

# **CULTURE MEDIA**

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# **CULTURE MEDIA**

For any microorganism to be cultured in laboratory, it is necessary to culture medium:

# Culture Media

- **Culture Medium:** Nutrients prepared for microbial growth
- **Sterile:** No living microbes
- **Inoculum:** Introduction of microbes into medium
- **Culture:** Microbes growing in/on culture medium

# The Requirements for Bacterial Growth

- Physical Requirements
- Chemical Requirements

# Physical Requirements

- Temperature
- pH
- Osmotic Pressure

# Chemical Requirements

- Carbon sources
- Nitrogen sources
- Phosphorus sources
- Oxygen sources
- Hydrogen sources
- CHONPS sources
- Trace Elements
- Water

# TYPES OF MEDIA

TABLE 6.2

**A Chemically Defined Medium for Growing a Typical Chemoheterotroph, Such as *E. coli***

Constituent	Amount
Glucose	5.0 g
Ammonium phosphate, monobasic ( $\text{NH}_4\text{H}_2\text{PO}_4$ )	1.0 g
Sodium chloride (NaCl)	5.0 g
Magnesium sulfate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ )	0.2 g
Potassium phosphate, dibasic ( $\text{K}_2\text{HPO}_4$ )	1.0 g
Water	1 liter

TABLE 6.4

**Composition of Nutrient Agar, a Complex Medium for the Growth of Heterotrophic Bacteria**

Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter

# Agar

- Complex polysaccharide
- Used as solidifying agent for culture media in Petri plates, slants, and deeps
- Generally not metabolized by microbes
- Liquefies at 100°C
- Solidifies ~40°C



# CLASSIFICATION OF CULTURE MEDIA

## I. According to composition:

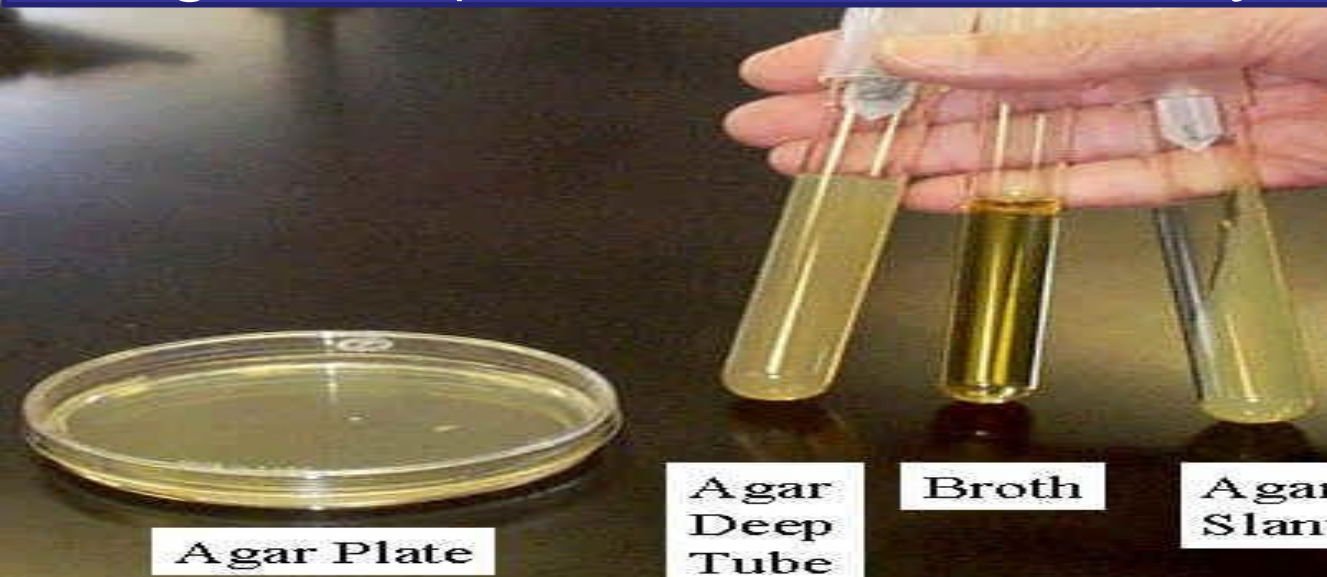
- Chemically Defined Media (**synthetic**): Extracts chemical composition is Known e.g. glucose inorganic salt phosphate for *E. coli*
- Complex Media (**non-synthetic**): chemical composition is not specifically defined; Extracts and digests of yeasts, meat, or plants e.g. Nutrient broth, Nutrient agar, McConkey, EMB  
Usually, bacteria are grown in complex media
- Tissue culture: for OIPs requiring living tissues for growth e.g. HeLa cell lines

