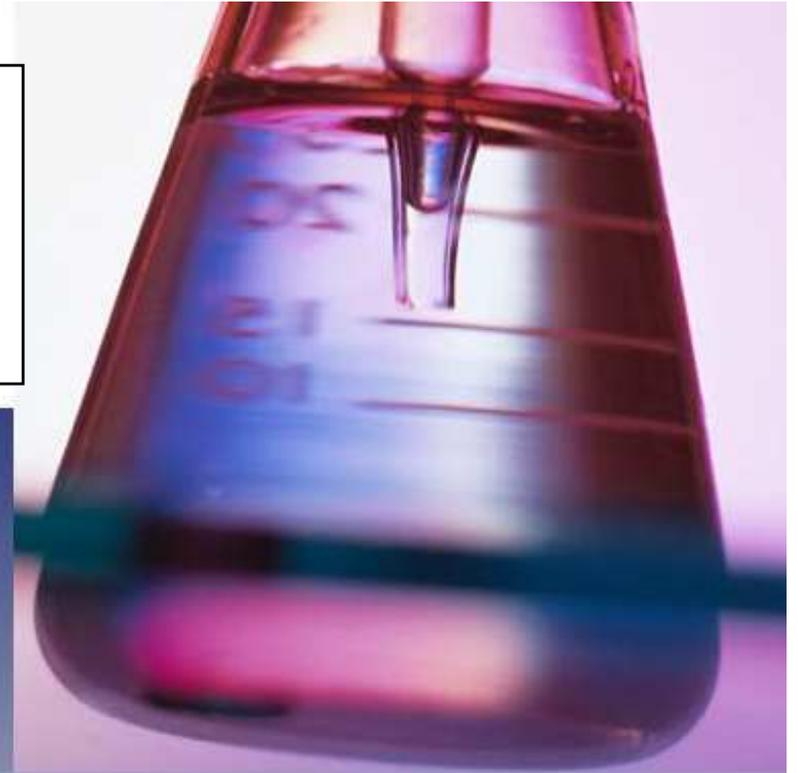


Dr Shyamal Kr Paul
Associate Prof, Microbiology
MMC



CULTURE MEDIA

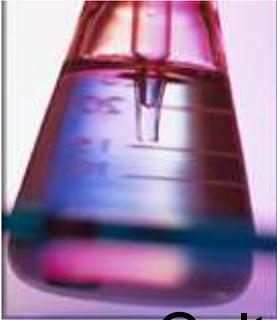


Essential requirements in culture media

- Any culture medium must contains:

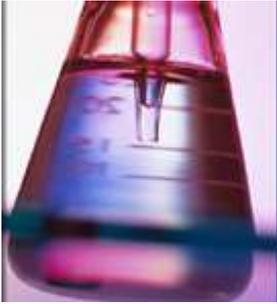
- A source of energy
- Sources of carbon, nitrogen, sulfur, phosphorus
- Minerals, e.g., Ca^{2+} , Mg^{2+} , Na^{+}
- Vitamins and growth factors
- Water



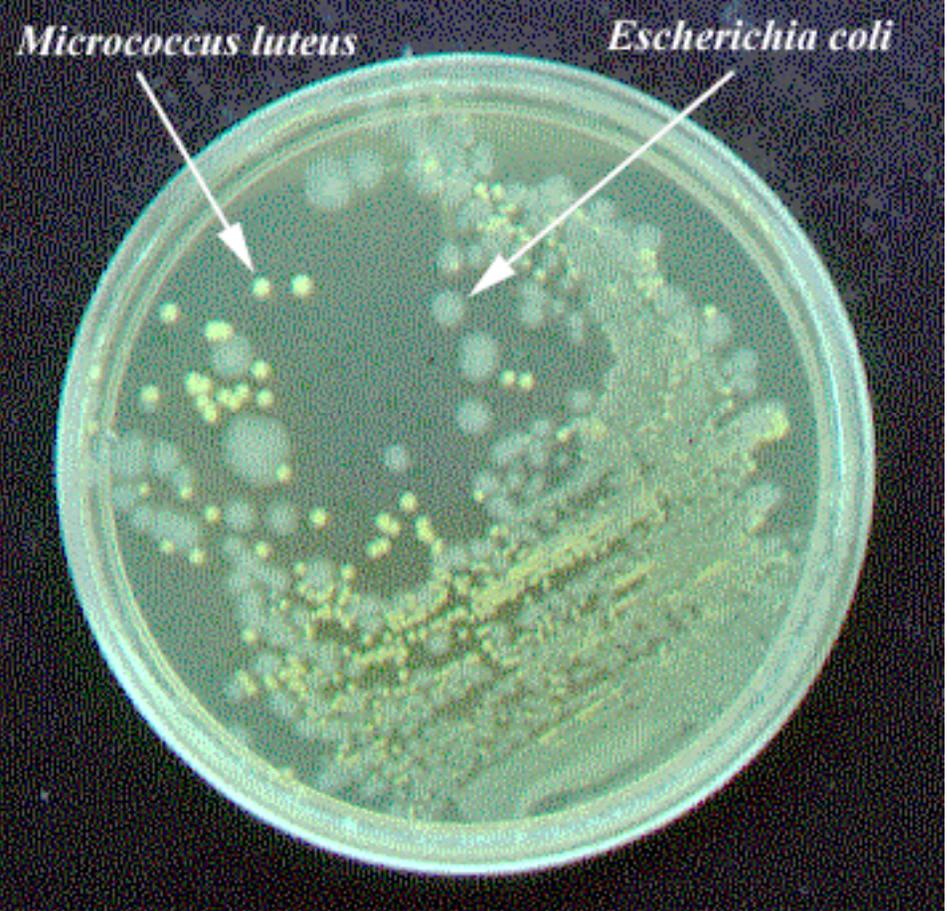


Culture Media

- Culture Medium:
 - Nutrients prepared for microbial growth
- Inoculum:
 - Suspension of microorganisms
- Inoculation:
 - Introduction of microbes into culture medium
- Culture: Microbes growing in/on culture medium
 - A pure culture contains only one species or strain
 - Mixed culture contains several species
 - Contaminated culture contains unwanted species of organisms



