# Culture Media

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# Outline

- Introduction
- Classification of Culture Media
  - By consistency
  - By function (selective, differential, enriched)
- Practical Demonstration/Activity
  - Streak plate technique overview
  - Incubation conditions

# Culture Media - Introduction

## What Are Culture Media?

Nutrient substances (solid or liquid) used to grow and isolate bacteria in the laboratory

## Why Do We Need Different Types of Media?

Different bacteria have different needs:

- Some bacteria grow easily anywhere
- Some bacteria are (fastidious) "picky eaters" - need special nutrients
- Some bacteria are overgrown by others - need selective environment
- We need to differentiate similar-looking bacteria

- **Solution:** Use DIFFERENT types of media for different purposes!

# Basic Components of Culture Media

**Essential ingredients all media contain:**

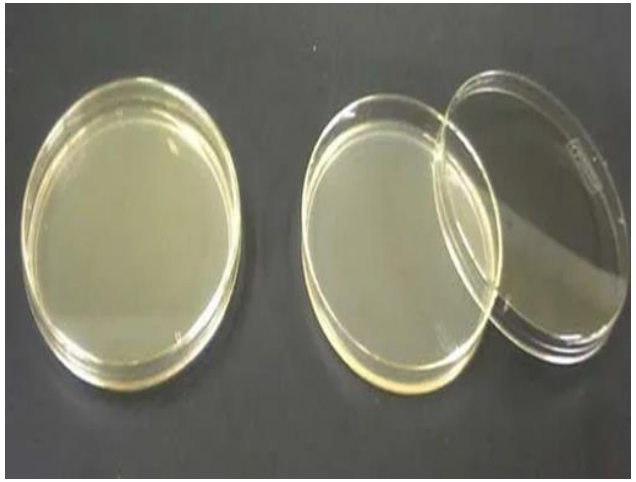
- **Water** - solvent, 95% of medium
- **Peptone/Protein source** - provides amino acids, nitrogen
- **Beef or yeast extract** - provides vitamins, minerals
- **Sodium chloride (NaCl)** - maintains osmotic balance
- **Agar** (for solid media) - solidifying agent, bacteria can't digest it

**Optional additions based on purpose:**

- Specific nutrients (blood, sugars, vitamins)
- pH indicators
- Selective agents (antibiotics, salts, dyes)
- Iron compounds

# Classification by Consistency

- **Solid media:** Agar (1.5-2%) - for isolation and colony morphology
- **Semi-solid media:** Agar (0.3-0.5%) - for motility testing
- **Liquid media (Broth):** No agar - for enrichment