- **Examples of elements added in complex media**

- **Beef extract** : concentrate of hot aqueous infusion of fresh beef
- **Peptone**: Spray dried hydrolysate of various proteins
- **Yeast extract**: Spray dried water soluble autolysis yeast cells

## II. According to Consistency:

- 📁 **solid**- with 1.5 to 3.0% agar
  - e.g. NA (Nutrient Agar)
- 📁 **liquid**- no solidifying agent
  - e.g. NB (Nutrient Broth)
- 📁 **semi solid**- with less than 1.5% agar
  - e.g. SIM (Sulfide Indole Motility Medium)



Semi-liquid medium



### **III. According to manner of Dispensing Formation:**

#### **1. Tubed**

**a. broth    b. semi-solid**

#### **2. Plated**

- **Slant Tubes:**



Tubes are containing a nutrient medium plus a solidifying agent, (agar-agar.) The medium has been allowed to solidify at an angle in order to get a flat inoculating surface .

- After autoclaving the media(in tube) for 20 minutes, the tubes are placed in a slanted position to allow the agar to solidify. These tubes are called **slants**



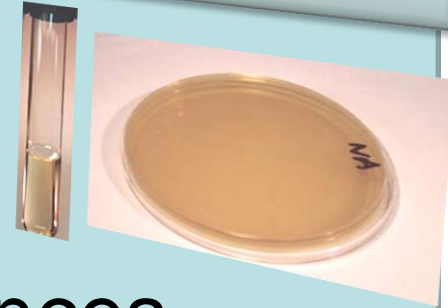
❖ Microorganisms grow on the surface of agar plates and slants

❖ Microorganisms grow on the surface of agar plates and slants

# IV. According to Function/Application:

 **Basal**/ordinary/general

General Media:  
Nutrient Broth and Nutrient Agar



 **Enriched**: with enrichment substances

 **Selective media**: with inhibitory substances

 **Differential media**: with indicators/dyes

 **Special Media for Biochemical Testing**

 **Media for Antimicrobial Susceptibility**

**Testing.**

# CLASSIFICATION OF CULTURE MEDIA

- **Selective Media:**
  - Suppress unwanted microbes and encourage desired microbes.
- **Differential Media:**
  - Make it easy to distinguish colonies of different microbes.
  - culture medium that includes elements, such as chemical indicators that produce observable differences between species of bacteria

- **Enrichment Media:**

Encourages growth of desired microbe: e.g. Selenite F broth favors the growth of *Salmonella* also prevents the growth of normal competitors like *E. coli*. *E. coli* does not die in the medium but they do not flourish like *Salmonella* does.



# Examples of selective media

- MacConkey agar{also can it as differential media}
- Hektoen enteric agar (HE)
- Mannitol salt agar (MSA)

# Examples of Differential media

- Eosin methylene blue (EMB)
- Blood agar
- MacConkey agar
- Mannitol salt agar (MSA)

## VI According to Use

- **Enriched Medium** – broth or solid, contains rich supply of special nutrients that promotes growth of a particular organism while not promoting growth of other microbes that may be (e.g. BAB & Chocolate agar)