Culture Media



12/30/15

pure culture of *S. albus*

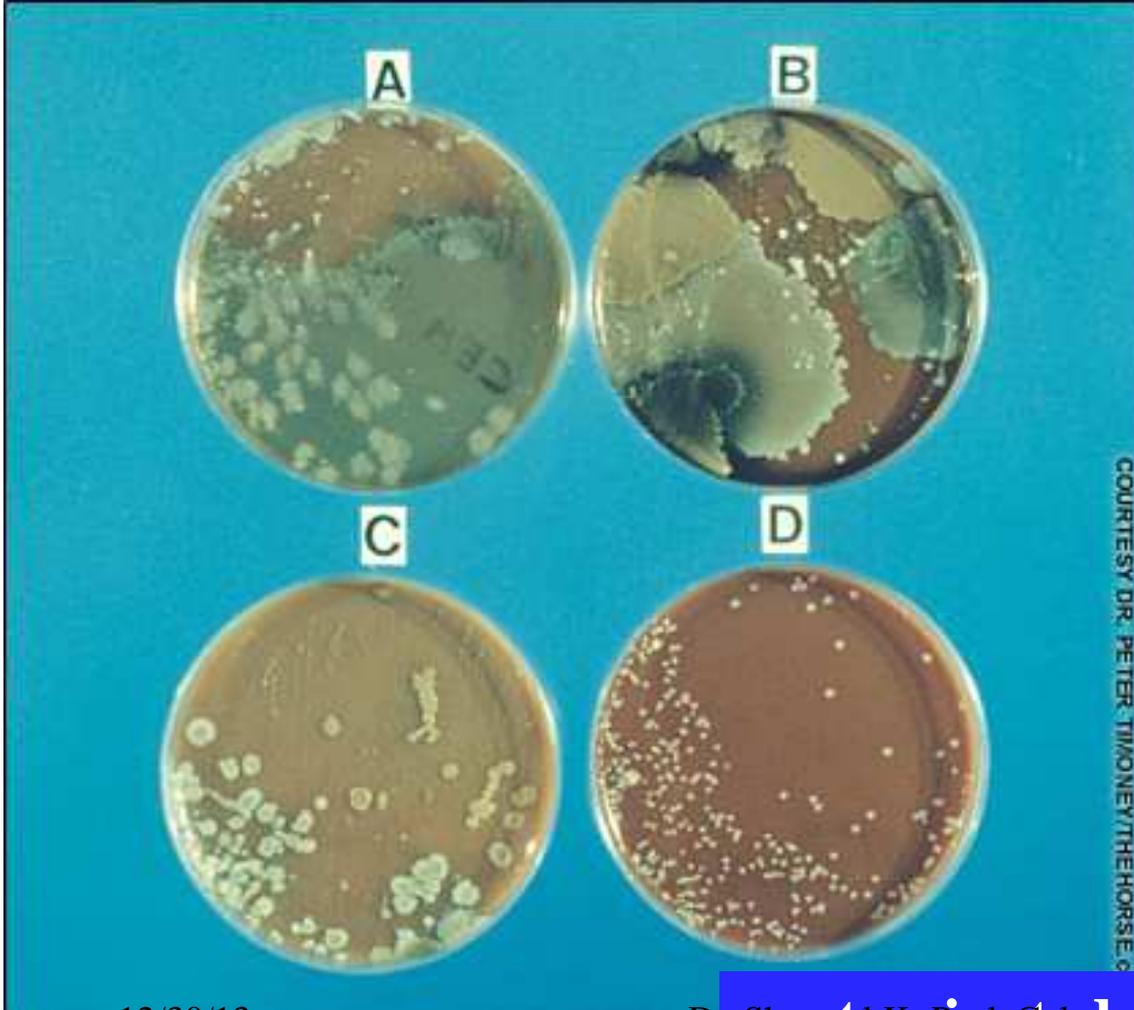
Dr. Shyamal Kr Paul, Culture media

mixed culture





Culture Media



COURTESY DR. PETER TIMONEY/THEHORSE.COM



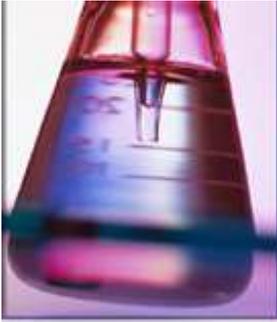
(C) 2007 Daniel Friedman - inspect-ny.com

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5



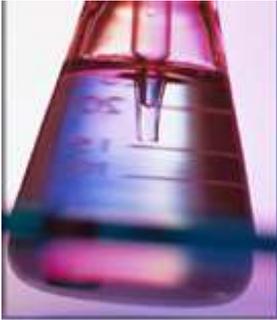
Culture Media

- Colony: visible growth of microbes on the surface of a solid medium
- A colony is a population of cells arising from a single cell or spore or from a group of attached cells
- A colony is often called a colony-forming unit (CFU)

12/30/13



Dr. Shyamal Kr Paul, Culture media



Classification of Culture Media

I. According to composition:

Chemically Defined Media (**synthetic**): Exact 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 ingredients 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 autolysed yeast cells

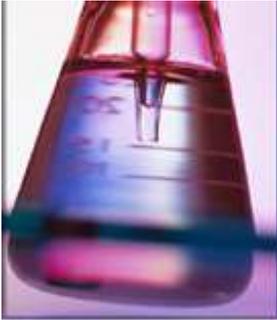


TABLE 6.2

**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 (K_2HPO_4)	1.0 g
Water	1 liter



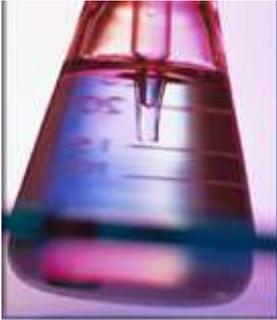
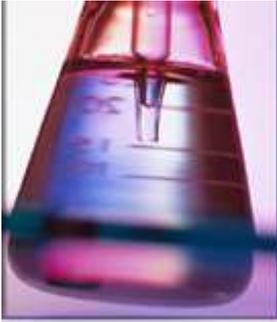


TABLE 6.4

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

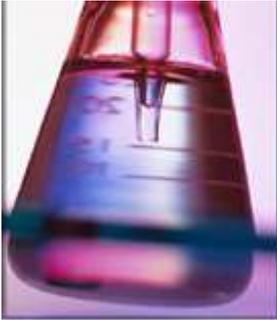
Constituent	Amount
Peptone (partially digested protein)	5.0 g
Beef extract	3.0 g
Sodium chloride	8.0 g
Agar	15.0 g
Water	1 liter





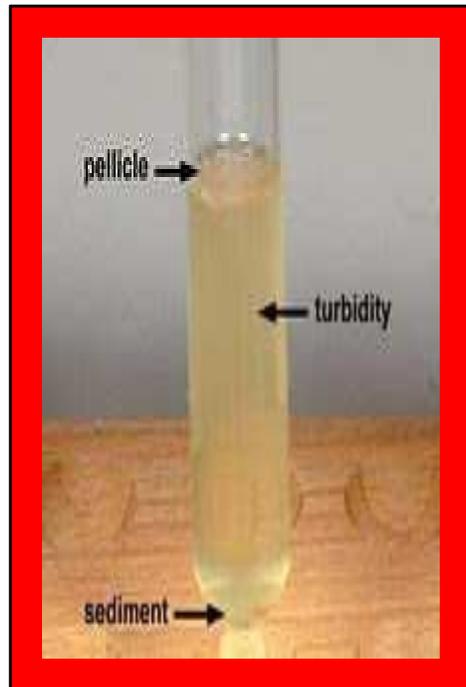
II. According to Consistency:

- 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)



III. According to manner of Dispensing/ Formation:

1. Tubed



a. broth



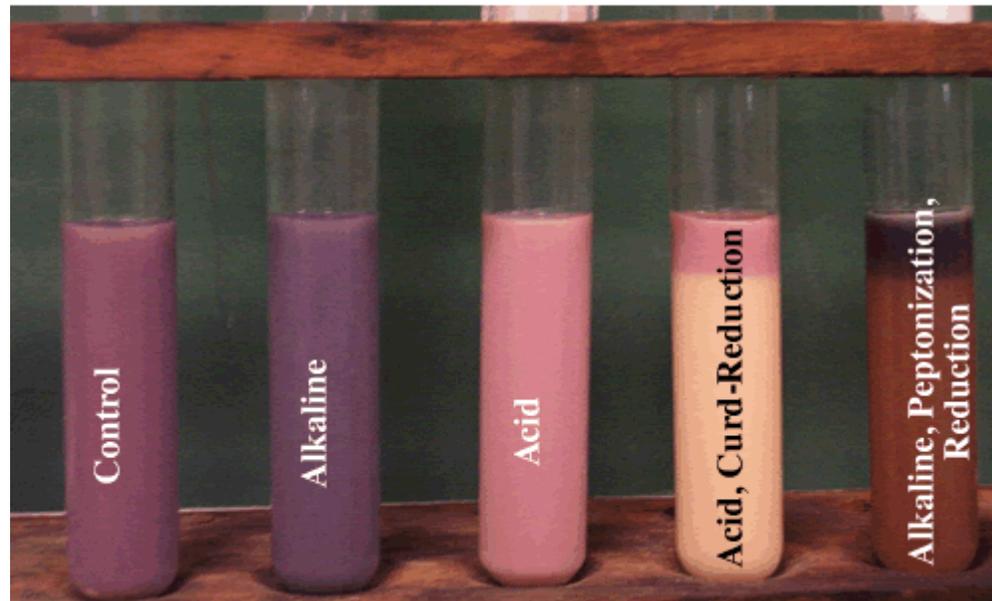
b. semi-solid



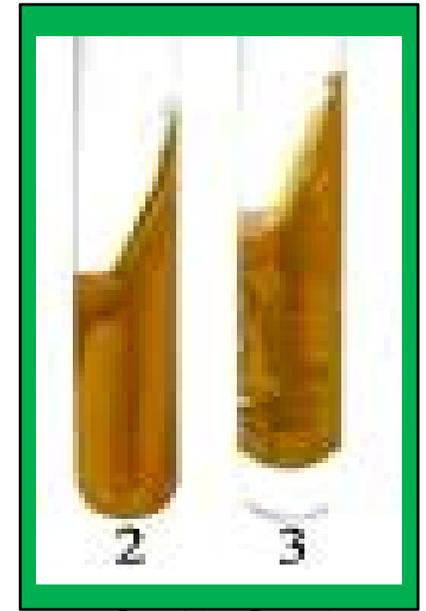
c. solid



slant/slope



butt/deep



butt-slant

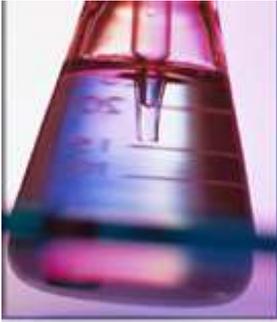
2. **Plated**

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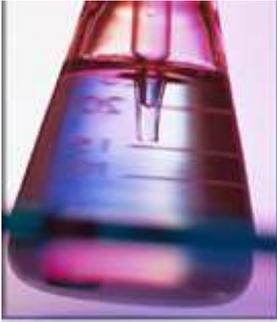
Dr. Shyamal Kr Paul, Culture media