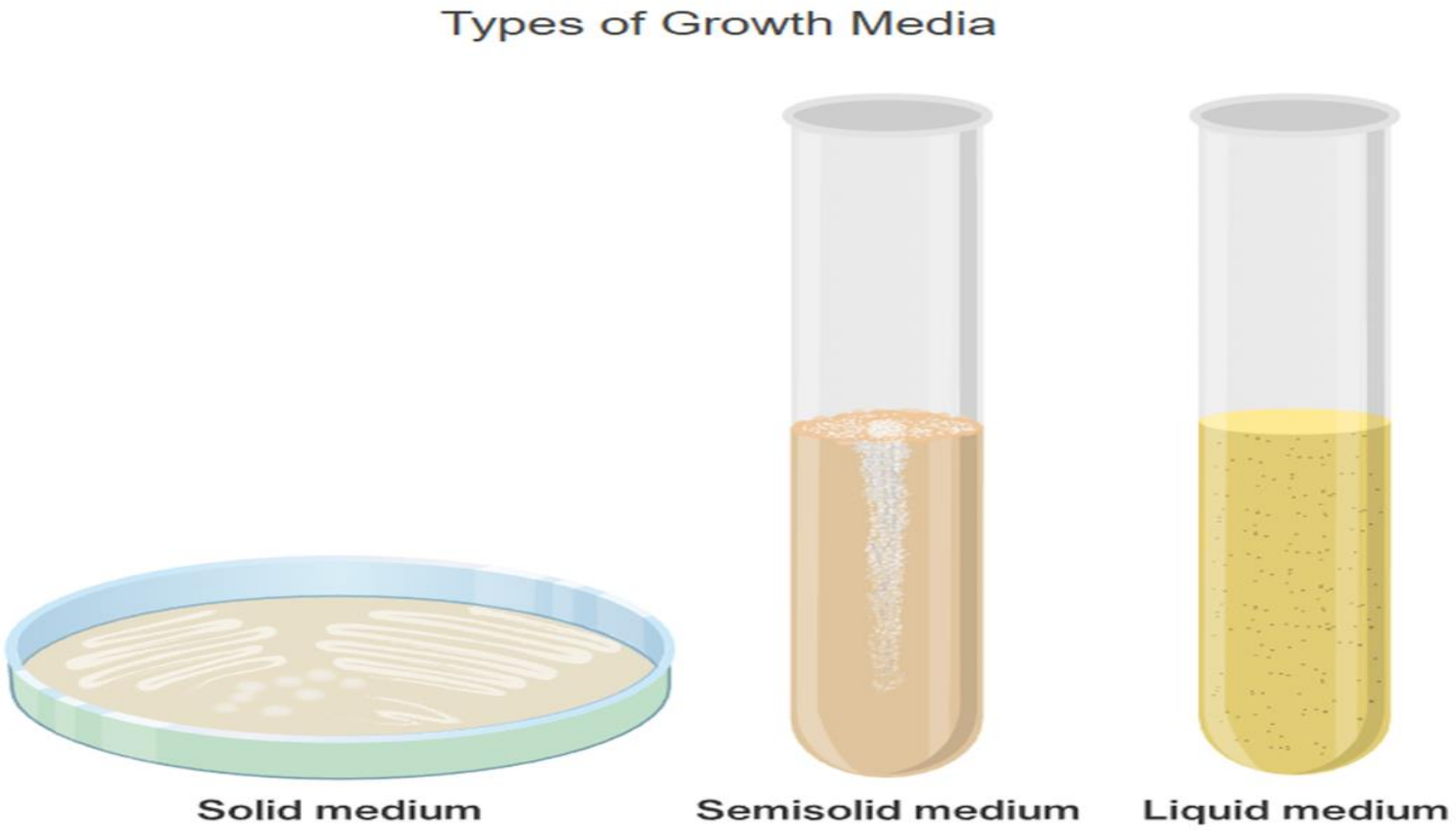


**Solid**

**Semi- Solid**

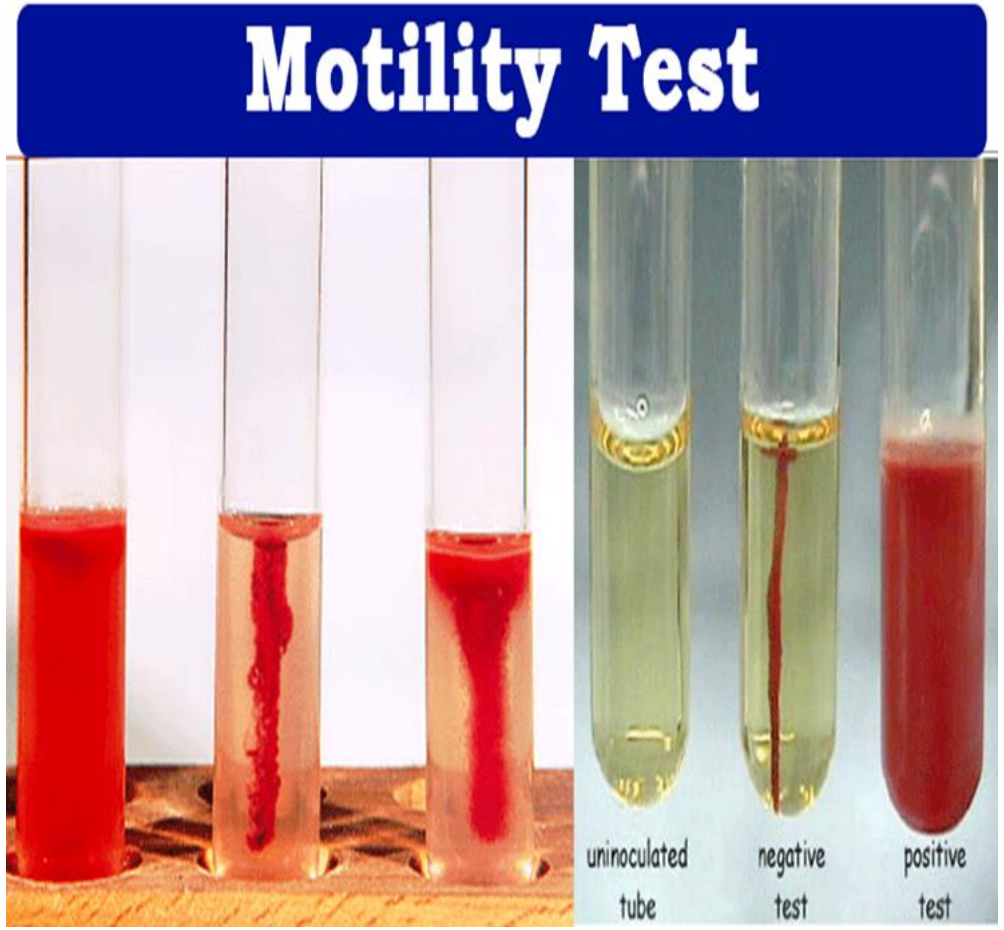

**Liquid**

# Classification by Consistency



# Semi-Solid Media for Motility Testing

- **Semi-solid media (0.3-0.5% agar)**
- **Principle:** Soft agar allows motile bacteria to migrate away from stab line
- **Method:** Stab inoculate in center with straight wire
- **Results:**
  - **Motile organisms:** Growth spreads throughout medium (turbidity/haziness)
  - **Non-motile organisms:** Growth only along stab line (sharp line)



# Classification by Function: Non-Selective Media

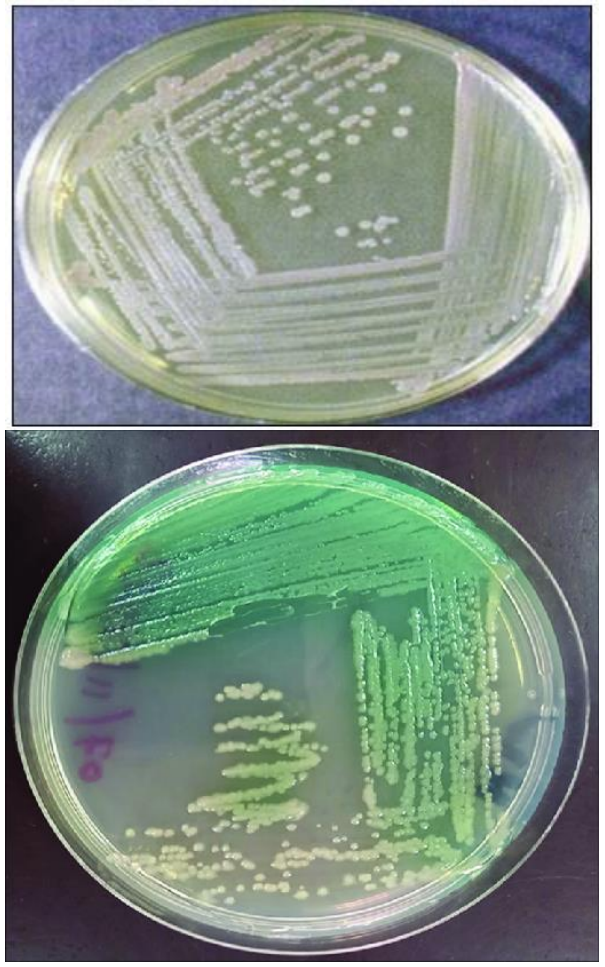
- Purpose: Support growth of MOST bacteria
- **No inhibitors** - almost any bacteria can grow

