

# CULTURE MEDIA

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# INTRODUCTION

- ◉ **Media/Medium**—The nutrient material in which bacteria grow.
- ◉ **Culture**-a bacterial growth in a medium
- ◉ **Broth**-a liquid nutrient medium
- ◉ **Broth culture**- a liquid nutrient medium with organisms growing
- ◉ **Agar plate**- round culture dish with solid nutrient media
- ◉ **Slant**— tube with solid media, with a slanted surface.
- ◉ **Colony**- Thought to contain only the descendants of a single bacterium



# CULTURE MEDIUM

- The growth of any microorganism, whether in its natural niche or in a laboratory, is dependent upon the presence of certain essential compounds in its environment.
- The environment which contains all these ingredients which are required for bacterial growth in microbiology laboratory is called a culture medium.

# COMPOSITION OF MEDIUM

Water

Agar

Peptone

Carbon

Sulfur

Nitrogen

Meat extract

Yeast extract

Mineral salts

# CLASSIFICATION OF CULTURE MEDIA

- ◉ Solid
- ◉ Semisolid
- ◉ Liquid

## **An Introduction to Agar**

- ◉ It is a component of the cell walls of several species of an algae (Sea Weed).
- ◉
- ◉ Chemically, agar is a polymer made up of subunits of the sugar galactose.
- ◉ Dissolved in boiling water and cooled, laboratory agar looks gelatinous.

- ◉ Why agar, as opposed to regular gelatin, is used for culturing bacteria.
  - It will not be degraded (eaten) by bacteria.
  - Agar is firmer and stronger than gelatin.
  - It's still possible, however, to use gelatin as a culture medium for bacteria if agar is unavailable.
- ◉ Agar is a gel at room temperature, remaining firm at temperature as high as 65 °C. Agar melts at approximately 85 °C, a different temperature from that at which it solidifies, 32-40 °C.
- ◉ Agar is typically used in a final concentration of 1-2% for solidifying culture media. Smaller quantities (0.05-0.5%) are used in media for motility studies (0.5% w/v) and for growth of anaerobes (0.1%) and microaerophiles.
- ◉ Although agar's chief use is as a culture medium for various microorganisms, particularly for bacteria, its other less well-known uses include serving as a thickening for soups and sauces, in jellies and ice cream, in cosmetics *etc.*