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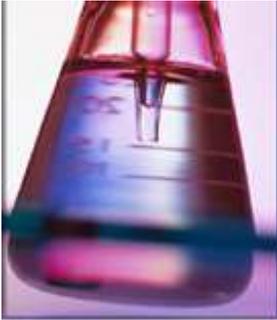
Agar

- 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 at 40°C



IV. According to Function/Application:

- **Basal**/ordinary/general
- **Enriched**: with enrichment substances
- Enrichment
- **Selective media**: with inhibitory substances
- **Differential media**: with indicators/dyes
- **Special Media for Biochemical Testing**
- **Media for Antimicrobial Susceptibility Testing**



Basic media

- These are media which may be used for cultivation of most ordinary microorganisms

- May be in liquid form ex:

Nutrient broth : composed of beef ext+
Peptone+ NaCl

- May be in a solid form ex:

Nutrient agar: similar to nutrient broth
but supplemented with 1-2% agar



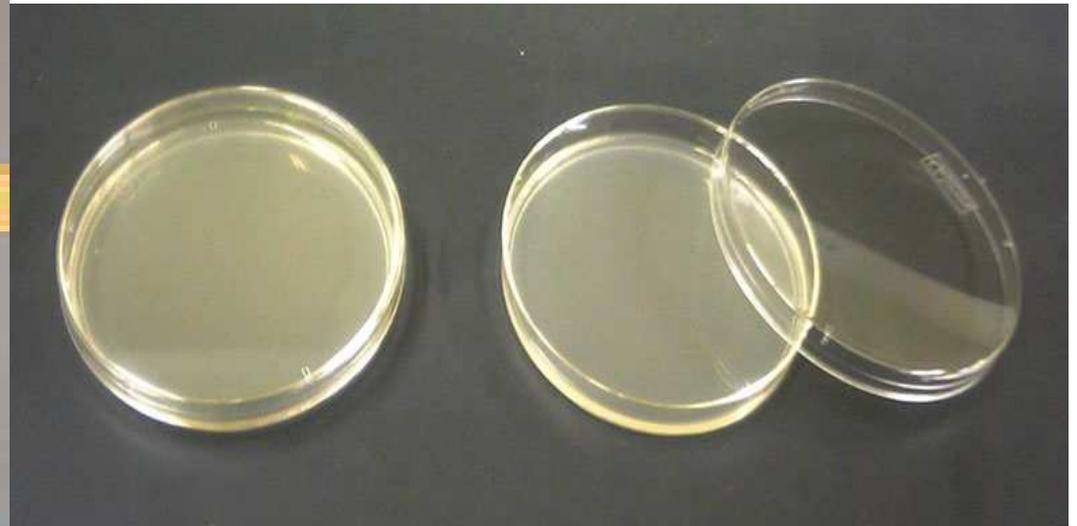
Solid culture media



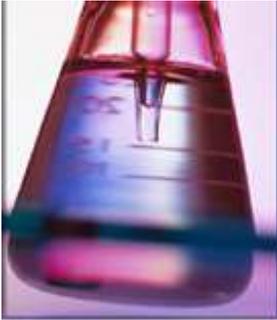
deep



Slant



**Plate
(Petri dish)**

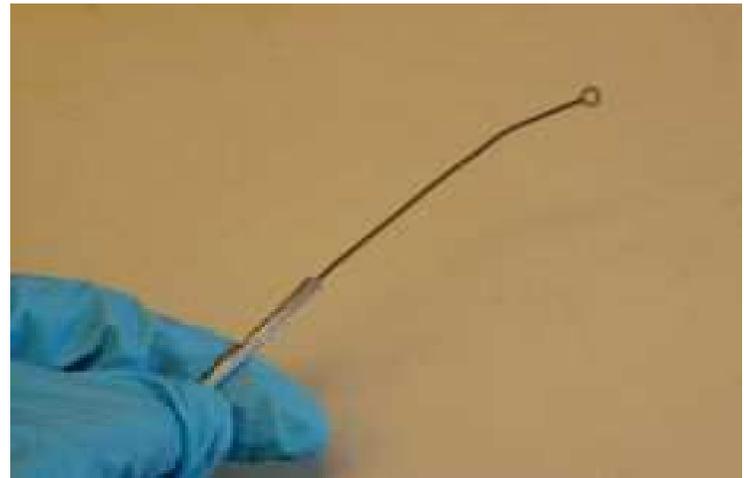


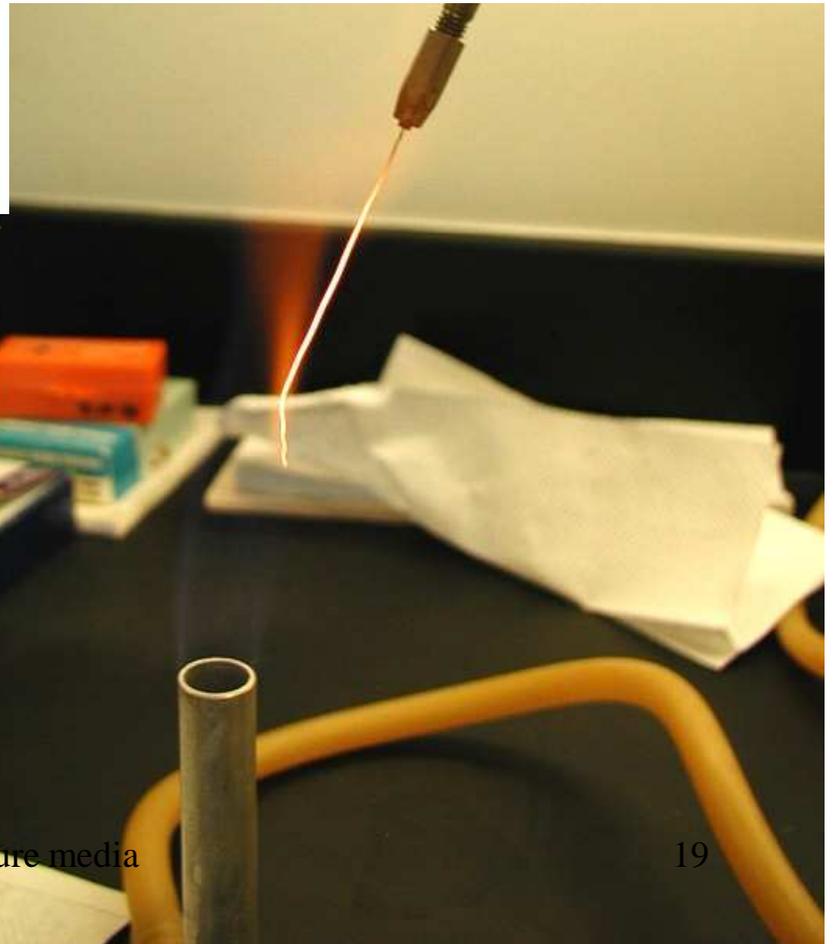
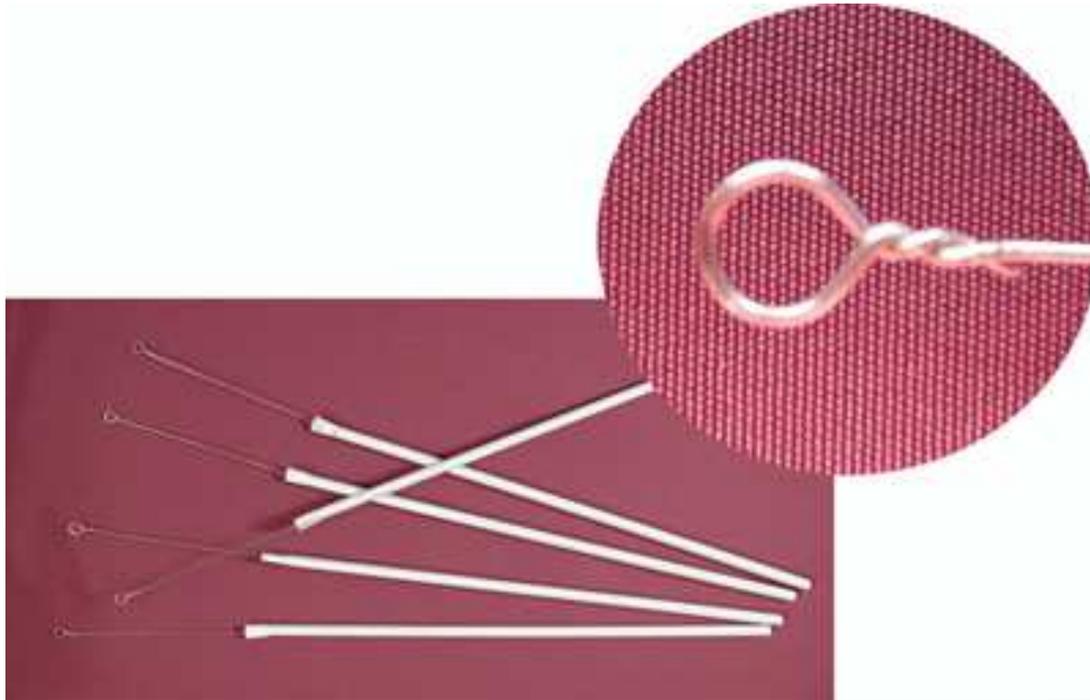
Inoculation

- Inoculation:
- **Surface:**Streaking
- using an inoculation loop
- **Seed:**

Suspension of M.O
is put in the plate

Then agar is poured or M.O is mixed with agar at
suitable temperature.



