

# **Practical Bacteriology**

## **Laboratory Five**

Types of Culture media

# Microbiological culture

Method of multiplying microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions



# Important Reasons For Bacterial Culturing

- ❑ Isolating a bacterium from sites in body normally known to be sterile indication of its role in the disease process.
- ❑ Culturing bacteria is also the initial step in studying its morphology and its identification.
- ❑ Bacteria have to be cultured in order to obtain antigens from developing serological assay for vaccines
- ❑ Certain genetic studies and manipulations of the cells also need that bacteria be cultured in vitro.
- ❑ Culturing on solid media is another convenient way of separating bacteria in



# Composition of culture media:

- ❑ **Provide similar environmental and nutritional conditions that exist in its natural habitat**

❑ An artificial culture medium must provide all the nutritional components that a bacterium gets in its natural habitat.

❑ A culture medium contains water, a source of carbon & energy, source of nitrogen, trace elements and some growth factors

- ❑ The pH of the medium must be set accordingly





# How is media made?

- When lab personnel make media they measure out a quantity of **dry powdered nutrient media**, add **water** and **check the pH**.
- They pour the media into bottles, cap it and **autoclave**.
- This is a process similar to home canning techniques in food preservation.
- The autoclave exposes the media to high temperature (121°C) and pressure (15 psi) for 20 minutes.
- Once the media is **autoclaved** it is considered \_\_\_\_\_ (all life forms killed).



## Classification:



Bacterial culture media can be classified  
in at least three ways

1. CONSISTENCY

2. NUTRITIONAL COMPONENT

3. FUNCTIONAL USE



# **Classification of culture media on the basis of consistency**

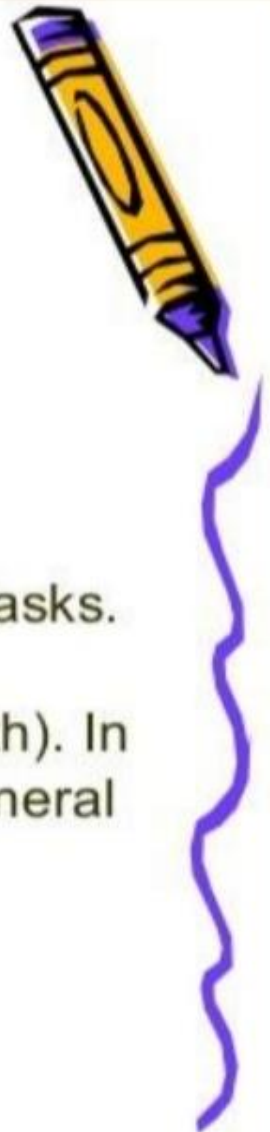
- 1) Liquid or broth (without agar)**
- 2) Semi solid media 0.5% agar (used for testing motility)**
- 3) Solid media (gelatinous) 1.5-2% agar**

# 1) Classification based on consistency:

## A. Liquid media :

These are available for use in test-tubes, bottles or flasks.

Liquid media are sometimes referred as "**broths**" (e.g nutrient broth). In liquid medium, bacteria grow uniformly producing general turbidity





# 1) Classification based on consistency:

B.Solid media:

An agar plate is a **Petri dish** that contains a **growth medium** (typically **agar** plus nutrients used to **culture microorganisms**).

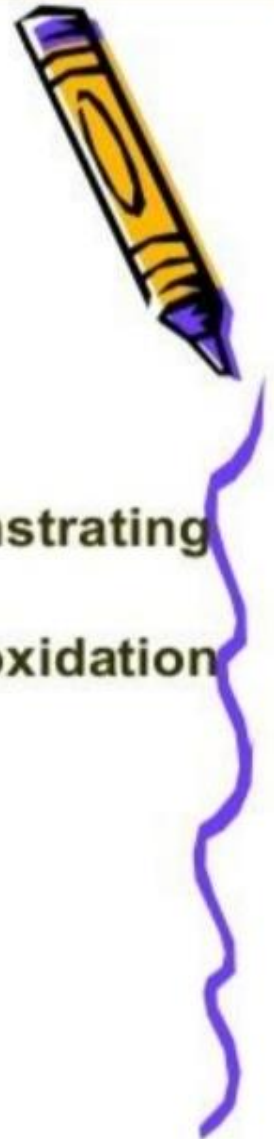
Agar is the most commonly used solidifying agent