## A. Basal/General Purpose Media

- Basic medium: peptone + beef extract + agar
- Simple, inexpensive
- For non-fastidious bacteria
- Example: Nutrient Agar

## B. Enriched Media

- Contain additional nutrients for fastidious organisms



The growth of Pseudomonas aeruginosa on nutritional agar

# Classification by Function: Non-Selective Media- Enriched Media

**Types:**

1. **Blood Agar**: Sheep blood (5%) + nutrient agar
   - Rich medium - supports fastidious organisms
   - Also serves as differential medium (haemolysis patterns)

2. **Chocolate Agar:** Blood agar heated until brown (some blood components released)
   - **Even richer** than blood agar
   - **Uses:** Fastidious organisms like *Haemophilus influenzae*

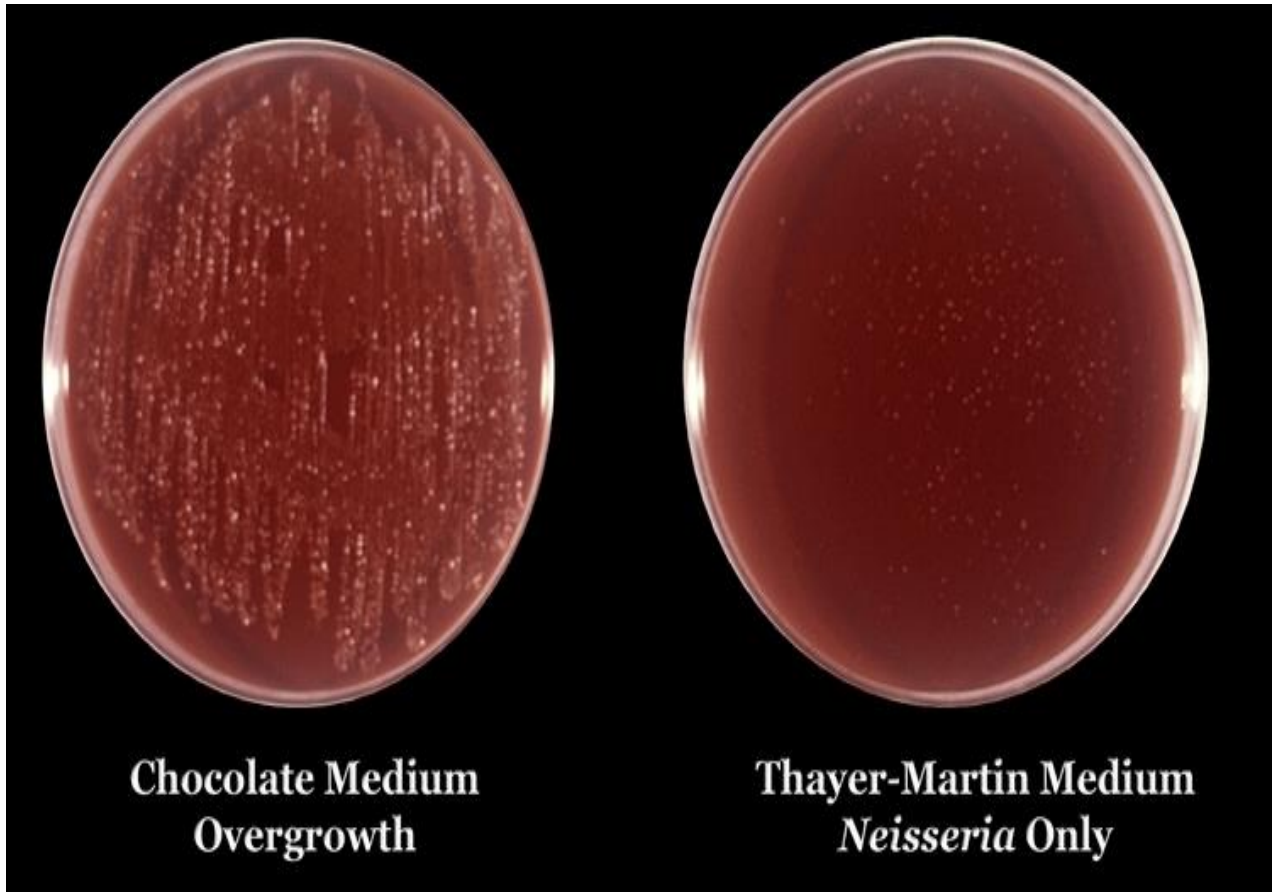
# Classification by Function: Selective Media

- **Purpose:** INHIBIT growth of unwanted bacteria, allow desired bacteria to grow
- **Contains inhibitory substances (antibiotics, dyes, salts, pH)**
- **Concept:** Mixed sample → Selective media → Only desired bacteria grow