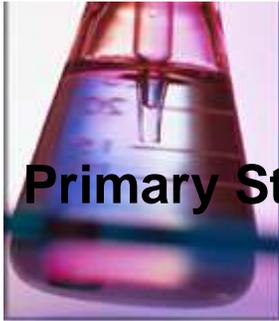


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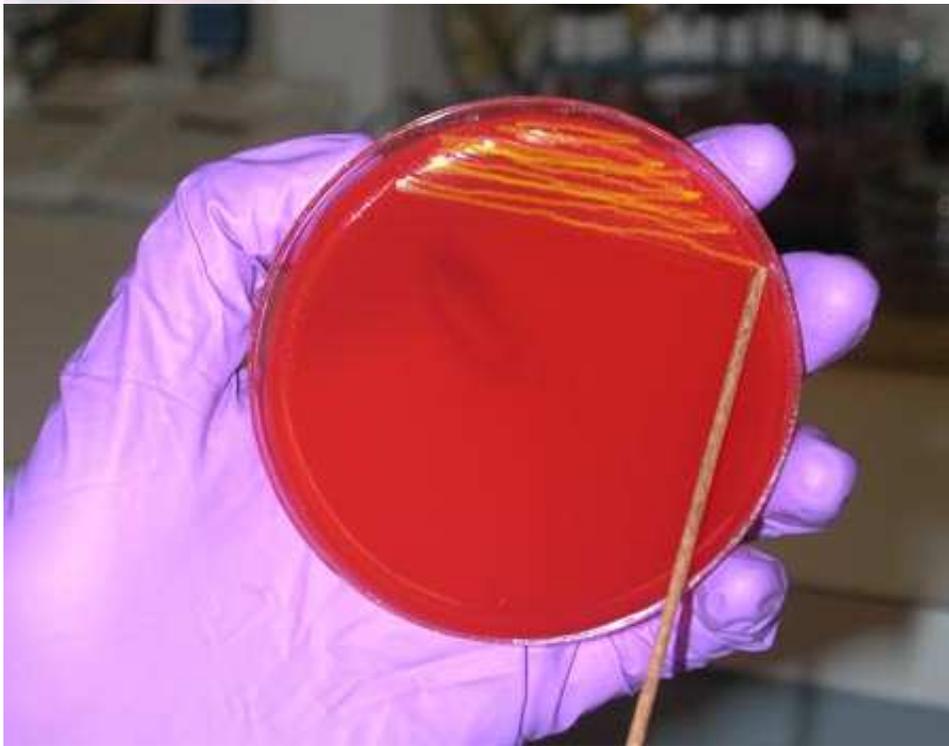


Dr. Shyamal Kr Paul, Culture media

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Primary Streak (shown in Yellow).



Hold the plate as though you are going to "paint" the surface of the agar . If you are right-handed, hold the plate in your left hand, and the inoculating loop in your right - as through you would a paint brush. Hold the plate in your right hand, and inoculating loop in your left if you are left-handed.

Dip your inoculating loop (sterile swab, or sterile stick as shown in the above picture) into a broth culture, or touch it to the material you want to spread - an isolated colony or swab an area for which you want to quantify the microbial species present.

Go back and forth a number of times in a small area of the Petrie plate. The goal is to spread your material completely over this initial area of the plate.



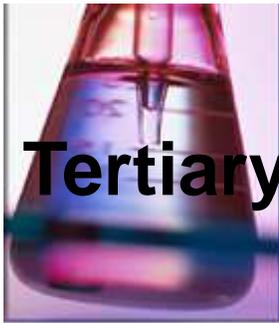
Secondary Streak (shown in Blue).



Sterilize your inoculating loop, or use a fresh, sterile inoculating stick or swab. Squelch it in an unused area of the agar before streaking to cool it. If you were to use the original, unsterile loop, you will not be diluting the individual microbes you plates in the first streak.

Pick up the plate and rotate it 1/4 of a turn to your left (if right-handed), or to your right (if left handed).

Run the loop through the previous streak 2-3 times, then draw it along 1/3 of the remaining plate, as shown by the blue line in the above image.



Tertiary Streak (Orange)



Rotate the plate another 1/4 turn and sterilize your inoculating loop or take a fresh, sterile stick or swab. Squelch a heated inoculated loop in an unused area of the agar before streaking to cool it.

Run the loop through the previous, secondary streak 2-3 times, and draw the streak over a remaining 1/3 of the plate, as shown.



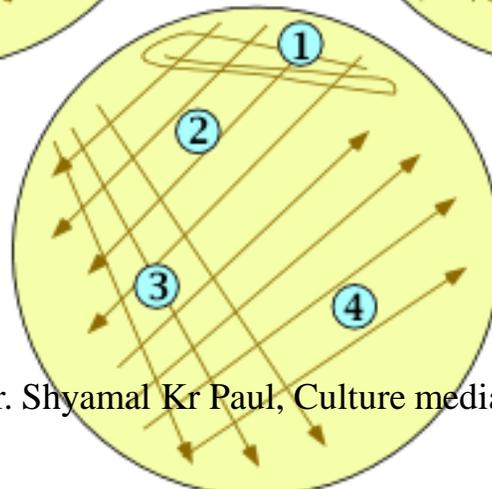
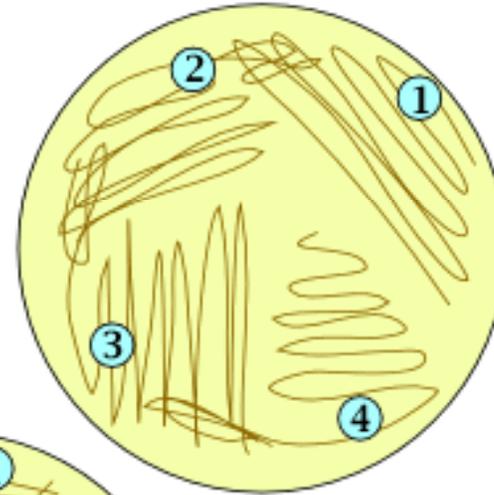
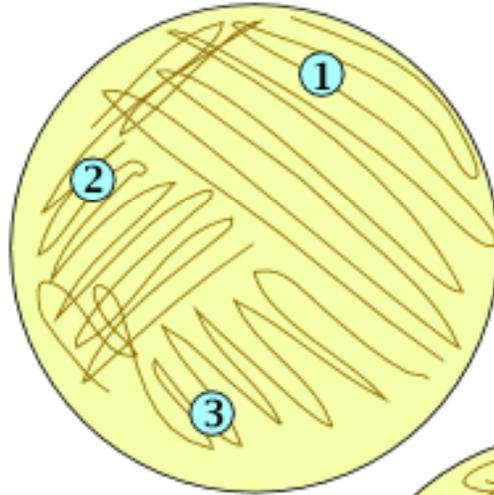
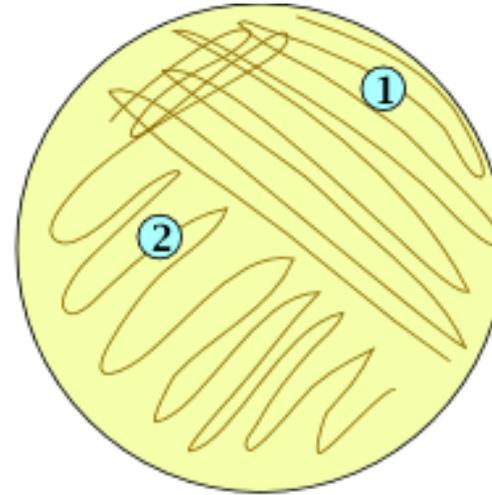
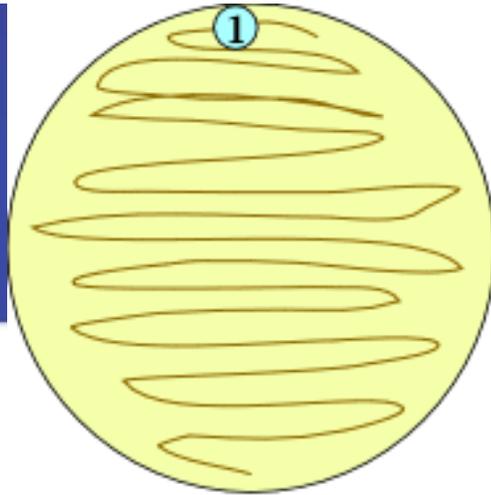
Quaternary Streak (Black)



Rotate the plate another 1/4 turn and sterilize the inoculating loop. Squelch a heated inoculated loop in an unused area of the agar before streaking to cool it.