# 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(7).

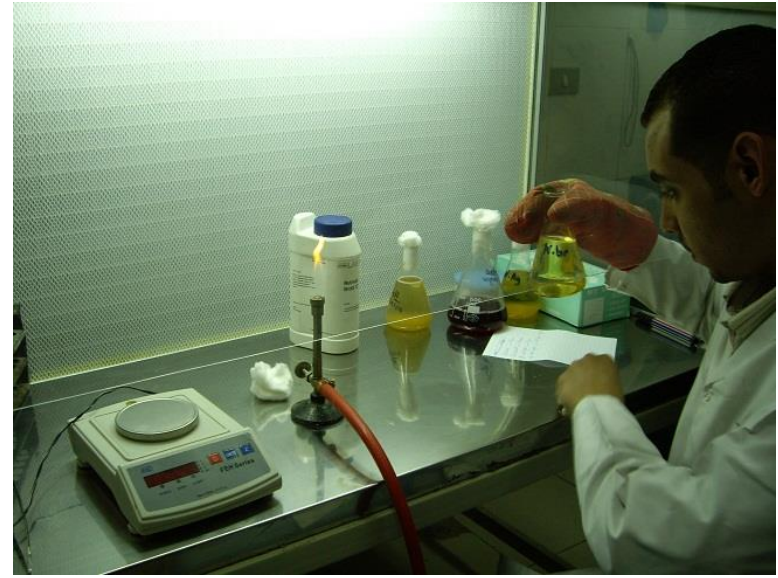
The weight

They dispense the media into bottles(flask,tube).

cap it and **autoclave**. The autoclave exposes the media to high temperature (121°C) and pressure (15 psi) for 20 minutes.

- Once the media is **autoclaved** it is sterile

*(all microorganism forms killed)*



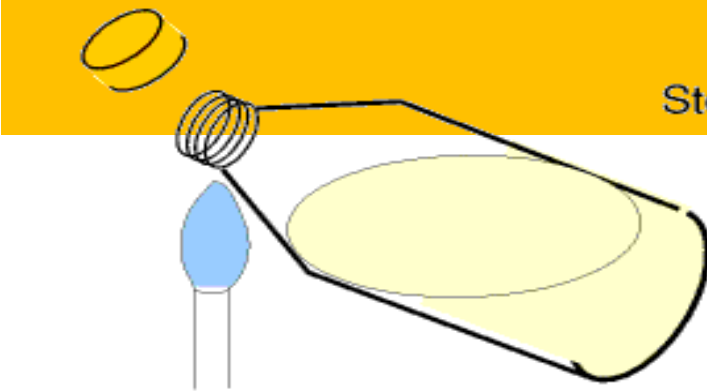


# Aseptically pouring agar plates



## "Pouring a Plate"

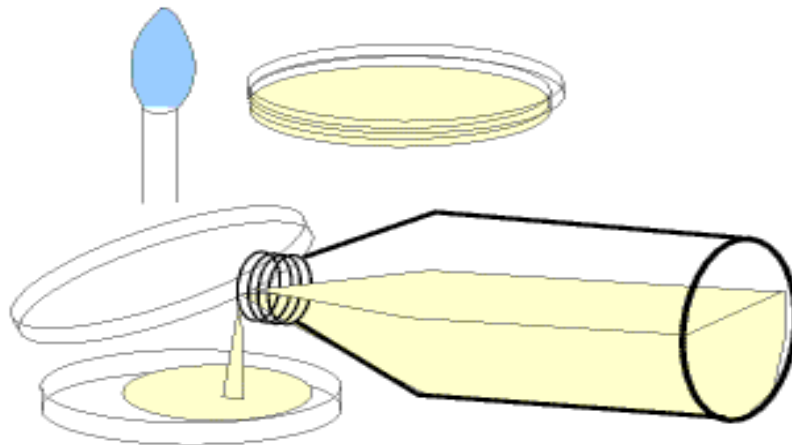
Sterilised molten agar is poured in and left to set.