# Classification by Function: Selective Media- Example

**Thayer-Martin Agar (For *Neisseria*)**

- Chocolate agar + **antibiotics** (vancomycin, colistin, nystatin)
- Antibiotics inhibit normal flora
- **Use:** Isolate *N. gonorrhoeae*, *N. meningitidis* from genital/throat specimens




**Rectal specimen**

# Classification by Function: Differential Media

- Allow multiple bacteria to grow, but distinguish between them based on biochemical properties
- Contains indicators that produce visible changes based on bacterial metabolism

**Concept:**

Different bacteria → Different metabolic activities → Different appearance on plate

**Examples:** Blood Agar (Also differential!)

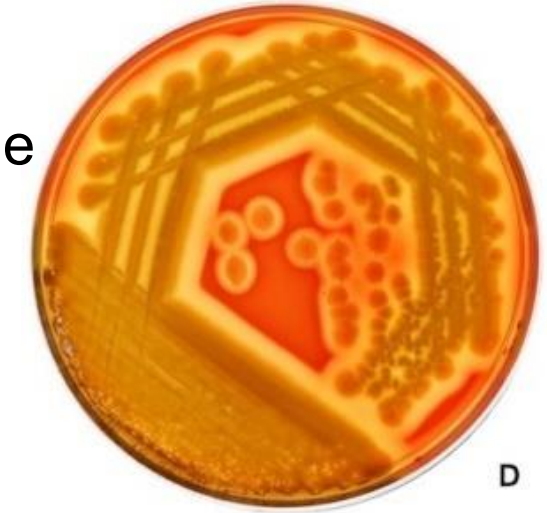
# Classification by Function: Differential Media- Example

**Alpha (α) hemolysis:**

- Partial lysis of red blood cells, the bacteria's enzymes convert hemoglobin to methemoglobin.
- Appear as a greenish or brownish discoloration surrounding the bacterial colony.
- Example: *Streptococcus pneumoniae*


**Beta (β) hemolysis:**

- The bacteria produce hemolysin enzyme that completely lyse the red blood cells.
- A transparent, clear zone around the bacterial colony
- Example: *Streptococcus pyogenes*



# Classification by Function: Differential Media- Example

**Gamma (γ) hemolysis:**

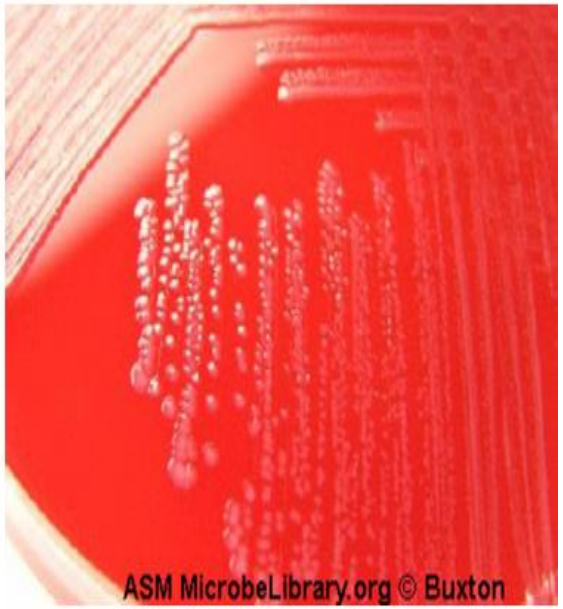
- No hemolysis, meaning no lysis of red blood cells occurs.
- No change in the blood agar. The area around the colony remains opaque and red, the bacteria do not produce hemolysins enzyme.
- Example: *Enterococcus faecalis*

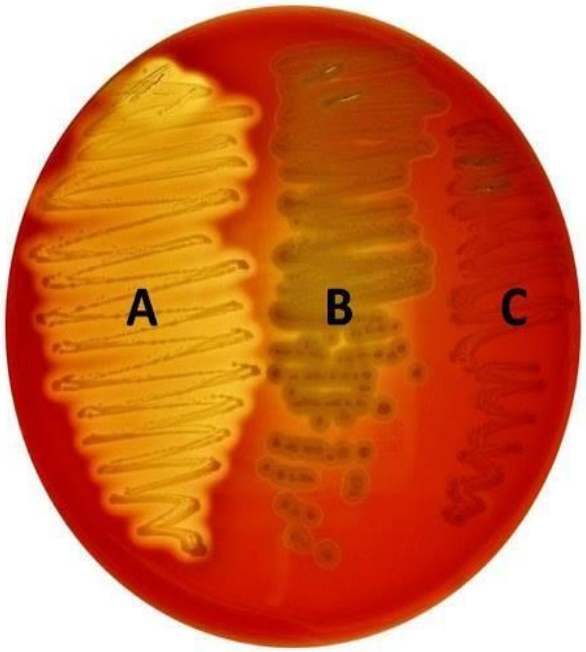
# Classification by Function: Differential Media- Blood Agar

Alpha hemolysis
(partial)


Beta hemolysis
(complete)


Gamma hemolysis
(none)