Run the loop through the previous tertiary streak 2 times and draw over the remaining free space in the plate, being careful not to contact the primary streak (yellow).

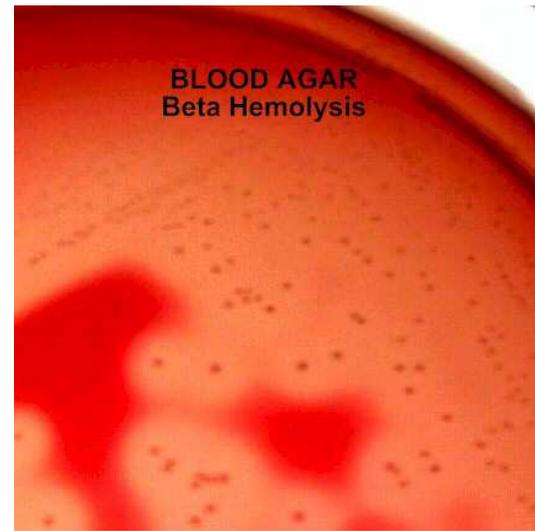
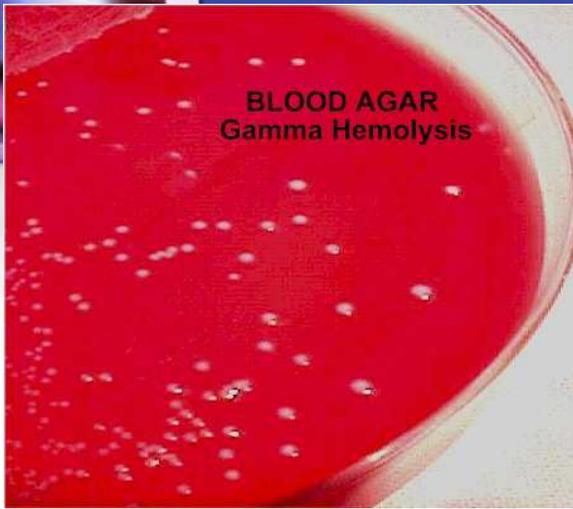
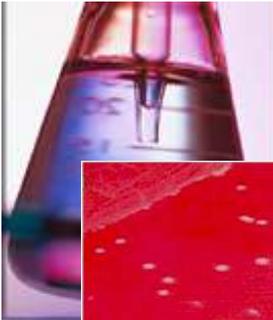
Incubate the plate as needed, and check 18-24 hours later for growth!

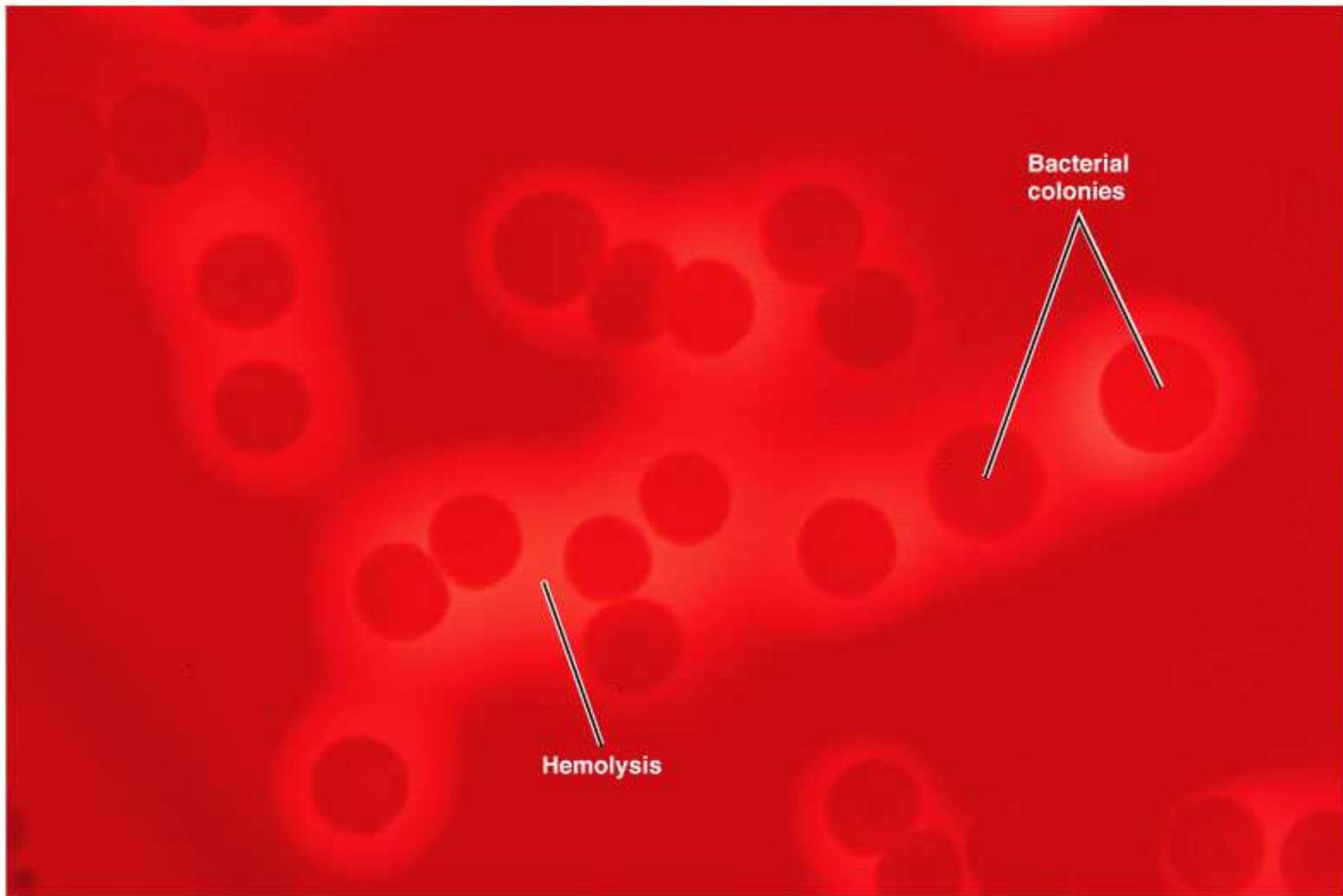




Blood agar plate (BA)

- Nutrient agar with 5% sheep blood
- Cultivation of fastidious and non fastidious bacteria.
- Differential – Identify hemolysis - Some bacteria secrete enzymes that lyse red blood cells (hemolysins) such that a clearing around the colony appears.
 - β hemolysis- complete clearing (white hemolysis)
 - α hemolysis – incomplete clearing (green hemolysis)
 - γ hemolysis- no hemolysis





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beta/complete hemolysis



alpha/partial hemolysis

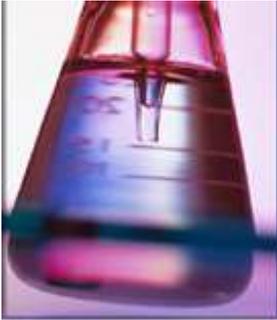


gamma/no hemolysis

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Dr. Shyamal Kr Paul, Culture media

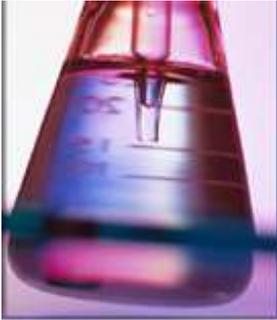


According to Use

- 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 present (e.g BAP & chocolate agar)