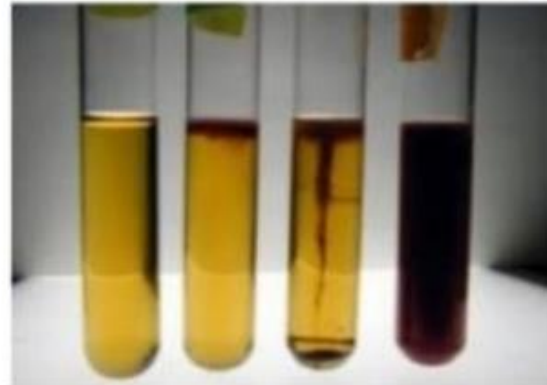
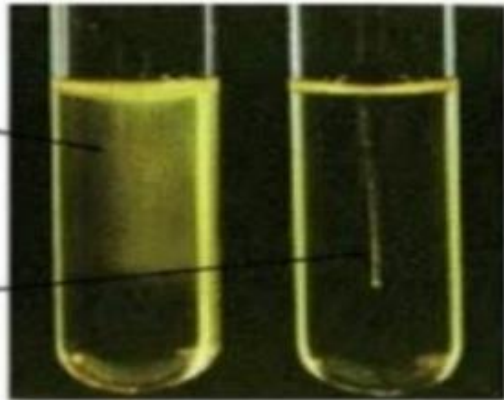
# 1) Classification based on consistency:

## C) Semi-solid agar:

Such media are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains. Hugh & Leifson's oxidation-fermentation



# Examples of Semi-solid media



# **Classification based on functional use or application**

- A. Basal media**
- B. Enriched media**
- C. Enrichment media**



## **D. Differential media**

### **➤ Blood agar plate**

- 1. Beta – haemolytic**
- 2. Alpha – haemolytic**
- 3. Gamma haemolytic (Non – haemolytic)**

### **➤ MacConkey agar**

**E. Selective and selective – differential media**

- **MacConkey agar**
- **Mannitol salt agar**

**F. Transport media**

**G. The Preservation Culture media**

## 2) Classification based on nutritional component:

### ➤ Simple media:

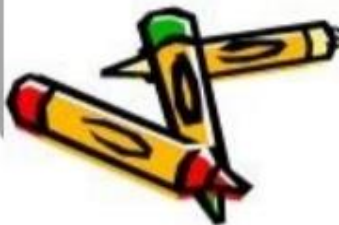
Simple media such as peptone water, nutrient agar can support most non-fastidious bacteria.

### ➤ Complex

**media:** Complex media such as blood agar have ingredients whose exact components are difficult to estimate

### ➤ Synthetic media:

Specially prepared media for research purposes where the composition of every component is well known.



### 3) Classification based on functional use or application:

Basal media  
Enriched media  
Selective media  
Enrichment media  
Differential media  
Transport media  
Anaerobic media





### 3) Classification based on functional use or application:

- Basal media

Basal media are basically simple media that supports most non-fastidious bacteria

Examples of Basal media:

Peptone water, nutrient broth and nutrient agar



### 3) Classification based on functional use or application:

#### **Enriched media**

Addition of extra nutrients in the form blood, serum, egg yolk etc to basal medium makes them enriched media