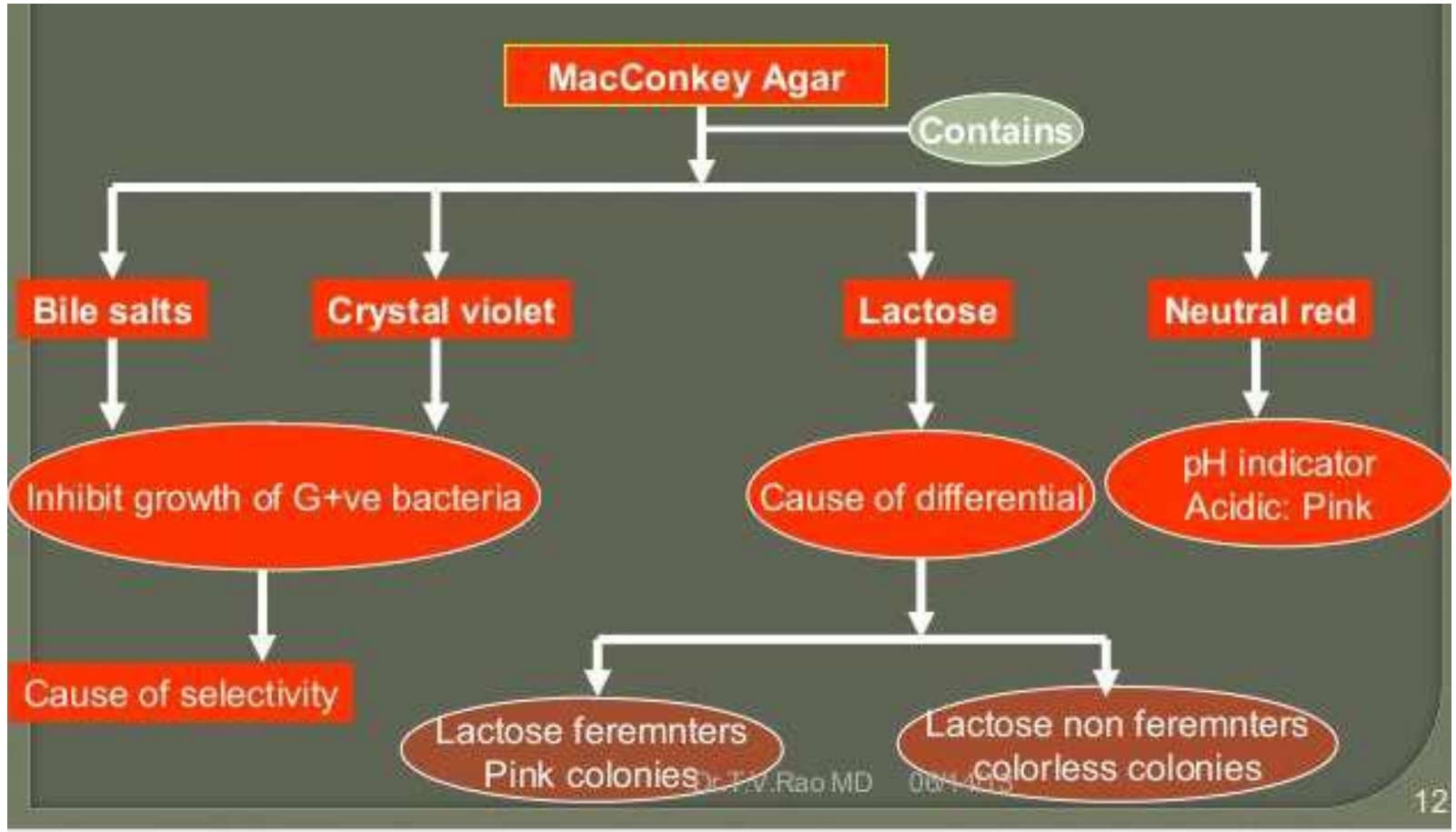


# Classification by Function: Selective and Differential Media- MacConkey Agar

**MacConkey Agar (For Gram-negative bacteria)**

- Contains:
  - **Bile salts** + **Crystal violet dye** → inhibit Gram-positive bacteria
  - Lactose + pH indicator
- **Result:** Only Gram-negative bacteria grow
- **Use:** Isolate enteric bacteria (*E. coli*, *Salmonella*, *Shigella*) from stool