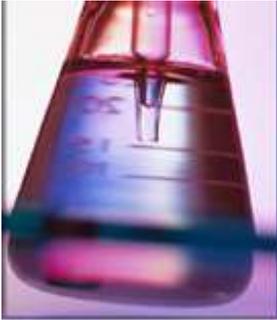
Blood Agar





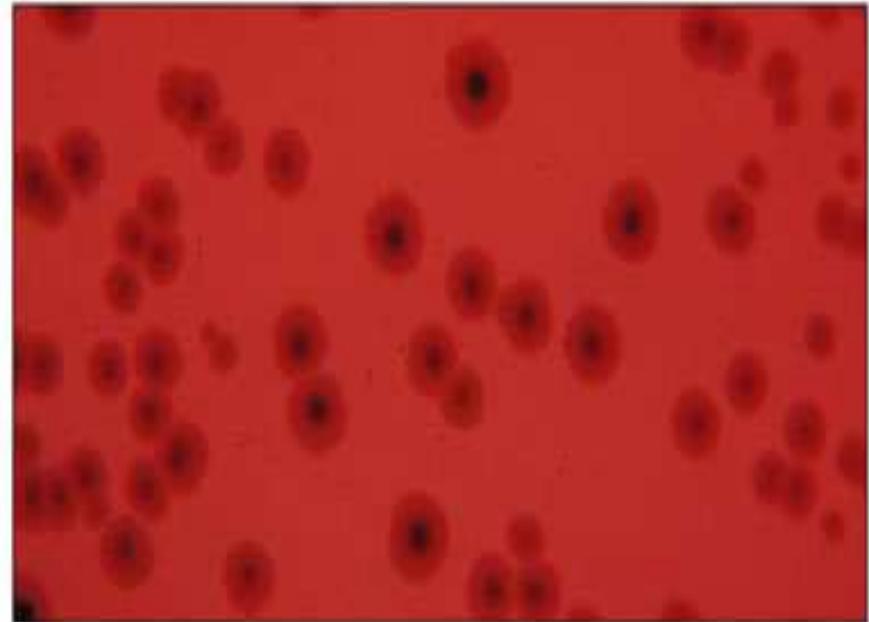
Specialized Media

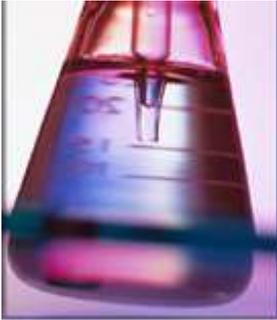
- Enrichment Broths
 - “encourage” the growth of a particular type of microbe;
 - Addition of “nutrients” enrich for microbial group of interest
 - Ex. Cellulose broth- enriches for microbes which degrade cellulose
 - Ex. Petroleum Broth- enriches for microbes which could eat an oil spill.



Selective Media

- Suppress unwanted microbes and encourage desired microbes





SELECTIVE MEDIA

- With **inhibitors** to prevent growth of unwanted organisms and favor desired organisms

e.g. A. to inhibit growth of Gram positive organisms

1. Gentian violet
2. bile salts
3. Na desoxycholate

B. to inhibit growth of Gram negative organisms

1. K tellurite
2. Na azide

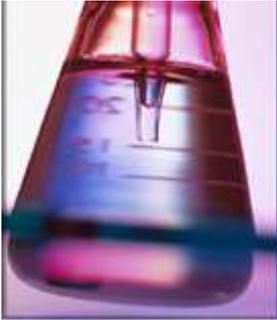
C. to inhibit swarming growth of Proteus

1. Chloral hydrate
2. alcohol

D. to inhibit contaminants or invaders

12/30/13 use antibiotics like penicillin, streptomycin or Malachite Green



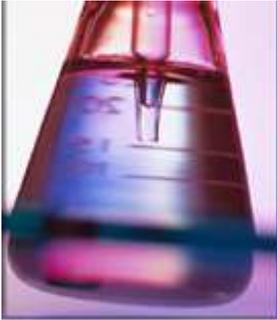


SELECTIVE MEDIA

■ Indicators are added to demonstrate Hydrogen Sulfide production, CHO fermentation, pH

- e.g. 1. Thayer- Martin: for growing Neisseria organisms
2. Petragnanni Medium } for growing fungi
3. Sabouraud's agar }
4. TCBS (Thiosulfate Citrate Bile Salt Agar): for growing Vibrio organisms
- pathogenic *V. cholerae*- yellow
 - non-pathogenic *V. parahemolyticus*- green
5. MSA (Mannitol Salt Agar) → fermenters(*S.aureus*-yellow)
- non-fermenters(*S.epidermidis*-pink)
6. EMB (Eosin Methylene Blue) } for Gram - enteric bacilli
7. McConkey: lactose, CV, }
bile salt, neutral red





SELECTIVE MEDIA

9. SSA (Salmonella Shigella Agar) Salmonella –
colonies with black center
Shigella- without
black center



Mannitol Salt Agar (MSA)

- Both selective and differential medium.
- High salt concentration - inhibits most bacteria.
- Selective for *Staphylococcus sp.*
- Differentiate between *Staphylococcus sp.* by the sugar **mannitol** fermentation .
- Mannitol **fermentation** produce **acids** that change the medium pH.
- Peach color- neutral- no fermentation
- Bright yellow- Acidic – mannitol fermentation (*Staph.*



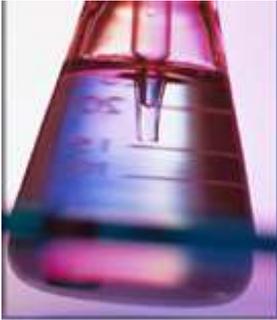


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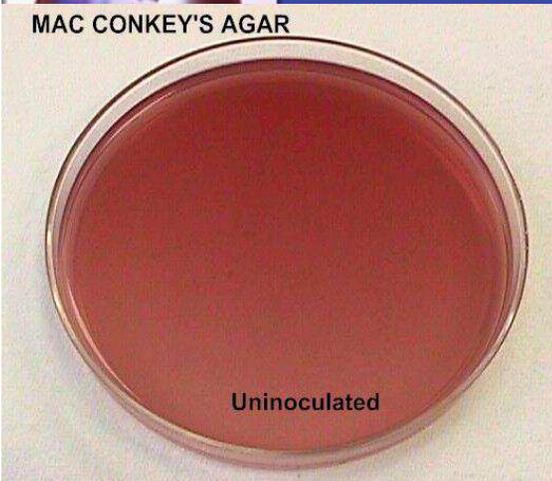
MacConkey Agar (MAC)

- Selective and differential medium.
- **Selective** - Gram positive bacteria are inhibited by the presence of bile salts and crystal violet inhibitors in the medium. Most of gram negative bacteria will grow.
- **Differentiate**- Between Gram negative bacteria by their ability to **ferment lactose**.
- **Pink colonies**- Bacteria that ferment lactose (precipitation of some salts in media by acid production).
- **Pale colonies**- Non fermenters