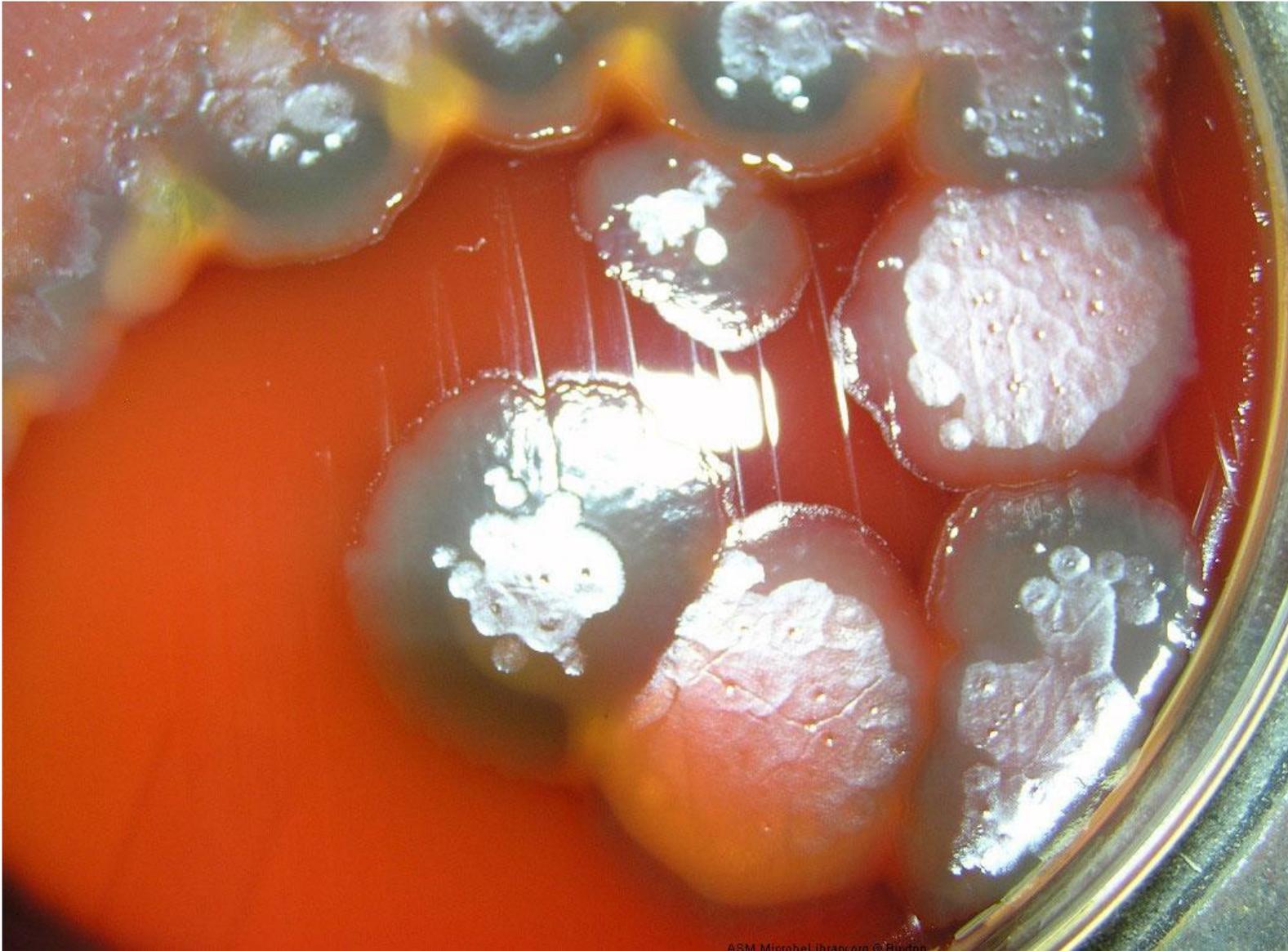
# SOLID CULTURE MEDIUM

- Used mainly in Petri dishes as plate cultures, can also be used in bottles or tubes as slant or slope cultures.
- Colonies which form on solid medium along with changes in the surrounding help to identify bacteria and differentiate one type of bacteria from another.

# COLONIAL APPEARANCE



# COLONIAL APPEARANCE



Pigment production

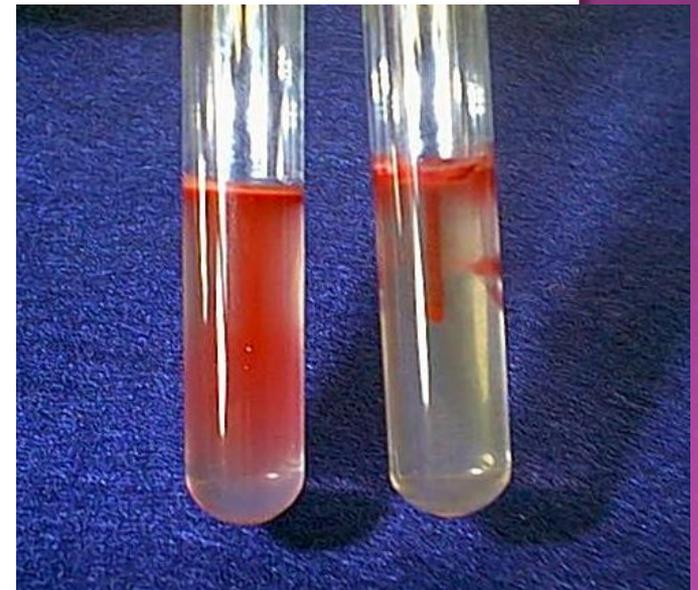
# FLUID CULTURE MEDIUM

- ◉ Multiplication and growth of bacteria is usually described in 4 stages
- ◉ Growth is shown by turbidity in medium
- ◉ Fluid media are used mainly as enrichment media, biochemical testing media, and blood cultures



# SEMI-SOLID CULTURE MEDIA

- ⦿ Contain up to 0.5% w/v of Agar to a fluid medium
- ⦿ Used as
  - Transport media
  - Motility media

