buffled Flasks



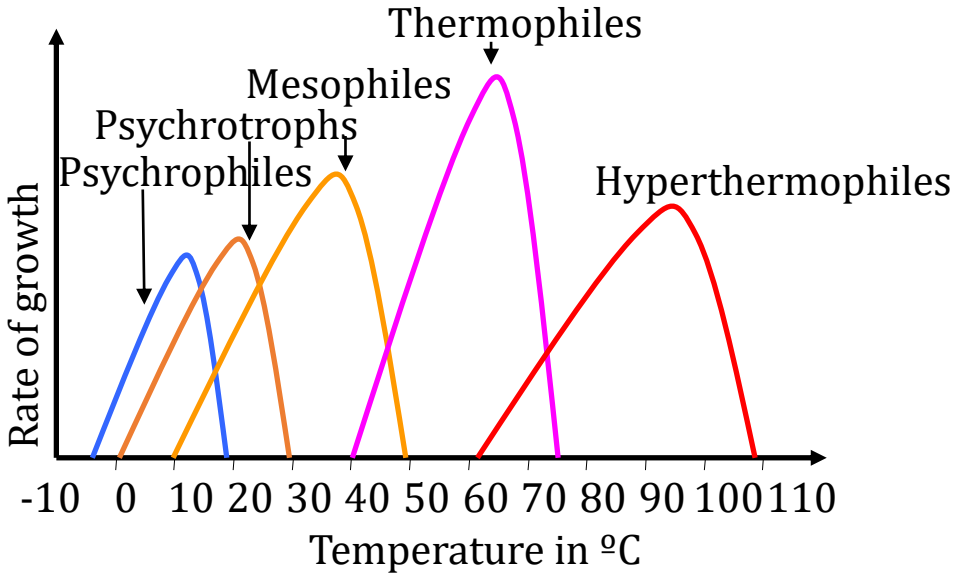
Growth requirements

Physical

- Temperature
- pH
- Osmotic pressure
- Moisture & desiccation

Chemical

- Carbon source
- Nitrogen, sulfur phosphorus
- Oxygen



Temperature

Most pathogenic bacteria are mesophiles
And grow optimally at 37 ^{0}C
(human body temperature)

Psychrophiles (cold loving)

- True psychrophiles
  (optimum growth at 15 ºC)
- Psychrotophs
  (optimum growth at 20-30 ºC)

Mesophiles (moderate temperature loving)

Thermophiles (heat loving)

Hyperthermophiles (tolerate extreme temperatures)

pH

Most medically important bacteria grow at neutral or slightly alkaline pH (7.2 to 7.6)

Very few bacteria grow below pH 4

Lactobacilli grow in acidic pH; cholera *vibrio* grow in alkaline pH

Growth media includes chemical buffers to prevent acid production

Foods are preserved by acids produced by bacterial fermentation

Moisture and desiccation

Moisture is essential - 80% body weight is water

Effect of drying varies by organism

- *T pallidum*, gonococcus are very susceptible
- Tubercle bacilli, staphylococci may survive for weeks
- Bacterial spores survive several years

Lyophilization

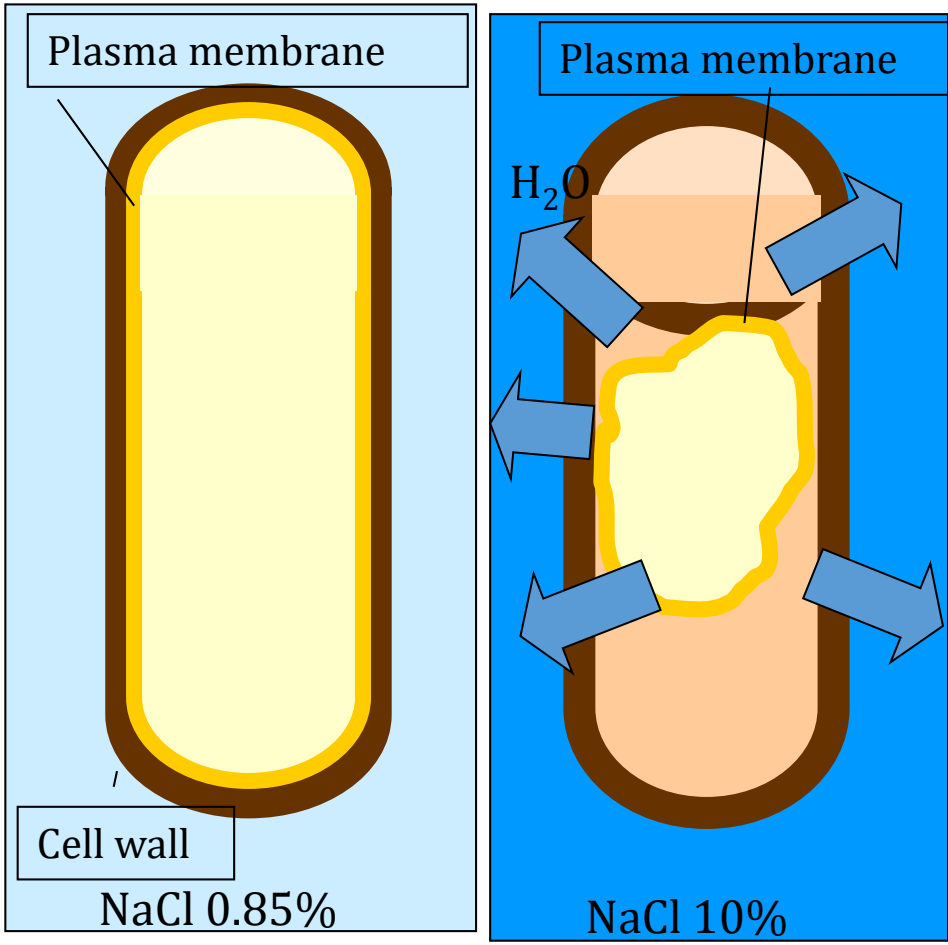
- Freeze dry process that protects bacteria