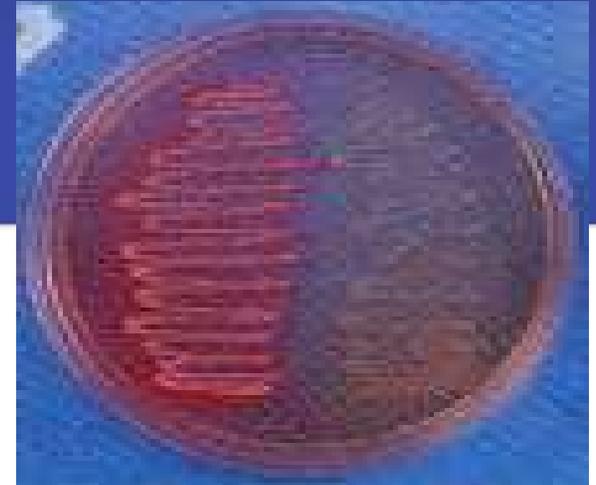




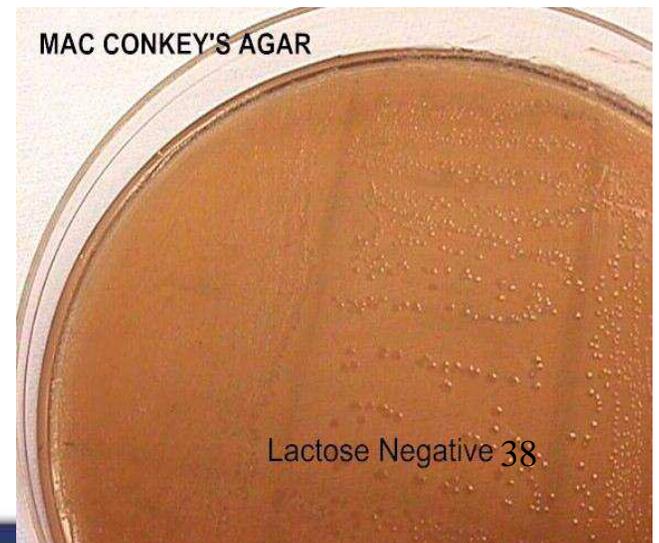
MAC CONKEY'S AGAR



Uninoculated



Lactose Positive



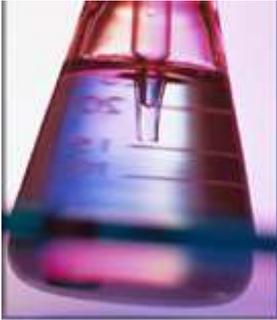
MAC CONKEY'S AGAR

Lactose Negative 38

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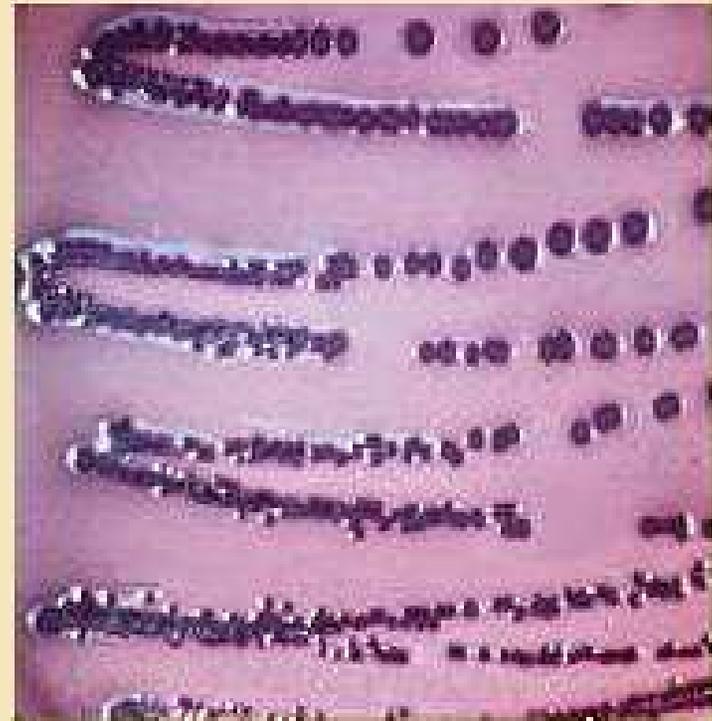