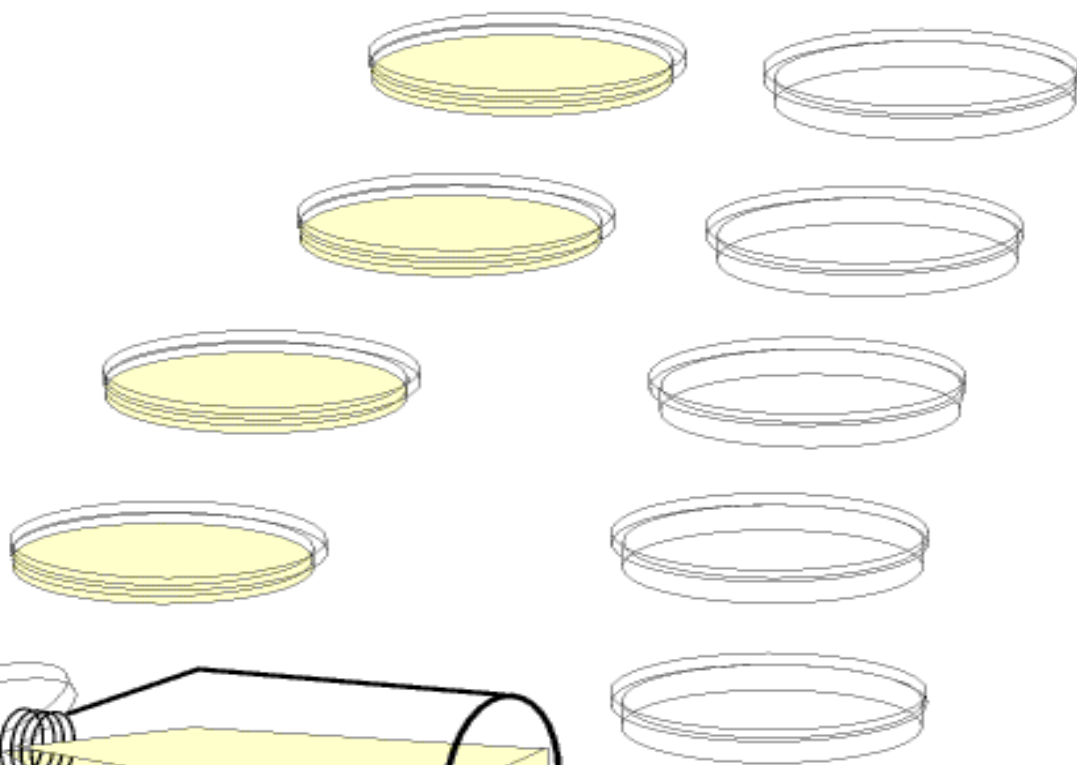
Neck of agar bottle is passed through flame



Petri dish lid is opened as little as possible, angled and kept over the base.



Each Petri dish hold about 20 ml, so 200ml will do for 10.



- Using a marker, **label** **To write** information on culture tubes or **Petri plates** (the name of the microorganism you are growing, your group symbol)





- All labeling is done on the **bottom** of the agar plate

1. Initials
2. Date (mm/dd/yy)
3. Code # or letter



Agar plates are stored upside down to prevent condensation.



- **Sterilize the inoculating loop .**
- Place all inoculated material in **incubator**  
**Culture tubes** should be stored **upright** in **plastic beakers**, while **Petri plates** should be **incubated upside-down** (lid on the bottom )



# Blood Agar

- Contains 5-10% mammalian blood (usually sheep or horse)
- Contains meat extract, NaCl, and agar
- Used to detect hemolytic activity
- $\beta$ -hemolysis ~ complete lysis of RBC
- $\alpha$ -hemolysis ~ partial lysis
- $\gamma$ -hemolysis ~ no lysis



- This media is **differential** because:
- Certain bacteria produce enzymes (hemolysins...) that act on the red cells to produce either:
- ***Beta hemolysis: Enzymes lyse the blood cells completely, producing a clear area around the colony.***
- ***Alpha hemolysis: Incomplete hemolysis produces a greenish discoloration around the colony***
- ***Gamma hemolysis: No effect on the red cells.***



gamma



gamma



beta



alpha



# MacConkey agar

- It contains; bile salts  
(to inhibit Gram-positive bacteria)
- Crystal violet dye
- Neutral red dye (which stains microbes fermenting)
- Lactose
- peptons