Osmotic pressure

High osmotic pressure (hypertonic) removes water causing plasmolysis – inhibits growth i.e. salt as preservative

Low osmotic pressures (hypotonic) cause water to enter and can cause lysis

Bacteria are more tolerant to osmotic variations because of the mechanical strength of the cell wall



Oxygen

Obligate aerobes

Only aerobic growth, oxygen required

Facultative anaerobes (most human pathogens)

Greater growth in presence of oxygen

Obligate anaerobes

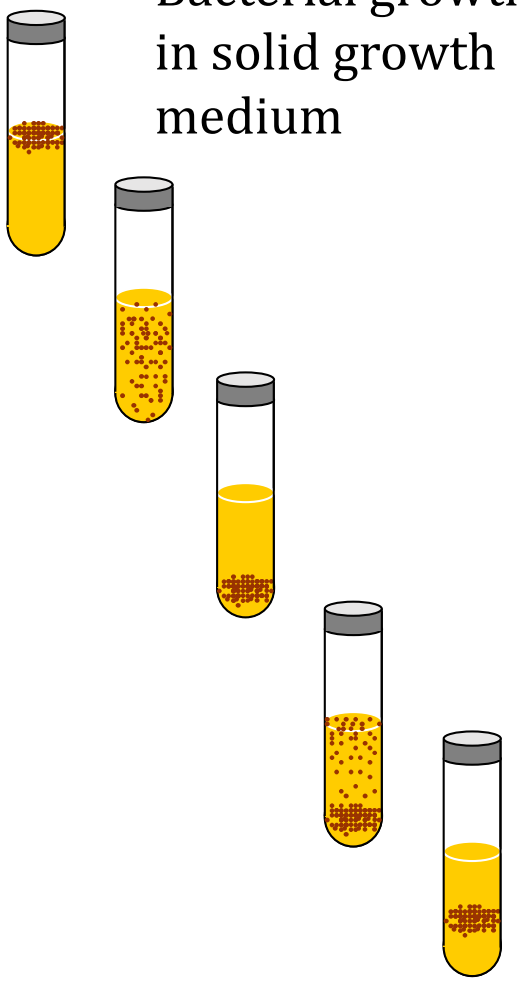
Only anaerobic growth, cease with oxygen

Aerotolerant anaerobes (e.g., *C. perfringens*)

Only anaerobic growth, continues with oxygen

Microaerophiles (e.g., *M. tuberculosis*)

Only aerobic growth with little oxygen



Bacterial growth in solid growth medium

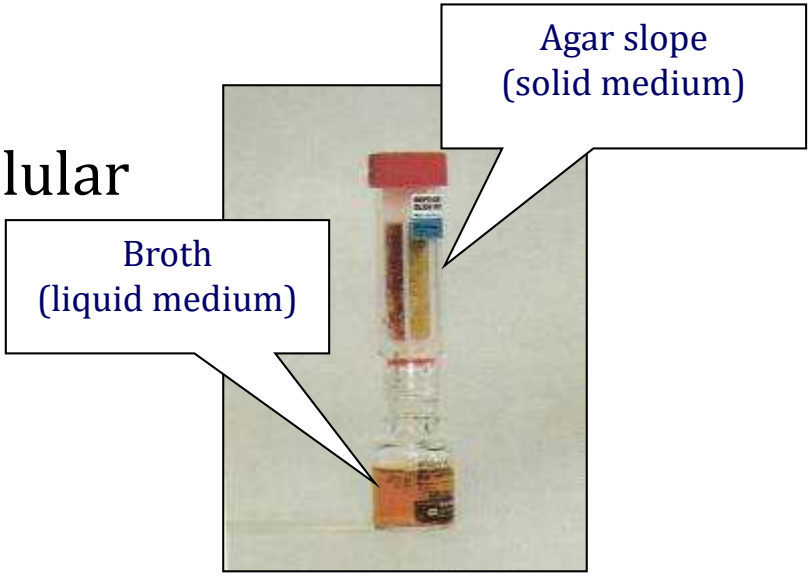
Types of bacterial culture media

Solid, semisolid, liquid, biphasic

Simple media, special media (enriched, selective, enrichment, indicator/ differential, transport) synthetic media

Aerobic and anaerobic media

Cell culture for obligate intracellular bacteria (e.g., *Chlamydia spp*)



Biphasic culture medium

On Consistency:

1. Solid Media. Advantages of solid media: (a) Bacteria may be identified by studying the colony character, (b) Mixed bacteria can be separated. Solid media is used for the isolation of bacteria as pure culture. 'Agar' is most commonly used to prepare solid media. Agar is polysaccharide extract obtained from seaweed. Agar is an ideal solidifying agent as it is : (a) Bacteriologically inert, i.e. no influence on bacterial growth, (b) It remains solid at 37°C, and (c) It is transparent.

