Culture Media

Culture Media used in Microbiology

Culture Media

- Provide certain environmental conditions, nutrients & energy in order to grow and produce bacteria

- Different categories of media can be made according to the type and combination of nutrients

Culture Media

A) Physiological requirements:

C.HOPKENS

- C= Source of carbon
- H= Hydrogen
- O= Oxygen
- P= Phosphorus
- K= Potassium
- E= Electrolytes
- N= Source of nitrogen
- S= Sulfur

Culture Media

B) Environmental conditions:

1- Oxygen (O2)

Obligatory aerobe, obligatory anaerobe, facultative anaerobe, Aerotolerant, and microaerophilic.

Culture Media

2- Temperature

Thermophile, mesophile, and psycrophile

3- PH

Neutrophile, basophile, and acidophile .

4- Osmotic pressure

Isotonic, hypotonic, and hypertonic

Culture media can be classified according to their:

A) Physical states >>

- Solid media
- Semi-solid media
- Liquid media

B) Function (uses)>>

- Basic media
- Enriched media
- Enrichment media
- Selective media
- Differential media
- Transport media

1.Solid Media

- Contain 1.5-2% agar
- Mainly used in Petri dishes
- Could be used in bottles or tubes to make deep or slope cultures
- **Function:**

➢ Colony morphology, pigmentation, hemolysis can be seen

➢ Can be used to prepare pure culture

➢ Used for detecting of contaminant

- Ex: Blood agar

2.Semi-solid Media

- Contain 0.5% agar (small amount)
- Function:

➢ Used as transport media

➢ Used for motility testing

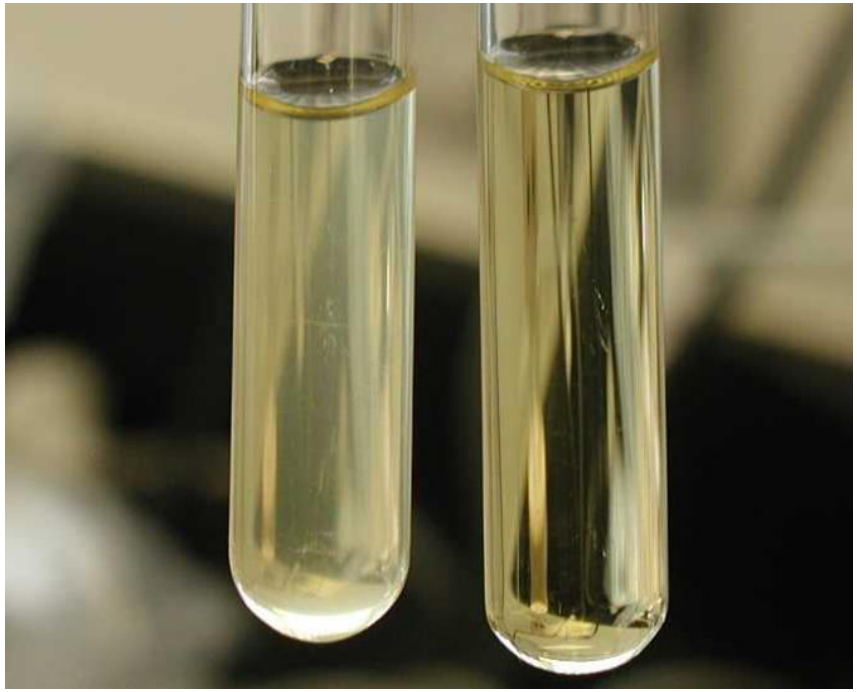
➢ Used for biochemical tests

- Ex: SIM

The organism in the tube on the left in the figure is motile and the organism in the tube on the right is nonmotile.

3.Liquid Media (broth)

- No agar
- Function:
  - ➢ Used as enrichment media
  - ➢ Used for biochemical tests
- The growth could be observed as turbidity
- Ex: nutrient broth



1.Basic Media

- Simple media (basic no additive)
- Support the growth of microorganism that do not have special nutritional requirement
- Ex: Nutrient agar and Nutrient broth.