MacConkey Agar
Contains
Bile salts
Crystal violet
Lactose
Neutral red
Inhibit growth of G+ve bacteria
Cause of differential
pH indicator
Acidic: Pink
Cause of selectivity
Lactose feremnters
Pink colonies
Lactose non feremnters
colorless colonies
Dr.T.V.Rao MD
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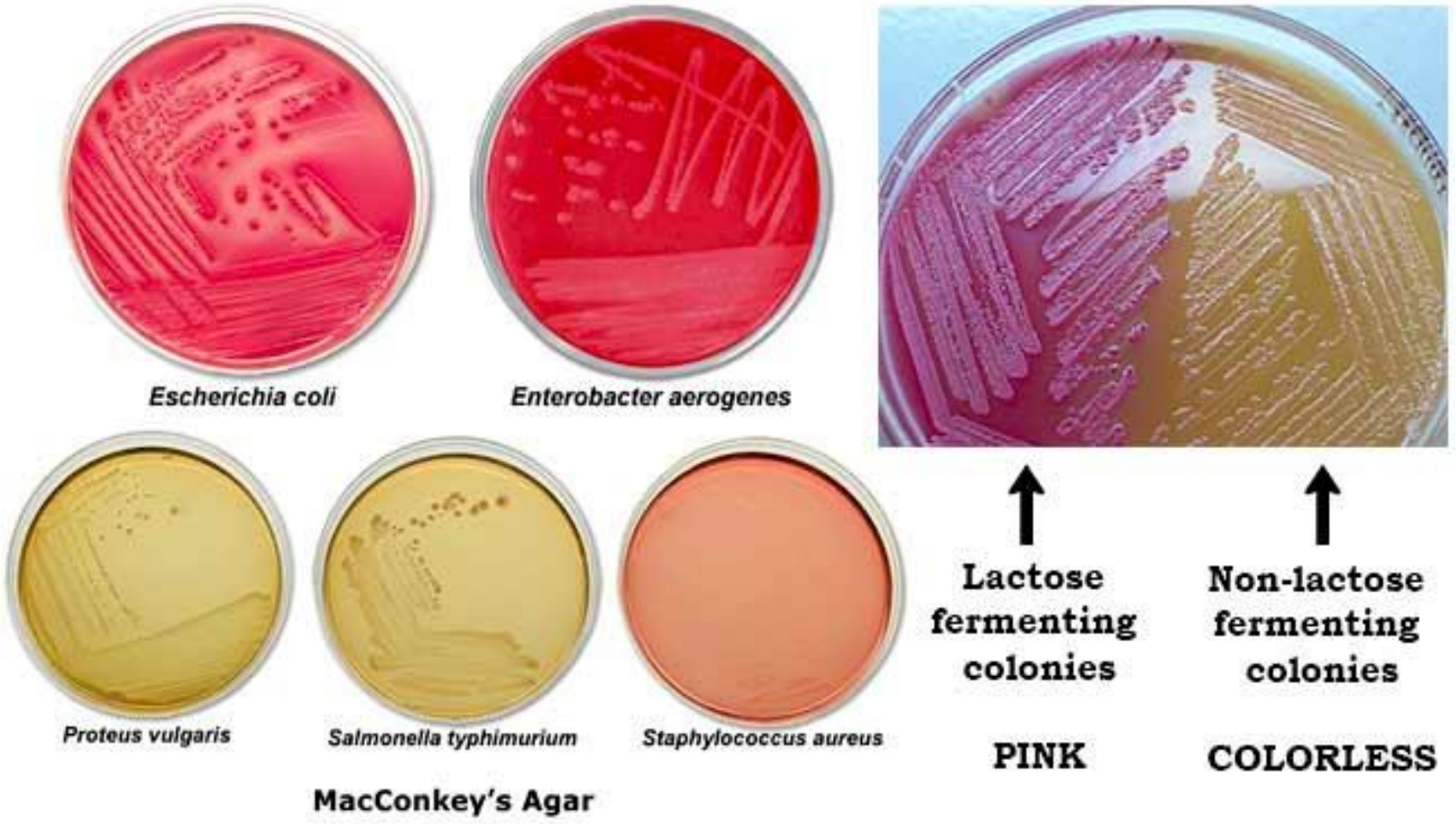
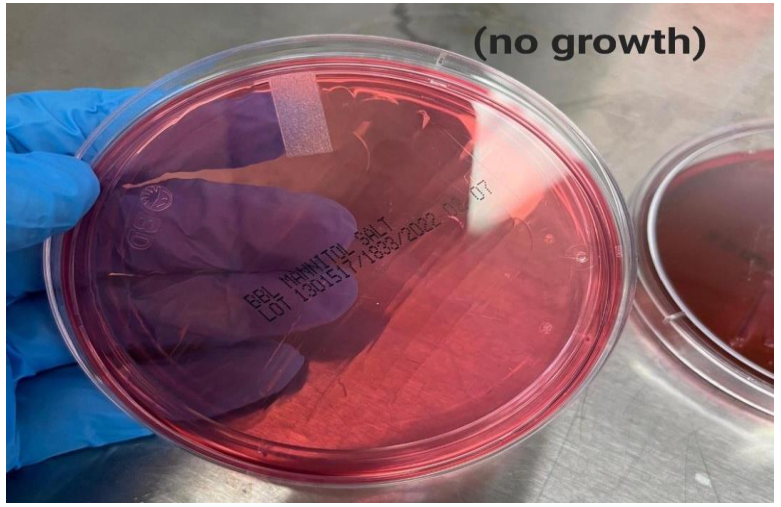
# Classification by Function: Selective and Differential Media- MacConkey Agar

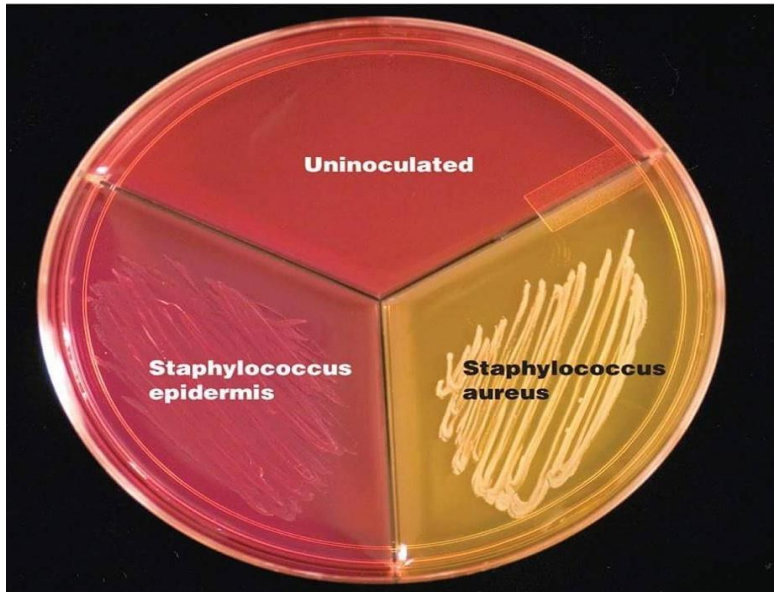


# Classification by Function: Selective and Differential Media- Mannitol Salt Agar

**Mannitol Salt Agar (For *Staphylococcus*)**

- Contains:
  - **7.5% NaCl** (high salt) → inhibits most bacteria
  - Mannitol + pH indicator
- **Result:** Only salt-tolerant bacteria (*Staphylococcus*) grow
- **Use:** Isolate *Staphylococcus* from skin/wound specimens