2. Liquid Media. It is used for profuse growth, e.g. blood culture in liquid media. Mixed organisms cannot be separated.

On Chemical Composition:

1. Routine Laboratory Media

2. Synthetic Media.

These are chemically defined media prepared from pure chemical substances. It is used in research work

ROUTINE LABORATORY MEDIA

These are classified into six types: (1) Basal media, (2) Enriched media, (3) Selective media, (4) Indicator media, (5) Transport media, and (6) Storage media.

ROUTINE LABORATORY MEDIA

1. **BASAL MEDIA:** Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water. Staphylococcus and Enterobacteriaceae grow in these media.
2. **ENRICHED MEDIA:** The media are enriched usually by adding blood, serum or egg. Examples: Enriched media are blood agar and Lowenstein-Jensen media. Streptococci grow in blood agar media.
3. **SELECTIVE MEDIA:** These media favour the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: MacConkey agar, Lowenstein-Jensen media, tellurite media (Tellurite inhibits the growth of most of the throat organisms except diphtheria bacilli). Antibiotic may be added to a medium for inhibition.

4. INDICATOR (DIFFERENTIAL) MEDIA: An indicator is included in the medium. A particular organism causes change in the indicator, e.g. blood, neutral red, tellurite. Examples: Blood agar and MacConkey agar are indicator media.

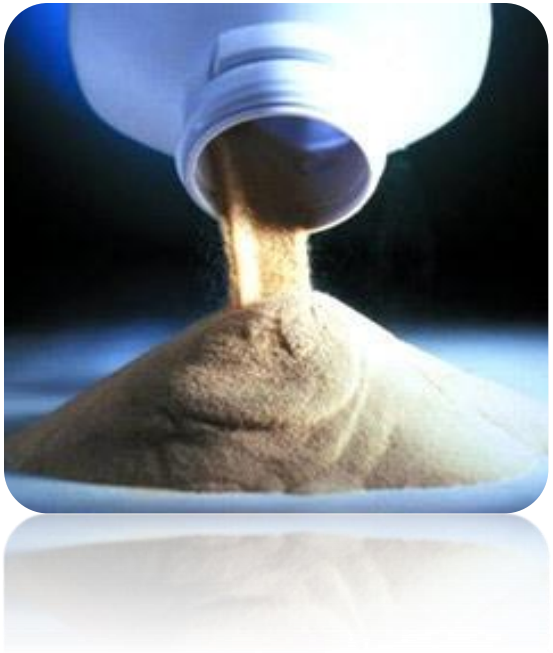
5. TRANSPORT MEDIA: These media are used when specie-men cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium.

6. STORAGE MEDIA: Media used for storing the bacteria for a long period of time. Examples: Egg saline medium, chalk cooked meat broth

BASIC REQUIREMENTS OF CULTURE MEDIA

NUTRIENTS :

- Energy source
- Carbon source
- Nitrogen source



MINERAL SALTS :

- Sulphates, phosphates, chlorides and carbonates of K, Mg and Ca
- A suitable pH- 7.2- 7.4

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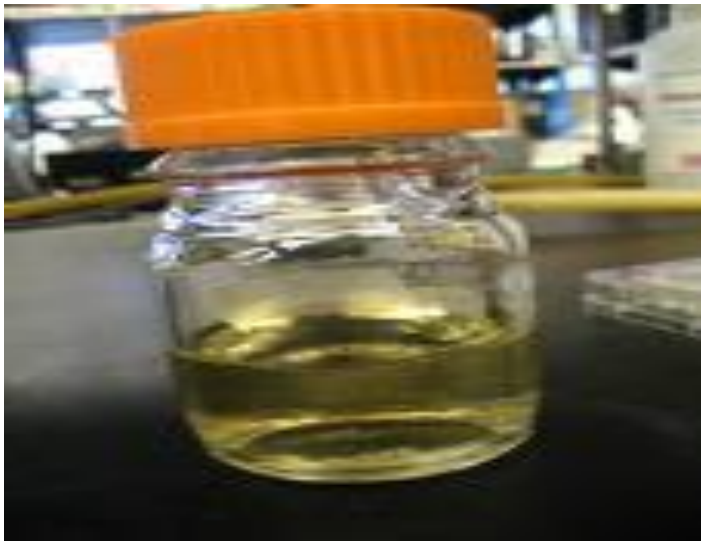
GROWTH FACTORS	
ARGININE	E.COLI
GLUTATHIONE	GONOCOCCI
CHOLESTEROL	MYCOPLASMA
ARYL SULPHATE, AMIDE	ATYPICAL MYCOBACTERIA
GLYCEROL	MYCOPLASMA HOMINIS
SULFONAMIDES	RIKETTSIA
TRYPTOPHAN	SALMONELLA TYPHI
L-CYSTEINE	LISTERIA MONOCYTOGENS
SODIUM CHLORIDE	VIBRIO PARAHAEMOLYTICUS
FACTOR X & V	H.INFLUENZAE