2.Enriched Media

- Contain simple nutrients & additional requirements such as: blood, serum, peptones, vitamins
- Support the growth of a wide variety of organisms, including fastidious and pathogenic organisms
- Ex: Blood agar, Chocolate agar

3.Enrichment media

- It is fluid medium that encourage the multiplication of wanted bacteria (discourage the growth of unwanted bacteria)
- Ex: Selenite broth used for salmonella

4.Selective Media

- This media contain substances that inhibit the growth of some microorganisms and allow others to grow
- Ex of inhibiting substances: bile salt, dyes, antibiotics
- Ex: Manitol salt agar is a selective media for *Staph aureus*

5.Differential media

- Used to differentiate microorganisms by using indicator or dyes
- Ex: CLED >> differentiate between LF (yellow) and NLF (blue)

6.Differential and Selective media

- Many cultures media are both differential and selective
- Ex: MacConkey agar, XLD agar and DCA

7.Transport media

- These types of media contains ingredients to prevent over growth of commensals and ensure the survival of aerobic and an aerobic pathogens
- Used when specimen cannot cultured as soon as collected
- Usually semi-solid
- Ex: Cary-Blair medium, Amies medium

8.Biochemical media:

- Used to test the biochemical activity of microorganisms, such as: TSI, SIM.

9.Standard media:

- Used as quality control & susceptibility test, such as: mueller-Hinton agar

Media Preparation

Preparation of solid culture media:

1. Weight the proper amount of ingredient which is in dehydrated form (powder) and put it in a flask
2. Add the proper amount of water to the flask
3. Do genital mixing and do not forget to label
4. Sterilize by autoclaving at 121c/15 psi for 15-30 min
6. Left the media to cool (45°C) then pour it in Petri dishes (25 ml) or slants under aseptic condition (near the flame and the bench is clean with disinfectant)
7. Keep the media in the fridge.

Preparation of liquid media:

1. Weight the proper amount of ingredient which is in dehydrated form (powder) and put it in a flask
2. Add the proper amount of water to the flask
3. Do genital mixing and do not forget to label
4. Dispense the media in tubes
5. Sterilize by Autoclaving

(some broth need sugar or chemical so after the autoclaving add the chemical (e.g. sugar) then we dispense in sterile tube)

6. Keep the media in the fridge.

Types of Culture Media

Blood Agar:

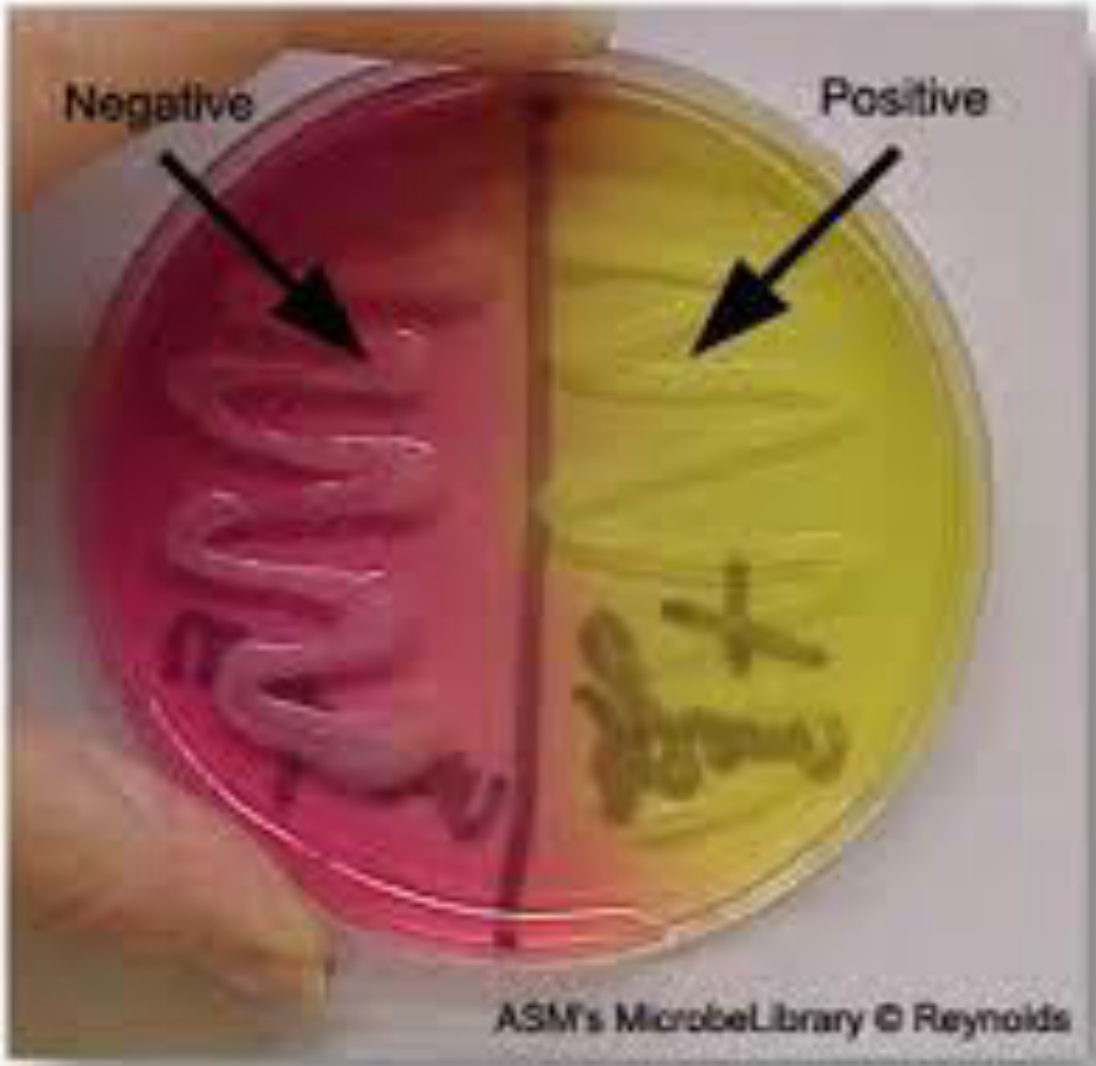
- Enriched medium (provide extra nutrient for bacteria).
- Differential media between α & β hemolytic streptococci.
- It is composed of nutrient agar + sheep or horse or human RBCs.

Chocolate Agar:

- Enriched media (contain lysed RBC).
- Used for growing of fastidious respiratory bacteria like: *Haemophilus influenza.*
- Not useful for fecal culture.

Mannitol Salt Agar:

- Selective and differential media
- Selective for Staphylococcus (Contains high salt concentration (7.5%) which inhibit most bacteria while Staphylococcus can grow)
- Differential between *S. aureus* and CN Staph
- Sugar: mannitol
- Indicator: phenol red
- *Staph aureus*>>> give yellow colonies


Negative
Positive
ASM's MicrobeLibrary © Reynolds

Enterics Culture Media

Enterobacteriaceae "Enterics"