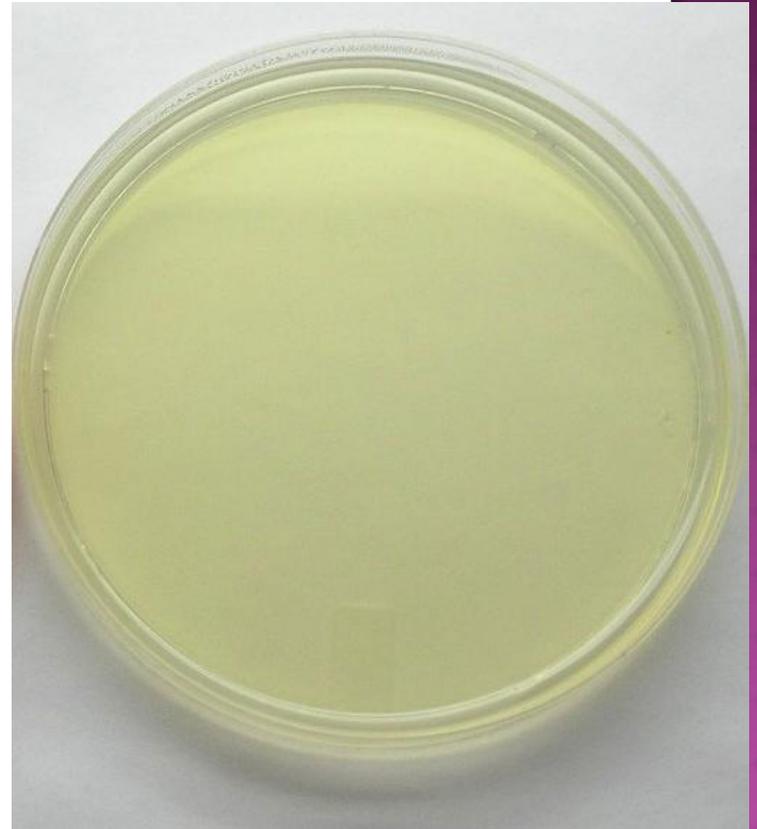


# TYPES OF CULTURE MEDIA

- ◉ Basic Media
- ◉ Enriched Media
- ◉ Selective Media
- ◉ Differential Media
- ◉ Transport Media
- ◉ Enzymatic media
- ◉ Multipurpose media

# BASIC MEDIA

- Simple Media
- Supports growth of Microorganisms that do not have special nutrient requirements e.g. Nutrient Agar and Nutrient Broth
- Uses
  - Preparation of Enriched Media
  - To maintain stock cultures of control strains
  - Subculturing pathogens from differential or selective media prior to performing biochemicals and serological tests
  - Used for antimicrobial sensitivity testing



# ENRICHED MEDIA

- ◉ Enriched with whole blood, lysed blood, serum, extra peptones, special extracts, or vitamins to support the growth of pathogens that require additional nutrients or growth stimulants
- ◉ The organism which requires enriched media or fastidious organisms e.g. *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and several *Streptococcal spp*

Uninoculated BAP



# CHOCOLATE AGAR

- Chocolate agar is an enriched medium which is used in culturing fastidious organisms such as *Haemophilus* species and *Neisseria*.
- It is comprised of sheep blood that provides the X and V factors necessary for *Haemophilus* growth.
- Chocolate agar, however, does not reveal hemolysis data, so species differentiation among the members of *Haemophilus* must be performed in another manner.



Uninoculated chocolate agar plate

# TRYPTIC SOY AGAR/BROTH

- ◉ Tryptic soy agar/broth is a basic medium used for culturing many kinds of microorganisms.
- ◉ Tryptic soy agar/broth is used mostly to generate a large supply of bacteria for certain biochemical tests.