# MacConkey's

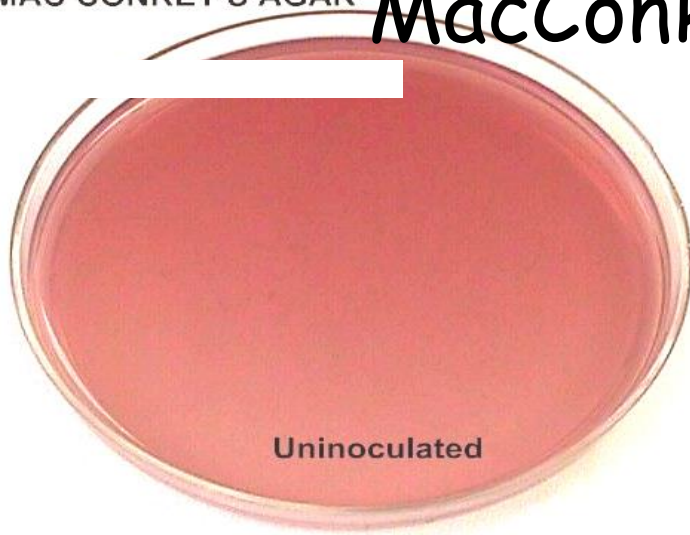
- MacConkey's is both a **selective** & **differential** media.
  - MacConkey's is **selective** media because it *inhibits* the growth of some organisms [Gram positive bacteria].
2. MacConkey's is **differential** media  
“lactose fermenters” bacteria will grow in red colonies while  
non-lactose fermenters” will be colorless and clear.

So if there are colonies of bacteria growing on MacConkey's, it's understood that they are Gram-

- If those colonies are colorless, they are not lactose fermenters.
- If the colonies have a pinkish appearance, they are lactose fermenters

MAC CONKEY'S AGAR

# MacConkey Agar



left: no lactose fermentation

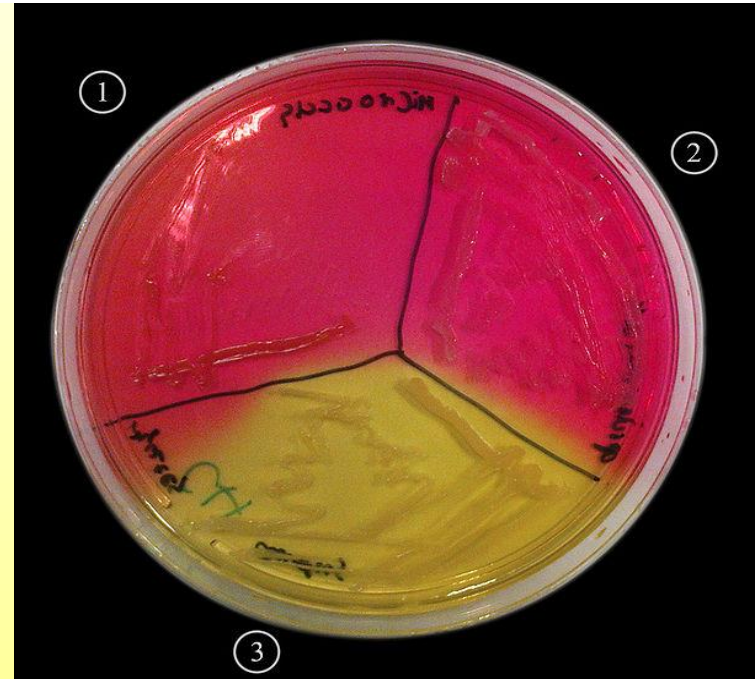
right: lactose fermentation





# Mannitol salt agar (MSA)

- It contains a high concentration (~7.5%-10%) of salt (NaCl), making it selective for *Staphylococci* and *Micrococcaceae*



*Micrococcus* sp(1). *Staphylococcus epidermis* (✓) and *S. aureus* (✓) colonies .

# Eosin methylene blue

- is a selective stain for Gram-negative bacteria
- inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose (e.g., *E. coli*) and those that do not (e.g., *Salmonella*, *Shigella*){as differential media}.