- Called "Enterics" because they're in intestine or gut of humans and animals

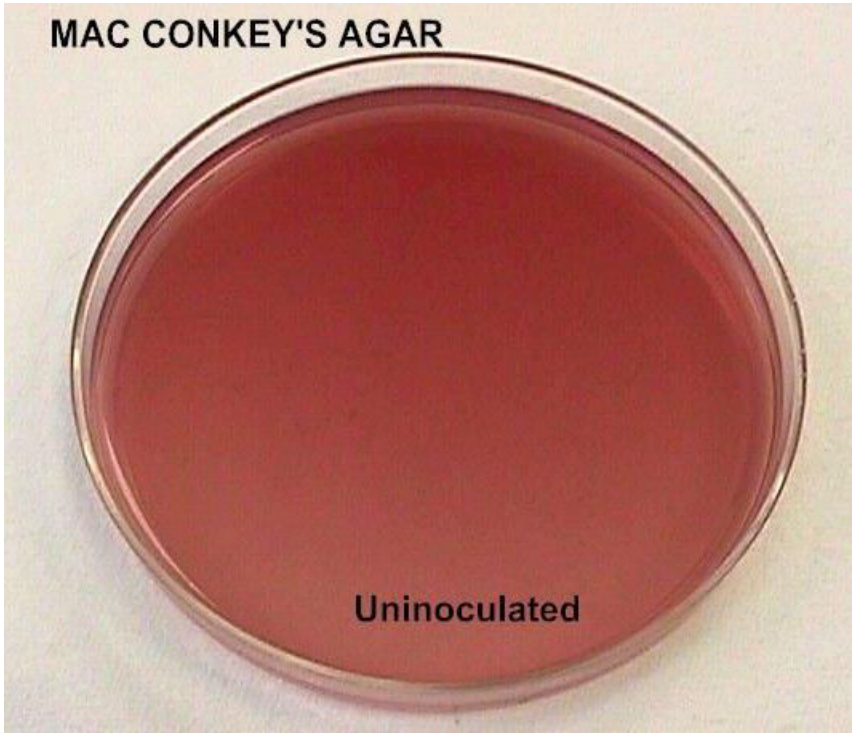
- Gram negative bacilli

- Enterics family included very large number of bacteria, Ex: *Escherichia coli (E.coli), Salmonella, Shigella, Klebsiella, Proteus, Enterobacter, Serratia,* and *Yersinia*

MacConkey Agar (Mac):

- It is selective and differential medium
- Bile salts & crystal violet inhibit the growth of gram +ve organisms
- Designed to isolate and differentiate enterics based on their fermenting characteristics
- Sugar: Lactose
- Neutral red is the pH indicator
- LF>> Pink colonies,,,, NLF>> Colorless colonies

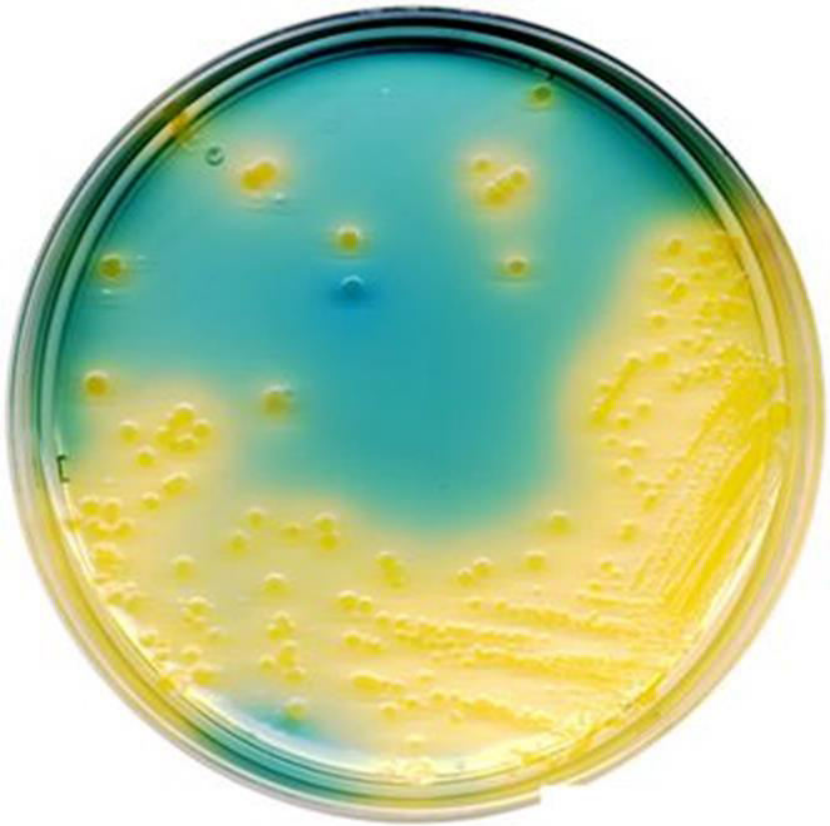
LF and NLF on Mac.