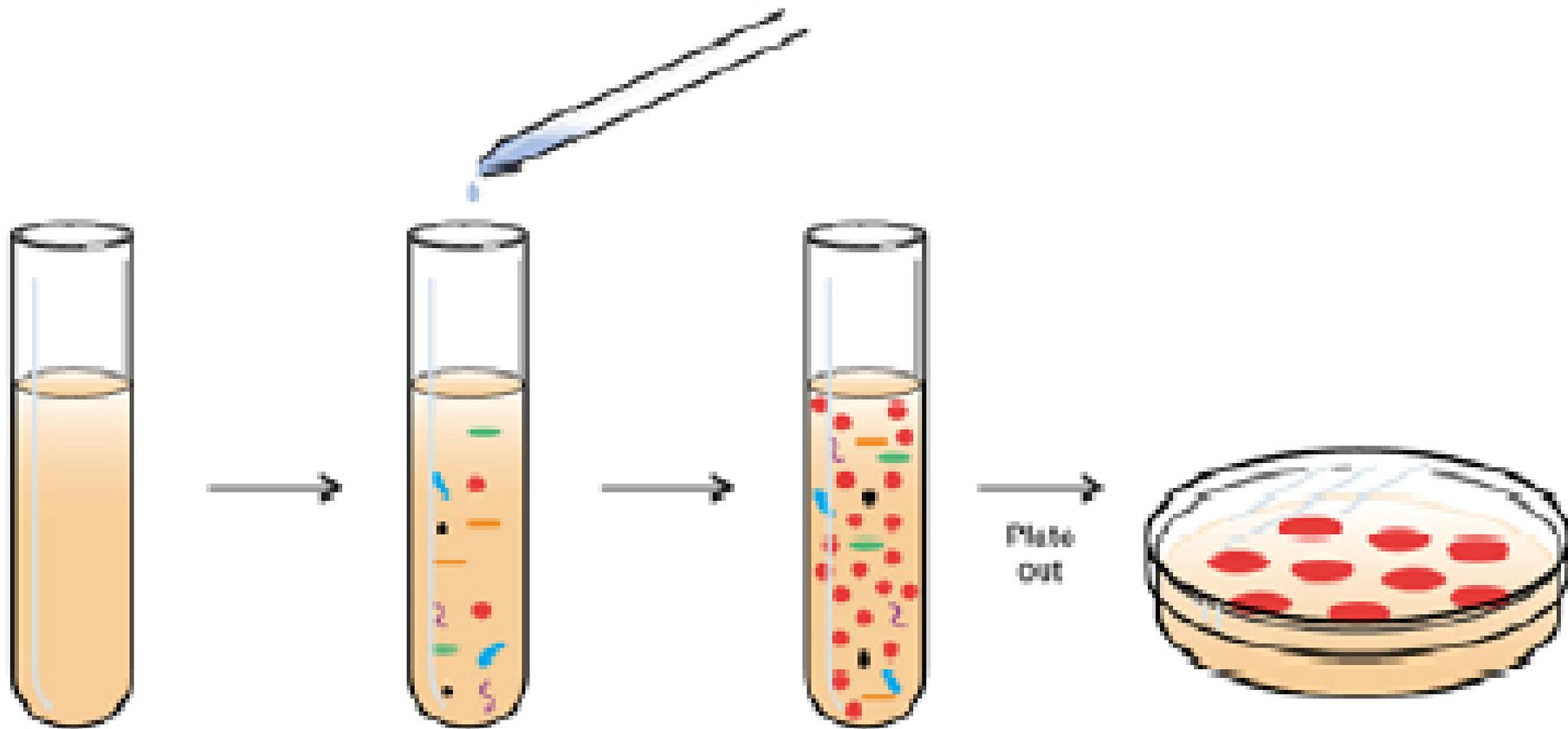
**Tryptic Soya Agar Slant**

# ENRICHMENT MEDIUM

- The term enrichment is used to describe fluid medium that increase the number of a pathogen by containing enrichments and substances that discourage the multiplication of unwanted bacteria
- Selenite F broth is used as a enrichment medium for *Salmonella* in faeces or urine prior to subculturing on XLD or other Enteric selective medium

# Types of Laboratory Culture Media (Enrichment)

# 6



Medium contains select nutrient sources chosen because few bacteria, other than the organism of interest, can use them.

Sample that contains a wide variety of organisms, including the organism of interest, is added to the medium.

Organism of interest can multiply, whereas most others cannot.

Enriched sample is plated onto appropriate agar medium. A pure culture is obtained by selecting a single colony of the organisms of interest.