# Chocolate Agar



- Is a non-selective, enriched growth medium
- Contains red blood cells, which have been lysed by heating



# Lowenstein-Jensen Medium

❖ For the cultivation and differentiation of *Mycobacterium* species



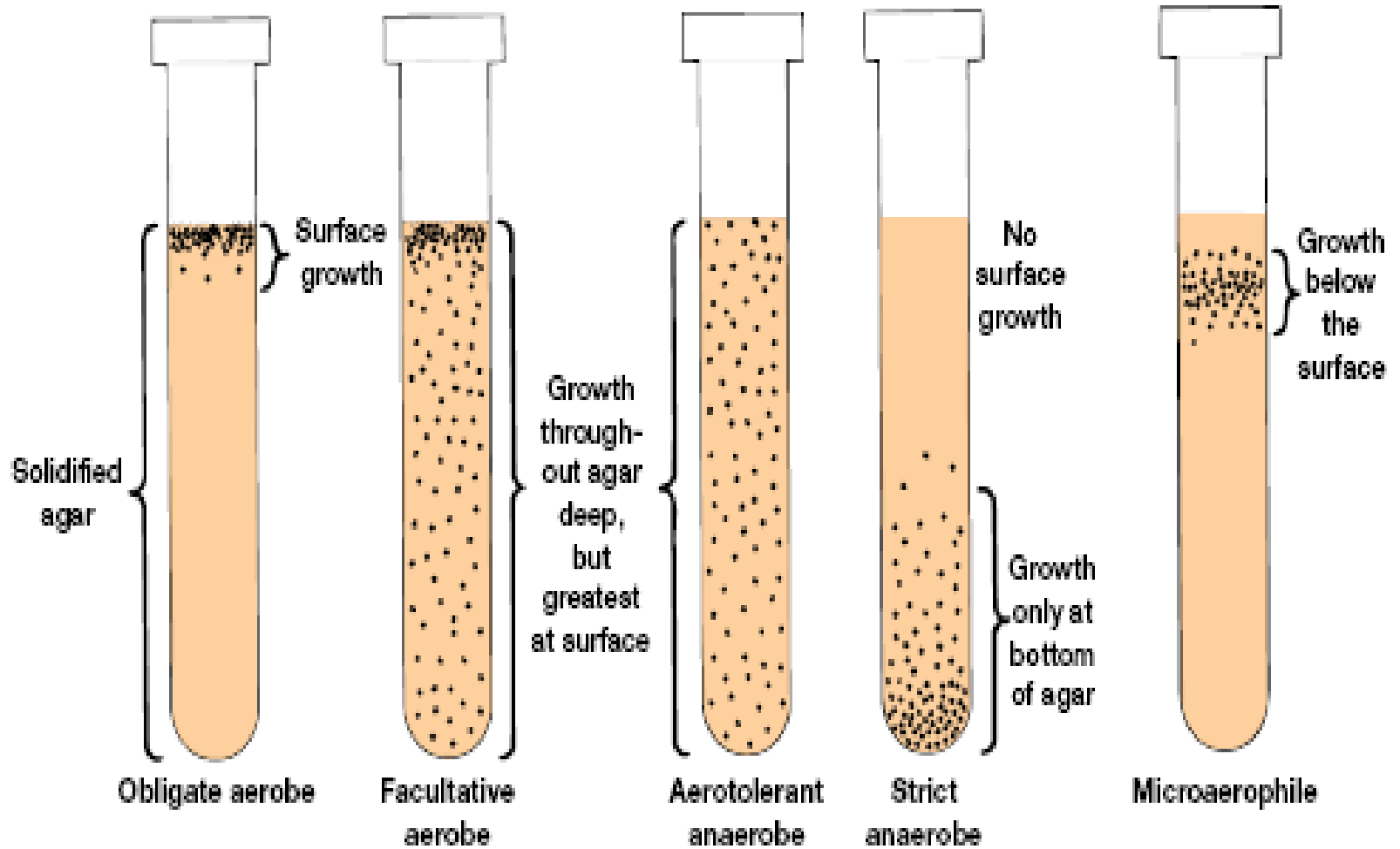
# Mueller-hinton Agar (MHA)