#### **Examples of Enriched media:**

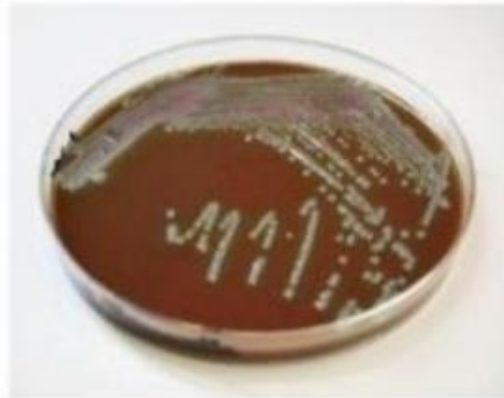
**Chocolate agar**

**Blood agar**



# CHOCOLATE AGAR

- **Chocolate agar** - is a non-selective, enriched growth medium. containing red blood cells that have been lysed by slowly heating to 80 °C. Chocolate agar is used for growing fastidious bacteria, such as *Haemophilus influenzae*



# BLOOD AGAR

- Blood agar plate (BAP)  
Contains mammalian blood (usually sheep or horse), typically at a concentration of 5-10%. BAP are enriched, differential media used to isolate fastidious organisms and detect hemolytic activity





### 3) Classification based on functional use or application:

#### Selective media

enrichment media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria

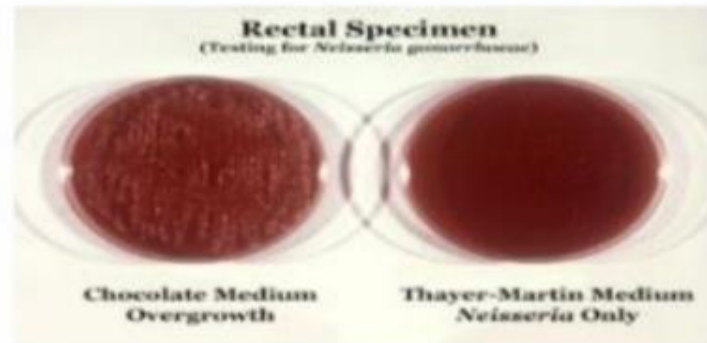
Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen.

to make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these



# Examples of Selective media

- Thayer Martin Medium  
selective for  
*Neisseria gonorrhoeae*



## Examples of Selective media

EMB agar is selective for gram-negative bacteria. The dye methylene blue in the medium inhibits the growth of gram-positive bacteria; small amounts of this dye effectively inhibit the growth of most gram-positive bacteria



### 3) Classification based on functional use or application:

#### Enrichment media

liquid media that also serves to inhibit commensal in the clinical specimen.

**Selenite F broth** and **alkaline peptone** water are used to recover pathogens from fecal specimens.





### 3) Classification based on functional use or application:

#### Differential media

Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony colour. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently coloured colonies.

#### Example of differential media

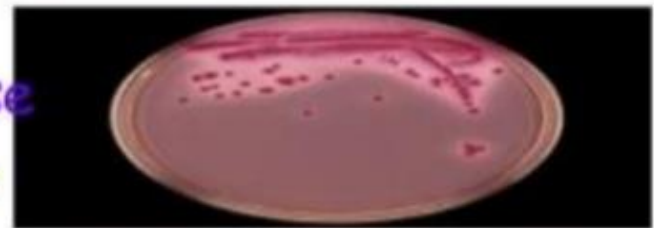
MacConkey's agar, CLED agar, TCBS agar, XLD agar etc





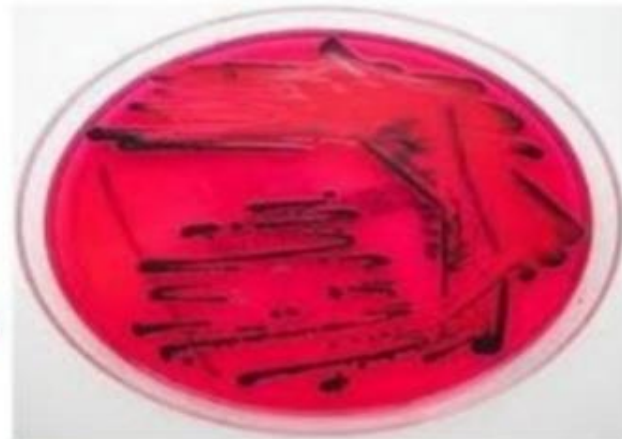
# MacConkey Agar

culture medium designed to grow Gram-negative bacteria and differentiate them for lactose fermentation. It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria).