# SELECTIVE MEDIUM

- ◉ The medium which contains substances that prevent or slow down the growth of microorganisms or unintended. e.g. XLD agar selects for *Salmonella* and *Shigella* by containing bile salts that inhibit the growth of many faecal commensals
- ◉ In recent years antimicrobials are increasingly used as selective agents in culture media e.g. Modified NYC media for *Neisseria gonorrhoeae* from urogenital specimens.

## Examples include

- ◉ Thayer Martin or New York City Medium for *N gonorrhoeae*
- ◉ Lowenstein -Jensen medium

# LOWENSTEIN -JENSEN MEDIUM

This is a medium selective for *Mycobacterium tuberculosis*. The medium contains malachite green, which inhibits the growth of other organisms and gives the medium its blue green color



# MANNITOL SALT AGAR (MSA)

- ◉ A common medium used for the isolation of pathogenic staphylococci is the MSA. The high salt concentration of this medium inhibits the growth of most other organisms.
- ◉ Pathogenic staphylococci not only grow on the medium, but they also produce acid from it. This acid production turns the pH indicator from red to yellow.
- ◉ Non-pathogenic staphylococci can grow on the medium but produce no acid from it.
- ◉ Selective and Differential



# DIFFERENTIAL MEDIUM

- ◉ The media to which indicators, dyes or other substances are added to differentiate microorganisms e.g. TCBS contain Bromothymol blue which differentiates sucrose fermenter from non-sucrose fermenter *Vibrio spp*
- ◉ Most but not all differential media distinguish between bacteria by an indicator which changes color when acid is produced following CHO fermentation.

DNase Agar

# SALMONELLA -SHIGELLA (SS) AGAR

- ◉ This medium is selective for salmonella and shigella. It contains the three times the normal concentration of bile salts, which inhibits *E.coli*.
- ◉ The indicator is **neutral red**. Salmonella also produce hydrogen disulphide which blackens the medium.



SS Agar

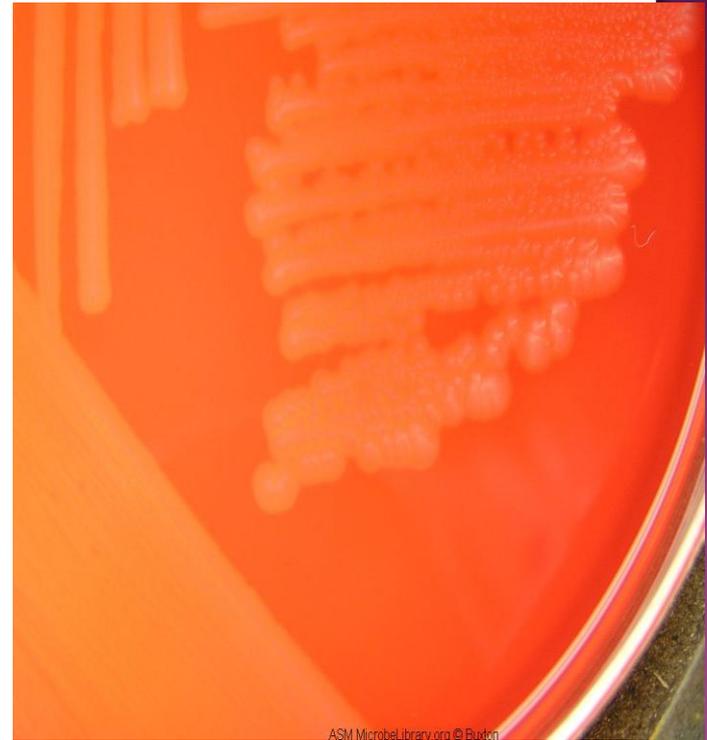
# CYSTEINE LACTOSE ELECTROLYTE DEFICIENT (CLED) MEDIUM

- ◉ This medium is selective for urinary tract pathogens. The indicator is bromothymol blue.
- ◉ The medium contains lactose and production of acid by bacteria converts the blue color of the medium to yellow.