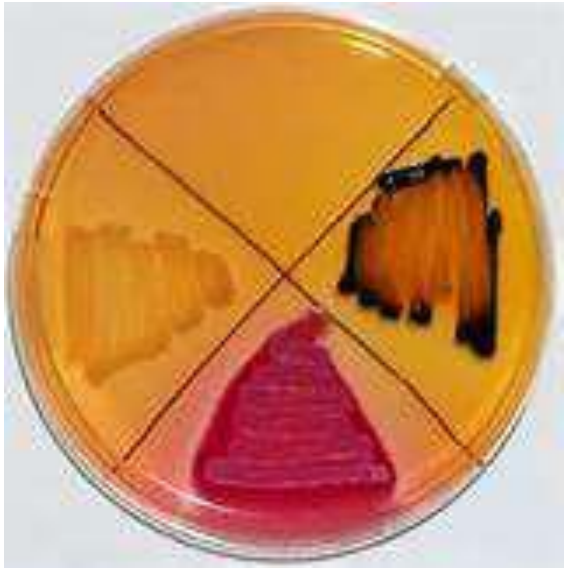


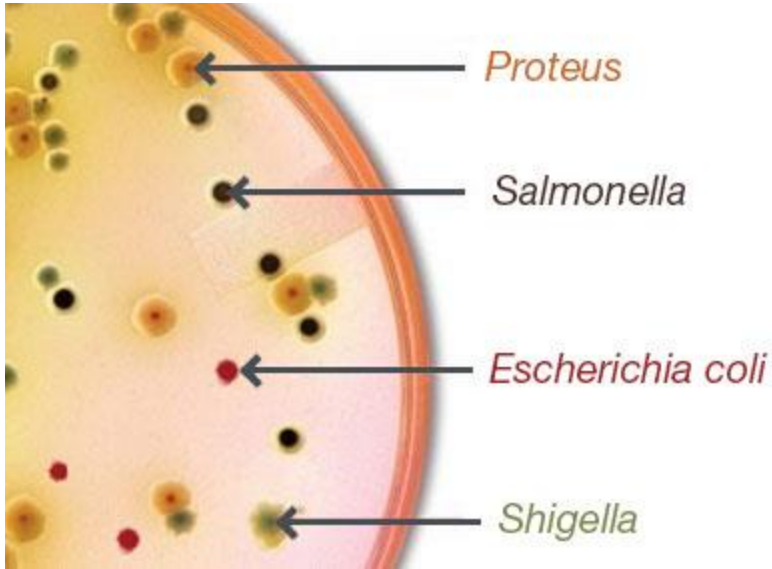




E. coli-
No growth
Pseudomonas
aeruginosa-
No growth
E. coli
Pseudo
Staph
Staphylococcus
aureus
-Yellow
colony
Mannitol Salt Agar (MSA)

# Salmonella Shigella agar (SS agar)

- **Purpose**
  - For isolation and differentiation of Salmonella & Shigella
- **Components**
  - **Bile salt:** inhibit the growth of gram-positive bacteria (selective
  - Lactose + pH indicator





- Why black colonies?
- Due to the production of FeS (ferrous sulfide forming black precipitate presented by black centered colonies)

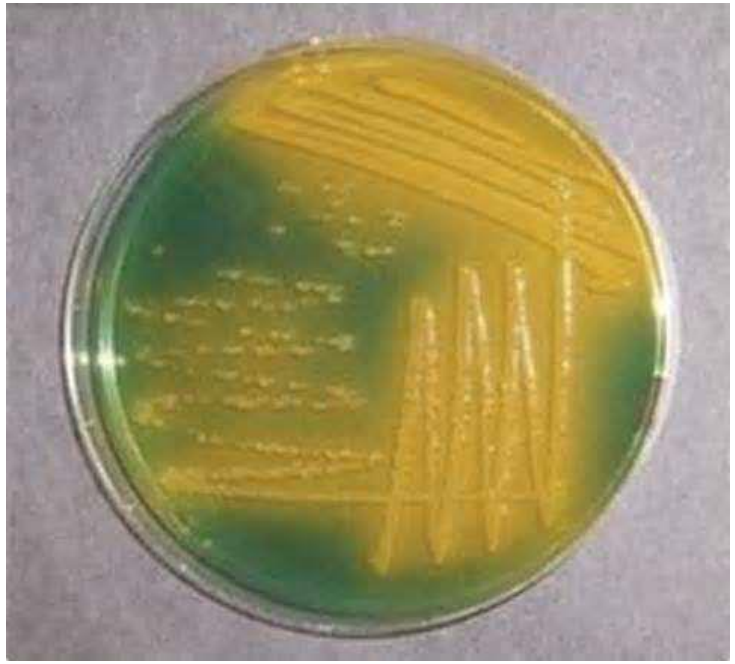
# Cholera identification

- Identification
  - Thiosulfate citrate bile salt sucrose agar or TCBS agar
  - The medium is alkaline (pH 8.6 ) which enhances the growth of Vibrio species
- Important components
  - Sucrose: sugar source
  - Bromothymol blue: pH indicator
    - pH< 6.0 yellow
    - pH> 7.6 blue

# Cholera identification

- Results
  - *Vibrio cholera:* Ferment sucrose smooth yellow colonies
  - *Vibrio parahemolyticus:* non sucrose fermenter, green colonies