SIMPLE MEDIA

Simple or basal media are culture media which contain the minimum adequate nutrition for non fastidious organisms



Example:- Nutrient broth/agar

Peptone water

Composition:-

Lab-Lemco -10gm

Peptone-10gm

NaCl- 5gm

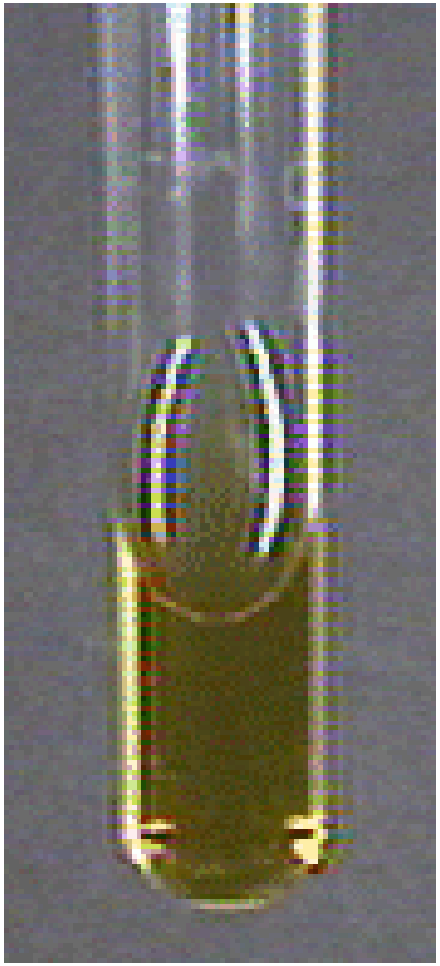
Distilled water-1000ml

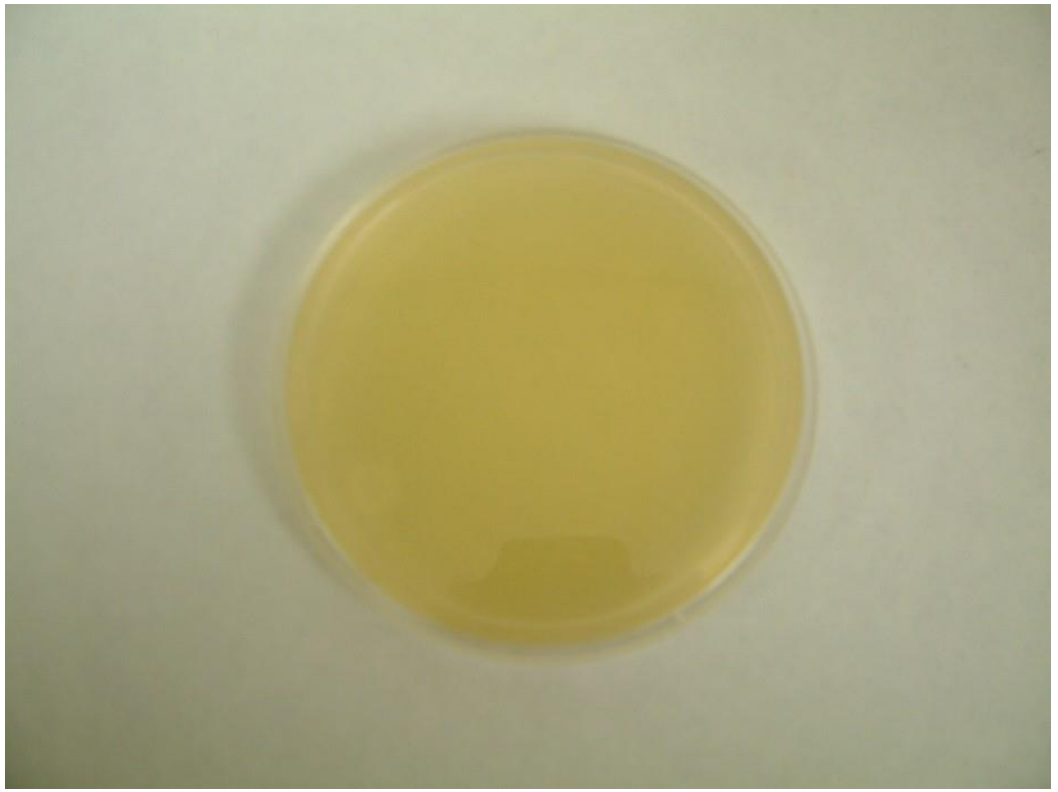
- When 2-3% agar is added ,then we have it as nutrient agar.