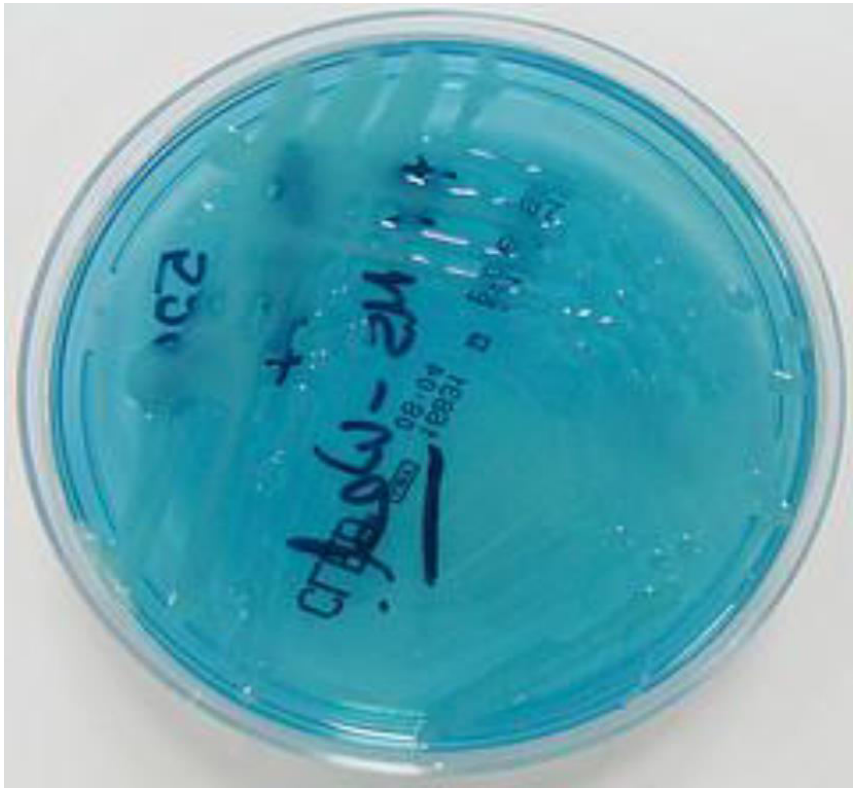
Cysteine Lactose Electrolyte Deficient Agar (CLED):

- A non selective, differential medium
- Supports growth gram –ve and contaminants
- Carbohydrates (sugar): lactose
- PH indicator: Bromothymol blue
- Prevent Proteus from swarming because the electrolyte deficient.
- LF>> **yellow** colonies,,, NLF>> **blue** colonies or colorless