- For antimicrobial susceptibility testing

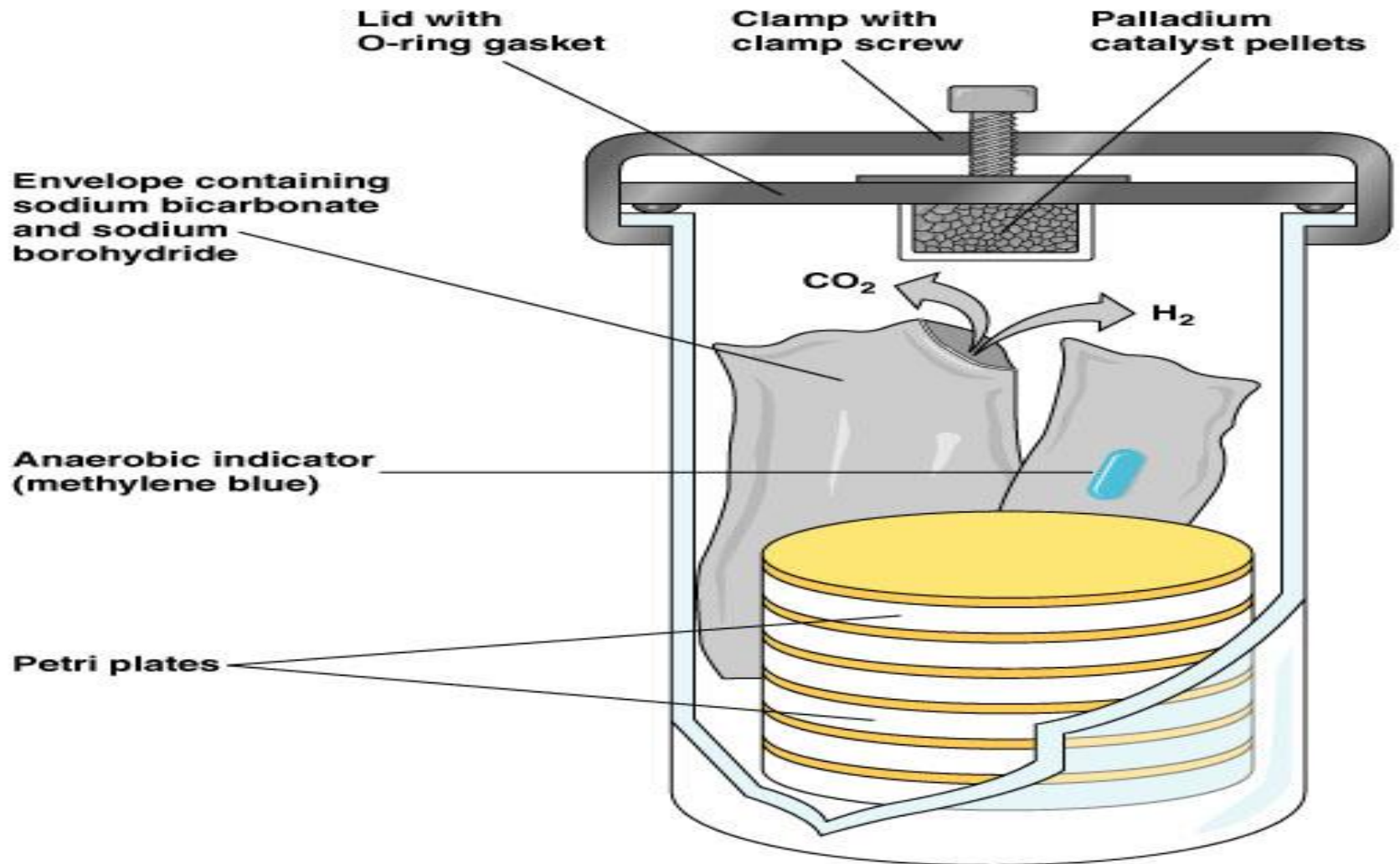
# Anaerobic Culture Methods

- Reducing media
  - Heated to drive off  $O_2$
  - Contain chemicals (thioglycollate or oxyrase) that combine  $O_2$