TCBS media


V. cholera


V. parahemolyticus

# Streaking Technique for Isolation

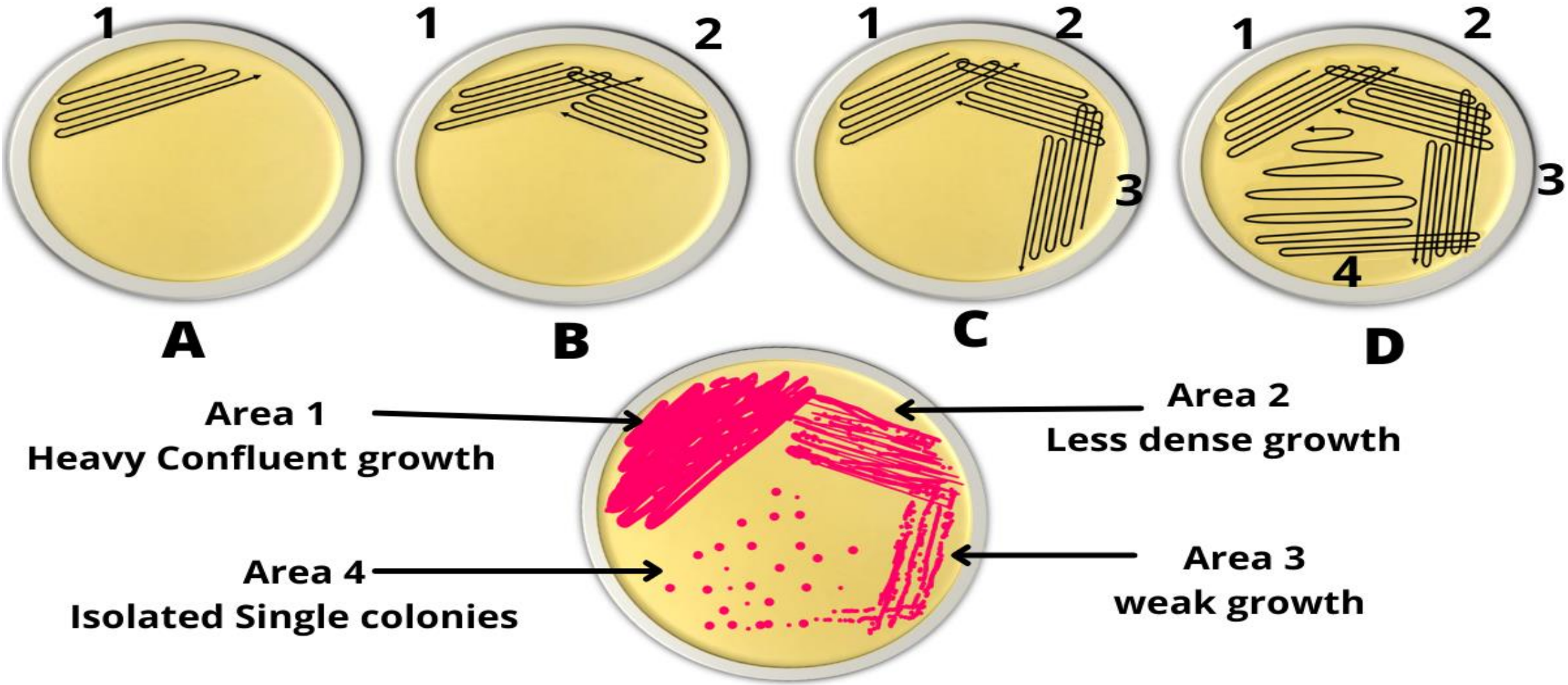
**Purpose:** dilute bacteria to obtain isolated single colonies

**Steps:**

- **Quadrant 1:** pick specimen, streak heavily in first quadrant
- **Quadrant 2:** streak 2-3 times from Quadrant 1 into Quadrant 2
- **Quadrant 3:** streak 2-3 times from Quadrant 2 into Quadrant 3
- **Quadrant 4:** streak 2-3 times from Quadrant 3 into Quadrant 4

**Key Points:** sterilize loop between quadrants (prevents carry-over)

# Streaking Technique for Isolation



# Incubation Conditions

**Standard Conditions:**

- **Temperature:** 35-37°C (body temperature)
- **Time:** 18-24 hours (routine), up to 48-72 hours for slow growers
- **Atmospheric Requirements:**
    1. Aerobic incubation: Room air (most organisms)
    2. $CO_2$ incubation (5-10%): Candle jar or $CO_2$ incubator as *Neisseria* species, *Streptococcus pneumoniae*
    3. Anaerobic incubation: Anaerobic jar/chamber

# Incubation Conditions: Anaerobic Environment

**The anaerobic jar method:**

It is a technique to create an oxygen-free environment for cultivating anaerobic microorganisms like *Clostridium perfringens*.

| **Component** | **Function** |
| --- | --- |
| **$H_2$ (from sachet)** | Reacts with $O_2$ to remove it |
| **Palladium catalyst** | Speeds up $H_2 + O_2 \rightarrow H_2O$ reaction |
| **$CO_2$ (from sachet)** | Provides required $CO_2$ for bacterial growth + maintains pressure |
| **Methylene blue** | Anaerobic indicator (colorless when no $O_2$) |

# Incubation Conditions: Anaerobic Environment

**How it works:**

1. Petri dishes containing the cultures are placed inside the jar.
2. Gas generation: A gas-generating sachet is added, which, when exposed to water, releases hydrogen and carbon dioxide.
3. Catalytic conversion: The hydrogen gas reacts with any oxygen present in the jar in the presence of a palladium catalyst, converting the oxygen into water.
4. Indicator check: An indicator strip (like methylene blue) is placed in the jar to confirm the process.
   - Methylene blue turns from blue to white (or colourless).

# Thank you