# XYLOSE LYSINE DEOXYCHOLATE AGAR

- XLD is used as a selective and differential medium for the recovery of *Salmonella* and *Shigella* species.



## Examples of Differential media

### Fungal media

- Dermatophyte test medium



### 3) Classification based on functional use or application:

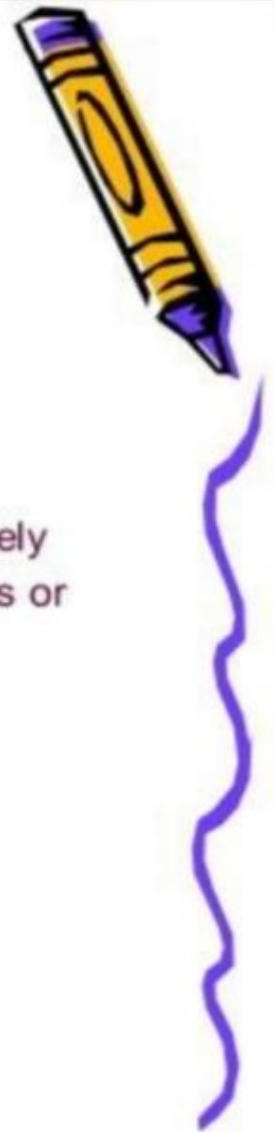
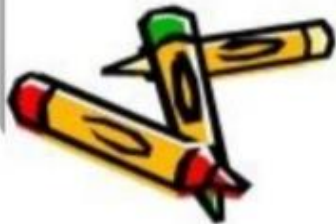
#### Transport media

Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. This can be achieved by using transport media.

#### Example of Transport media

Cary Blair medium for campylobacter species

Alkaline peptone water medium for v. cholerae.





# Some important criteria of Transport media

Transport media should fulfill the following criteria:

- temporary storage of specimens being transported to the laboratory for cultivation.
- maintain the viability of all organisms in the specimen without altering their concentration.
- contain only buffers and salt.
- lack of carbon, nitrogen, and organic growth factors so as to prevent microbial multiplication.
- transport media used in the isolation of anaerobes must be free of molecular oxygen.





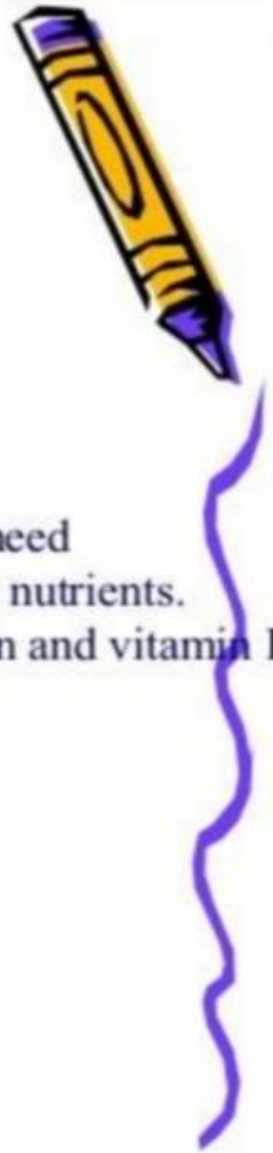
### 3) Classification based on functional use or application:

#### Anaerobic media

Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation–reduction potential and extra nutrients. Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K. Boiling the medium serves to expel any dissolved oxygen.

#### Example of Anaerobic media

Thioglycollate  
medium



*Thank you*