- For semisolid media – agar concentration is 0.2-0.4%

Uses:-

1. **This is basis of most of the media used in the study at common pathogenic bacteria.**
2. **It is used for subcultures of certain organisms.**





NUTRIENT AGAR

NUTRIENT BROTH

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PEPTONE WATER

TYPE : Basic liquid media

APPEARANCE : clear, colorless, watery, usually in test tube

Composition :

PEPTONE	10 g
SODIUM CHLORIDE, NaCl	5 g
WATER	1 litre

USES OF PEPTONE WATER

- The media is used chiefly as the basis for carbohydrate fermentation media.

- **Nutrient broths may contain a small amount of sugar derived from meat and it is essential that the basal medium to which various carbohydrates are added for fermentation tests should be free from natural sugars.**

  - ✓It is also used to test the formation of indole.
  - ✓Culture of organisms for demonstration of motility

COMPLEX MEDIA

Complex media have added ingredients for bringing out certain properties for bringing out certain properties or providing special nutrients required for growth of the bacterium in question.

SYNTHETIC MEDIA

✓These are prepared from pure chemicals and the exact compositions of medium is very well known.

Example :- Dubo's medium

SEMIDEFINED MEDIA

✓In these media the exact chemical composition of the constituents is not known because substances like meat and peptone are used.

✓Most of the culture media used for routine diagnostic work are semidefined culture media.

SPECIAL MEDIUM

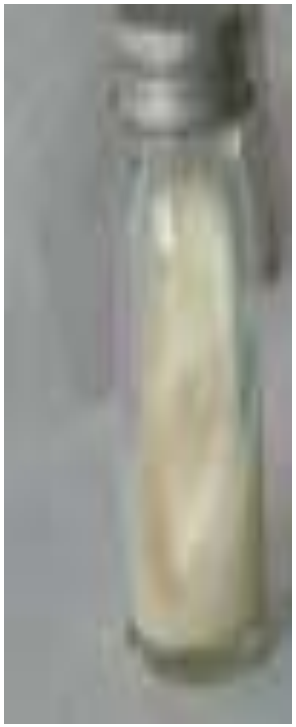
ENRICHED MEDIA

- When basal medium is added with some nutrients such as blood, serum or egg is called enriched media.
- They are used to grow bacteria which are more exacting in their nutritional needs.

Examples:-

Dorset's Egg Medium.

- ✓ It is a creamy coloured opaque slope kept in screw copped bottle
- ✓ Selective medium for isolation of *Mycobacterium tuberculosis*.
- ✓ Composition: Hen's egg, Nutrient broth



BLOOD AGAR

TYPE : Enriched media.

APPEARANCE : Red color.

COMPOSITION :

Sterile Nutrient agar + Defibrinated sheep blood

USES :

- Routine culture
- Widely used in medical bacteriology
- It is also an indicator medium showing the haemolytic properties of bacteria such as *Streptococcus pyogenes*.

CHOCOLATE AGAR

Also called Heated blood agar.

TYPE : **Enriched media.**

APPEARANCE : **Chocolate brown color.**

PROCEDURE

Melt the desired amount of nutrient agar.

Cool it in a water – bath at 75º C .

Add 10 ml of sterile blood .

Allow the medium to remain at 75º C.

Mixing the blood and agar by gentle agitation from time to time until the blood become chocolate brown in color, within about 10 min.

Then pour in plates.

USES

- ✓**CULTURE OF *Neisseria***
- ✓**CULTURE OF *Haemophilus influenzae***
- ✓**CULTURE OF *Pneumococcus***

ENRICHMENT MEDIA

❖ In this media, it has a stimulating effect on the bacteria to be grown or inhibits its competitors.

❖This result in an absolute increase in the number of wanted bacteria related to other bacteria.

❖**Example:-** Selenite F broth

✓It is enrichment medium for culture of *Salmonella typhi* and *paratyphi* bacilli from stool sample

✓Principle:- at neutral pH solution acid salinity has high toxicity to coli form group of bacteria and not to most of the salmonella groups.

SELENITE F BROTH

Alkaline peptone water

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SELECTIVE MEDIA

❖It is a medium in which certain substances are present which inhibit all other bacteria except the desired bacteria.

❖It encourages the growth of particular species from a mixed inoculum.