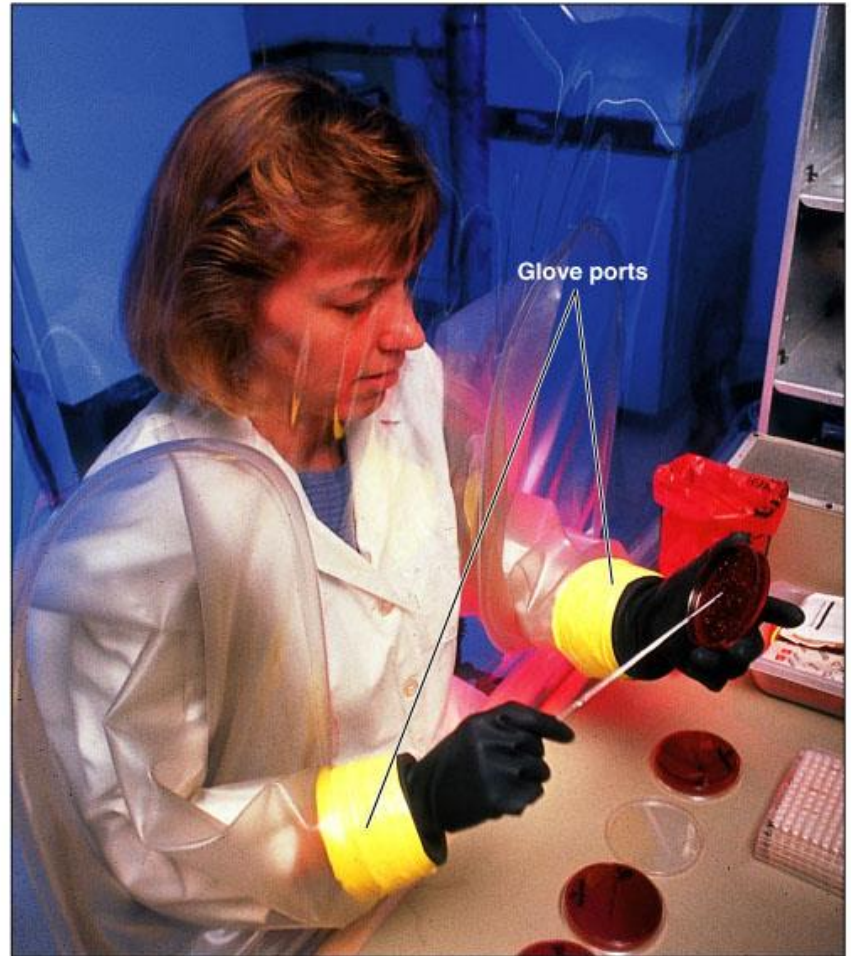




- Anaerobic jar

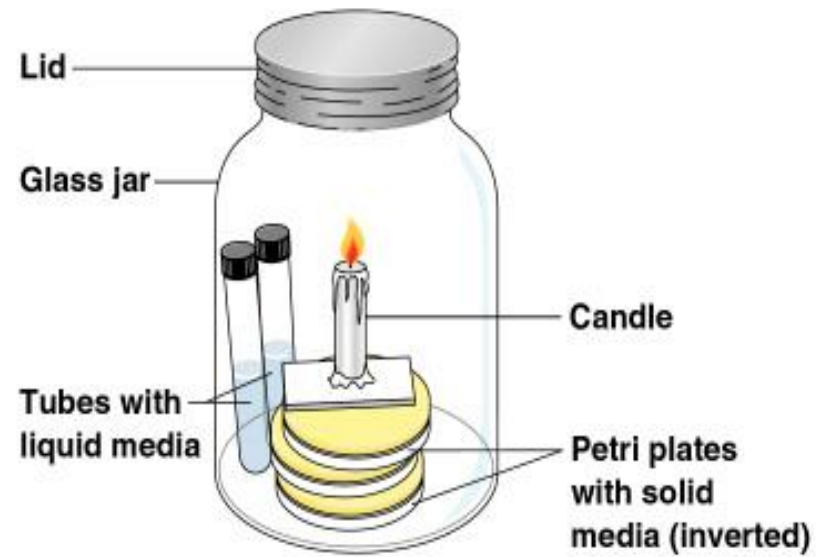


## ○ Anaerobic Chamber



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- Candle jar



- CO<sub>2</sub>-pack



Thank  
You



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