# BLOOD AGAR

- ◉ **Blood Agar** can also be used as differential medium when it differentiates hemolytic from non-hemolytic organisms
- ◉ Many culture medias are both differential and selective e.g. MSA, TCBS, McConkey, XLD, and DCA
- ◉ Enriched media may also be made selective and/or differential e.g. Crystal Violet Blood Agar is used as an enriched, selective and differential media for *Streptococcus pyogenes*



**Blood Agar**

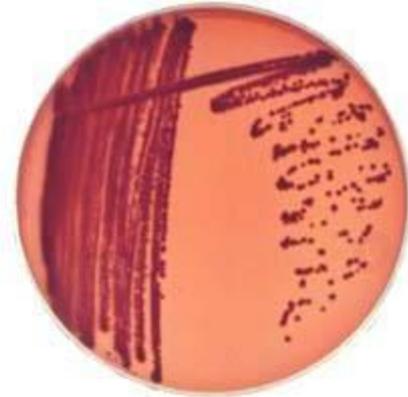
# BLOOD AGAR

- ◉ This is used to differentiate between hemolytic and non-hemolytic organisms.
- ◉ Hemolytic organisms produce hemolysis which degrade RBC's in the blood. The degradation may be complete or incomplete which appears in the medium as an area of clearing or of 'greenness' respectively.
- ◉ In the absence of hemolysins, no change is visible in the medium of the area around the colony.



# MCCONKEY AGAR

- MacConkey agar is probably the most popular solid differential medium in the world. It is mainly used in identification of lactose fermenting, Gram-negative enteric pathogens and for inhibiting growth of Gram-positive organisms.
- Bacterial colonies that can ferment lactose turn the medium red. This red color is due to the pH indicators response to the acidic environment created by fermenting lactose. Organisms that do not ferment lactose do not cause a color change



# TRANSPORT MEDIUM

- ◉ Semi solid medium
- ◉ Contain ingredients to prevent the over-growth of commensals and ensure the survival of aerobic and anaerobic pathogens when specimens can not be cultured soon after collection
- ◉ It is imported when transporting microbiological specimens from health centers to the district microbiology laboratory e.g. Cary Blair medium for preserving Enteric pathogens and Amies transport medium for ensuring the viability of *Gonococci* and other pathogens in the specimens collected on swabs

# ENZYMATIC MEDIA

- These are media used to observe the enzymatic reactions of organisms e.g. urease test, Indole test.



# MULTIPURPOSE MEDIA

- Three or more different substrates and two or more different reactions can be observed at the same time in a single medium.

## *Triple sugar iron (TSI) medium*

- In medium there is a solid, poorly oxygenated area on the bottom, called the **butt** and an angled, well-oxygenated area called the **slant**. The organism is inoculated into the **butt** and across the surface of the **slant**.
- Composition:*
  - Proteins*
  - Peptones*
  - Ferric sulphate*
  - Sodium thiosulphate*
- Sugars:*
  - Glucose, lactose, sucrose*



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# PRINCIPLE

- **If lactose (or sucrose) is fermented,**
  - A large amount of acid is produced, which turns the phenol red indicator yellow both in the butt and the slant.
- **If lactose is not fermented but the small amount of glucose is fermented;**
  - The oxygen deficient butt will be yellow, but on the slant the acid will be oxidised to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . And the slant will be red (neutral or red).
- **If neither lactose glucose is fermented**
  - Both the butt and the slant will be red.
- **Some organisms generate gas**
  - Which produce bubbles in the butt.
- **If  $\text{H}_2\text{S}$  is produced,**
  - The black color of ferrous sulphide is seen.
- **Results:** four different types of reactions are seen on TSI agar:



# MAINTENANCE MEDIA

- ⦿ These Media have added nourishment and are used for primary isolation of the organisms.
- ⦿ Then maintain organisms in a viable state, acting as reservoir of microorganisms.

## **Cooked meat broth (CMB).**

- ⦿ Maintenance medium for anaerobes and aerobes.
- ⦿ It also acts as a differential medium to observe the **saccharolytic** and **proteolytic** activity of organisms.

# SUBSTITUTING MEDIA

- These are medias in which one of the nutrients is substituted by another substrate to observe the enzymatic activity of an

organism, **citrate agar**:

Carbohydrates are replaced by sodium citrate as the sole source of carbon for the organisms.

Ammonium salts act as a source of nitrogen and the indicator is **bromothymol blue**.

Organisms that utilize citrate as a source of carbon, will break it down and utilize ammonium salts, producing ammonia.

This raises the pH of the medium and a blue color is produced.

Blue color indicates a positive test.