Example:- TCBS

-It is light green translucent medium kept in petridish

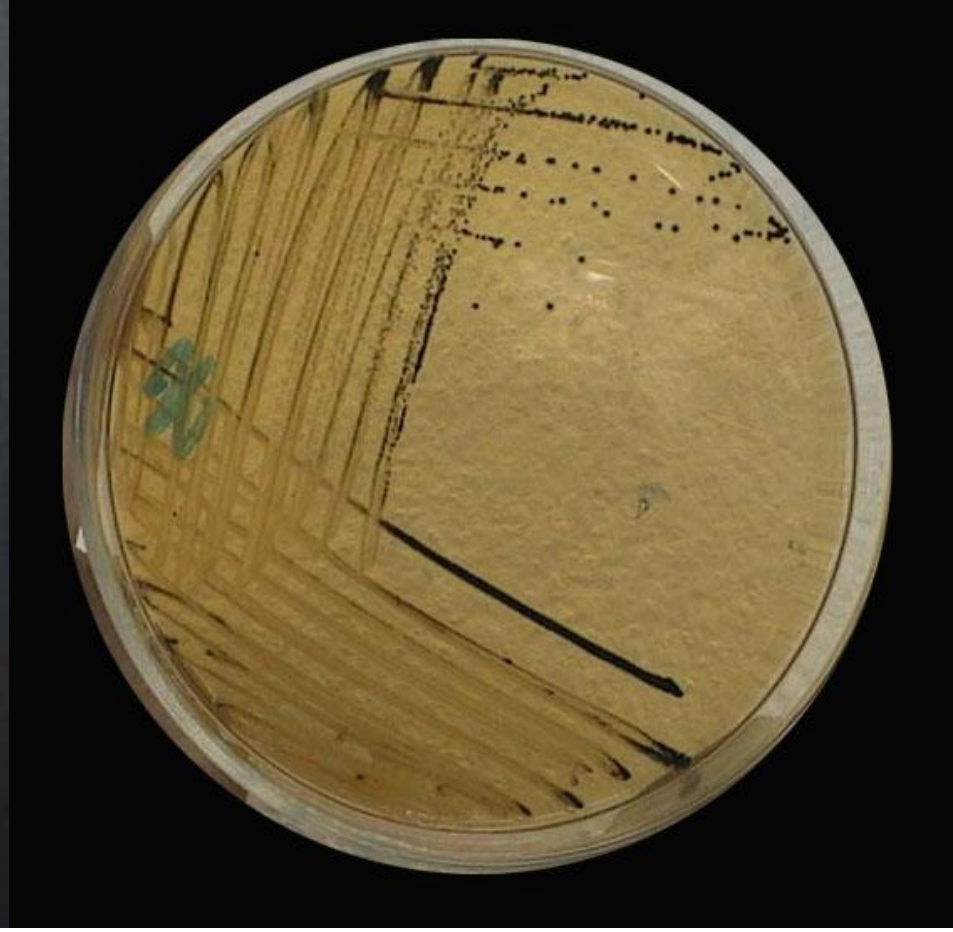
-It is selective medium for *Vibrio cholera*

-**Principle:-**

Bile salt inhibit the growth of normal commensals (unwanted bacteria).

✓*Vibrio chloerae* produce acid by fermentation of sucrose which acts on bromothymol blue (indicator) producing yellow colonies.

Salmonella Shigella Agar with DCA



Salmonella-Shigella agar plate (SS)

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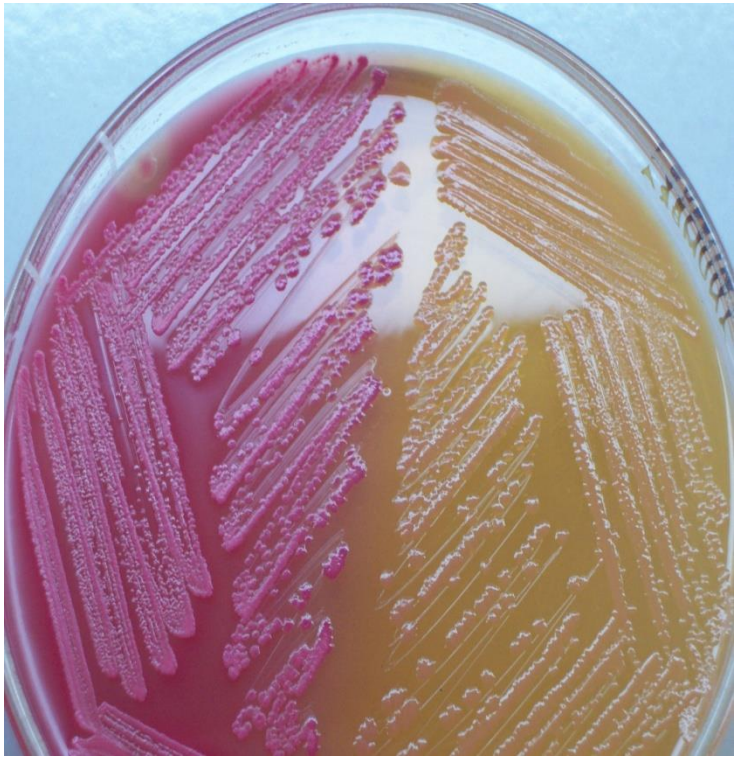
DIFFERENTIAL MEDIA

MAC CONKEY AGAR

MacConkey agar is a culture medium designed to grow Gram-negative bacteria. It is a useful medium for the cultivation of enterobacteriacea.



MacConkey agar showing both lactose and non-lactose fermenting colonies.

Lactose fermenting colonies are pink whereas non-lactose fermenting ones are colourless or appear same as the medium.

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It contains lactose and neutral red to distinguish the lactose-fermenting coliforms from the lactose non –fermenting salmonella and shigella groups.