LF on CLED



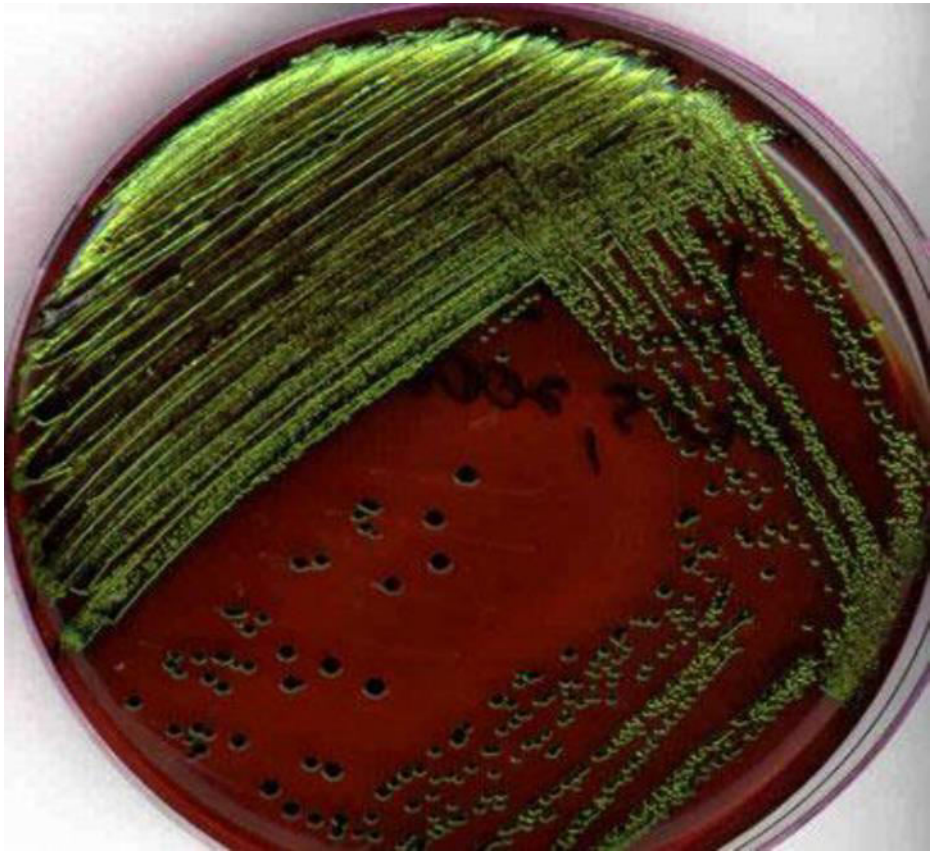
NLF on CLED



Eosin Methylene Blue Agar (EMB):

- A selective and differential medium
- Carbohydrates (sugar): lactose
- PH indicator: Eosin and Methylene blue
- Inhibiting agent: methylene blue
- E.coli: LF with characteristic “green metallic sheen”
- Klebsiella produces dark brown LF colonies
- NLFs produce colorless colonies

E. coli on EMB Showing Green Metallic Sheen



Deoxycholate Citrate Agar (DCA):

- A selective and differential medium
- Carbohydrates (sugar): lactose
- PH indicator: Neutral red
- Inhibiting agent: sodium deoxycholate and sodium citrate
- LF>> **pink** colonies ,,, NLF>> **colorless** colonies
- H2S producers give black-centered transparent colonies on DCA (salmonella). Shigella sp. produce colorless colonies without a black center.
- The reduction of ferric citrate to iron sulphide by the H2S>> black center

Hektoen Enteric (HE):

- A selective and differential medium
- Carbohydrates (sugar): lactose, sucrose and salicin
- PH indicator: Bromothymol blue
- Inhibiting agent: Bile salt
- LF>> orange to salmon colored colonies,,, NLF>> transparent colonies
- H2S producers give clear colonies with black enters (sometime referred to as the fish eye)

Xylose Lysine Deoxycholat Agar (XLD):

- A selective and differential medium
- Inhibiting agent: sodium deoxycholate
- PH indicator: phenol red,, Lysine is an a.a
- Sugars: xylose, lactose and sucrose
- Most Enterics, except shigella, ferment xylose
- Salmonella ferments xylose → acid product, **but** retains its red colonies because it decarboxylates lysine → alkaline product→ neutralizes acidity

- LF>> yellow colonies,,, NLF>> colorless (red) colonies
- Colonies of H2s producers (salmonella) are red with black centers.
- Shigella: red colonies without black centers
- Notes regarding Proteus on XLD:
  - XLD inhibits Proteus swarming but not as commonly used as CLED for Proteus
  - Proteus produces yellow colonies on XLD due to xylose fermentation NOT lactose fermentation