It contains **Bile salts** to inhibit non-intestinal bacteria and most Gram-positive bacteria, except *Enterococcus* and some species of *Staphylococcus* i.e. *Staphylococcus aureus*.

Neutral red dye : which stains microbes fermenting lactose.

Crystal violet dye : which also inhibits certain Gram-positive bacteria).

Gram-negative bacteria growing on the media are differentiated by their ability to ferment the sugar lactose.

Lactose fermenter cause the pH to drop and is detected by neutral red, (red at pH's below 6.8.) which appear as bright pink to red colonies on the agar.

Uses

Acting as a visual pH indicator, the agar distinguishes those Gram-negative bacteria that can ferment the sugar lactose (Lac+) from those that cannot (Lac-).

❖This medium is also known as an

"indicator medium"

"low selective medium".

Absence of electrolytes serves to inhibit swarming by Proteus species

Lac+

By utilizing the lactose available in the medium, Lac+ bacteria such as

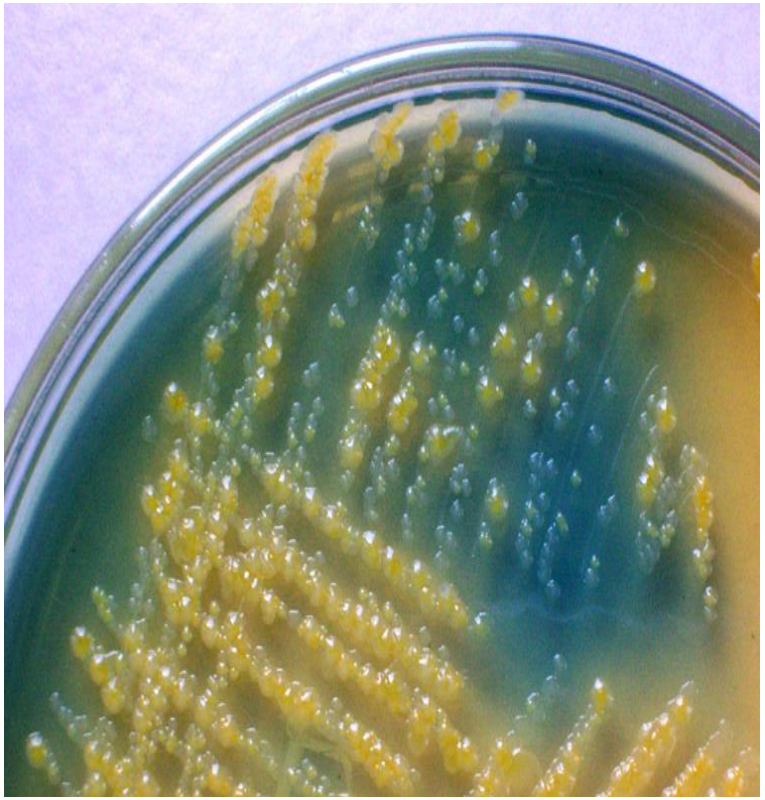
- ***Escherichia coli***
- ***Enterobacter spp.***
- ***Klebsiella spp.***

will produce acid, which lowers the pH of the agar below 6.8 and results in the appearance of red/pink colonies

CLED
(Cystine Lactose Electrolyte Deficient medium)

It is a valuable non-inhibitory growth medium used in the isolation and differentiation of urinary organisms.

Being electrolyte deficient, it prevents the swarming of *Proteus* species



Description:
Lactose & non lactose fermenters on CLED medium.

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INDICATOR MEDIUM

❖These media contain an indicator which changes colour when bacteria grow on them.

❖Example:- Wilson and Blair medium

✓For isolation of *Salmonella typhi* and *S. paratyphi*

✓They appear as black colonies

Principle:- The black colour of colonies is due to the ability of these organisms to reduce bismuth sulphite to sulphide in the presence of glucose coliforms are inhibited by brilliant green and bismuth sullphite

TRANSPORT MEDIUM

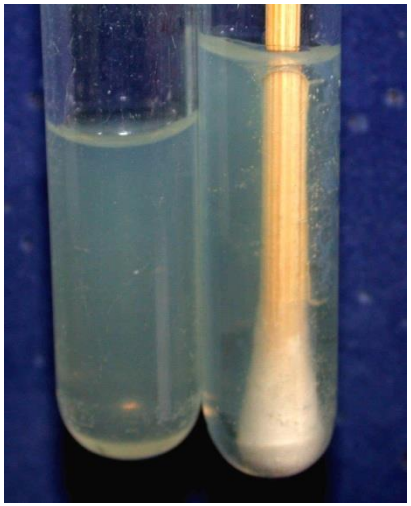
✓These are used for the temporary storage of specimens being transported to the laboratory for cultivation.

✓Such media ideally maintain the viability of all organisms in the specimen without altering their concentration.

✓Transport media typically contain only buffers and salt.

✓The lack of carbon, nitrogen, and organic growth factors prevents microbial multiplication.

✓Transport media used in the isolation of anaerobes must be free of molecular oxygen.



STUART TRANSPORT BROTH

CHARACTERISTICS OF TRANSPORT MEDIA:

It should be non-toxic

It should not promote or inhibit the bacterial growth

It should be easy to carry and transport

Examples:

1. **Venkatraman Ramakrishnan medium**
2. **Buffered glycerol saline transport medium**
3. **Cary and Blair medium**

Cary and Blair medium

Daffodil International University

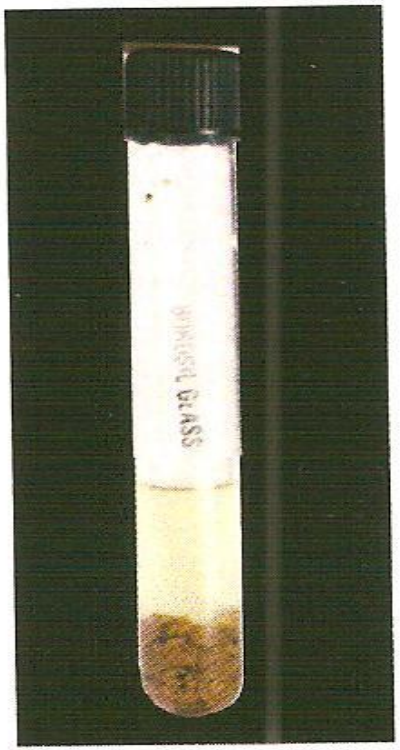
ANAEROBIC MEDIUM

These media are used to grow anaerobic organisms.

Examples:-

✓Thioglycollate broth

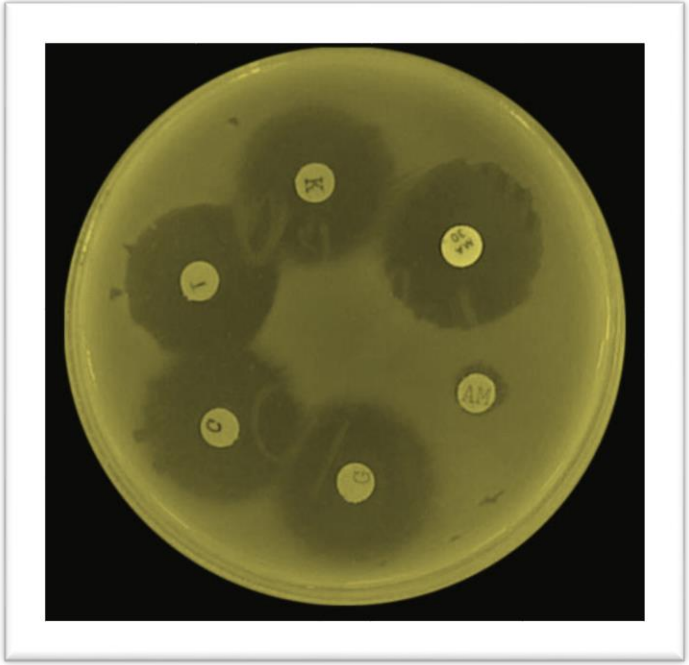
✓ Robertsons Cooked Meat Medium



Mueller-Hinton agar is an microbiological growth medium that is commonly used for antibiotic susceptibility testing.

Originally formulated for isolation of *Neisseria* species.

It is also used to isolate and maintain *Neisseria* and *Moraxella* species.



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Culture methods

Streak culture

Isolation of bacteria in pure culture from clinical specimen

Lawn culture

Antimicrobial susceptibility testing (disc diffusion), bacteriophage typing

Liquid cultures

Stroke culture

- To obtain pure growth for slide agglutination; biochemical tests

Stab culture

- Maintenance of stock cultures

Pour-plate culture

- Quantification of bacteria in liquid cultures, urine sample

Aero and /or hydrodynamic properties are very important characters in hydraulic transport and handling as well as hydraulic sorting of agricultural products. To provide basic data for the development of equipment for sorting and sizing of agro commodities, several properties such as: physical characteristics and terminal velocity are needed. The two important aerodynamic characteristics of a body are its terminal velocity and aerodynamic drag. By defining the terminal velocity of different threshed materials, it is possible to determine and set the maximum possible air velocity in which material out of grain can be removed without loss of grain or the principle can be applied to classify grain into different size groups. In addition, agricultural materials and food products are routinely conveyed using air. For such operations, the interaction between the solid particles and the moving fluids determine the forces applied to the particles. The interaction is affected by the density, shape, and size of the particle along with the density, viscosity, and velocity of the fluid. This chapter discusses briefly with the different aerodynamic properties and their methods of measurement.

Classical biotechnology

Industry today exploits early discoveries of the fermentation process for production of huge numbers of products

- Different types of beer
- Vinegar
- Glycerol
- Acetone
- Butanol
- Lactic acid
- Citric acid
- Antibiotics – WWII (Bioreactor developed for large scale production, e.g. penicilin made by fermentation of penicillium)
  - Today many different antibiotics are produced by microorganisms
  - Cephalosporins, bacitracin, neomycin, tetracycline……..)