Dr. Shyamal Kr Paul, Culture media

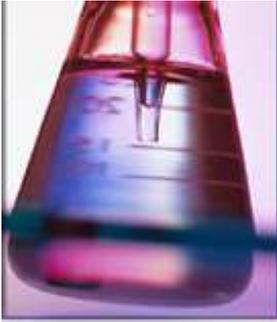


Eosine Methylene blue (EMB),

- Differentiatial between lactose fermenting and non fermenting enteric bacteria



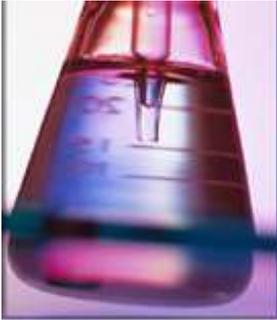
Lactose fermenter on EMB agar



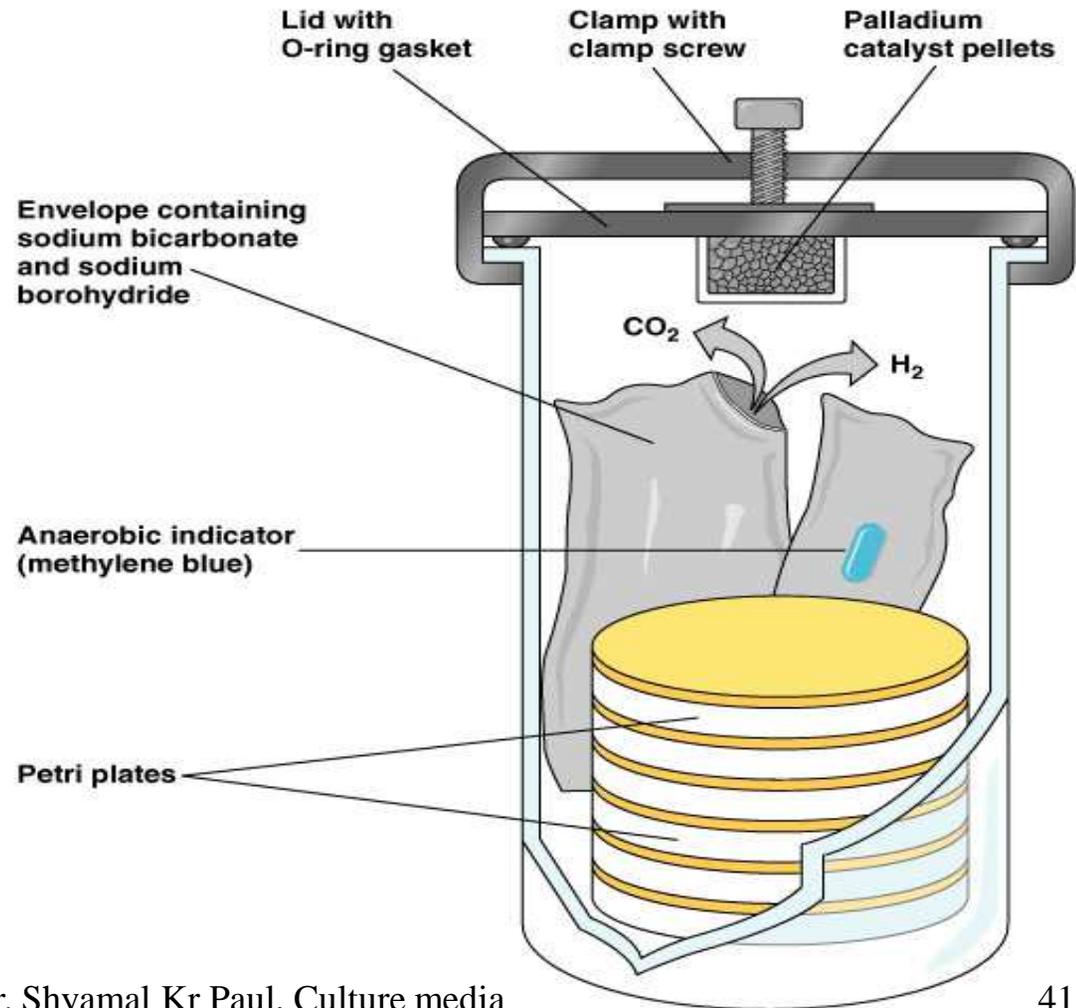
Anaerobic Culture Methods

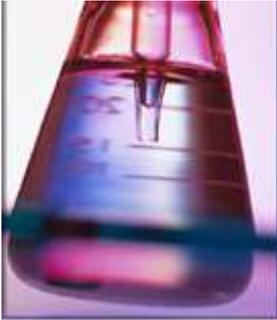
Reducing media

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

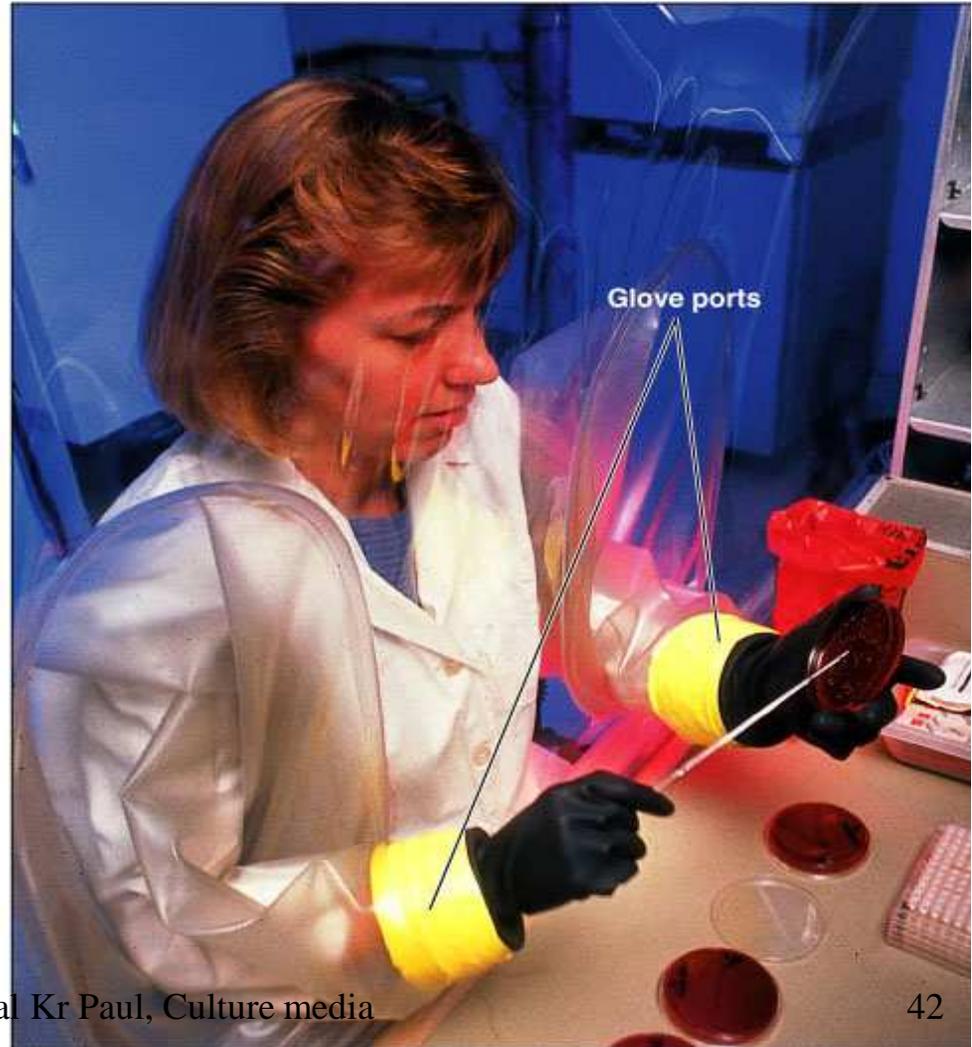


■ Anaerobic jar





■ Anaerobic chamber

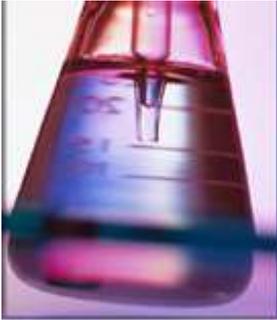


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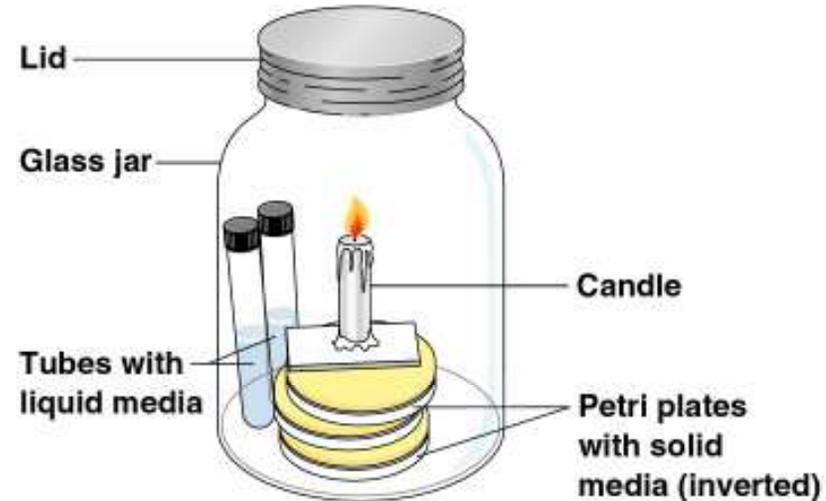
Dr. Shyamal Kr Paul, Culture media

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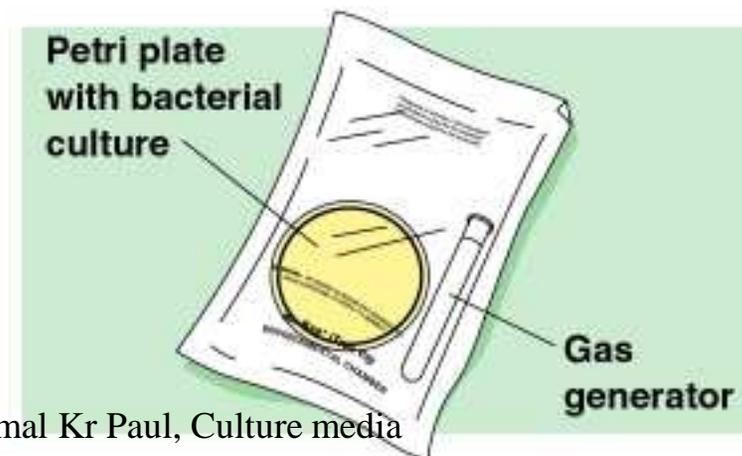


Capnophiles require high CO₂

■ Candle jar



■ CO₂-packet

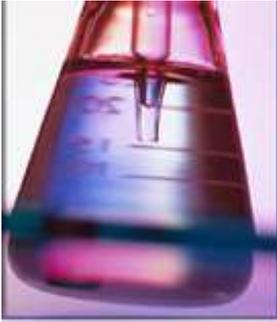


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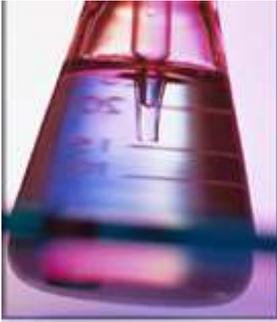
Dr. Shyamal Kr Paul, Culture media

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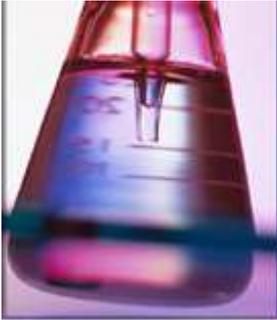
Culture Media to Isolate Common Members of Family *Enterobacteriaceae*

- **Eosin Methylene Blue (EMB)**
 - **Indicators:** aniline dyes (eosin and methylene blue)
 - **Inhibitors:** Eosin, methylene blue (inhibit gm (+) bacteria)
 - **Carbohydrate source:** lactose
 - LF= purple; *E. coli*=greenish metallic sheen; *Enterobacter*= fish-eye colonies
 - NLF= clear
 - LLF=yellow or pink after 48 hours of incubation



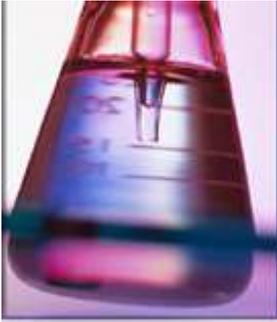
Family *Enterobacteriaceae*

- **Rapid LF:** *Escherichia*, *Enterobacter*, *Klebsiella*
- **Late LF:** *Citrobacter*, *Haffnia*, *Serratia*, *Salmonella arizonae*, *Shigella sonnei*, *Yersinia enterocolitica*
- **Non-LF:** *Proteus*, *Providencia*, *Morganella*, *Edwardsiella*, *Salmonella sp* except *arizonae*, *Shigella sp* except *sonnei*, *Yersinia sp* except *enterocolitica*



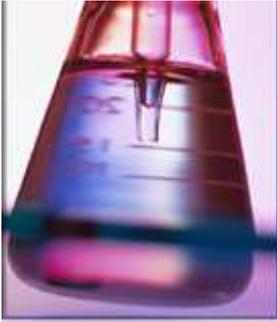
Culture Media to Isolate Common Members of Family *Enterobacteriaceae*

- **MacConkey agar**
 - Indicator: phenol red
 - Inhibitors: bile salts, crystal violet
 - Carbohydrate source: lactose
 - LF= pink-red
 - NLF= clear/colorless

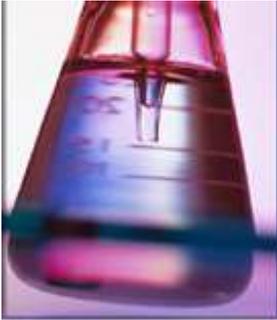


- **Salmonella-Shigella Agar (SSA)**
 - **Indicator:** neutral red
 - **Inhibitors:** bile salts, brilliant green agar
 - **Sulfur source:** sodium thiosulfate
 - Sulfide producers= colonies with black centers (example: *Salmonella*)
 - Non-sulfide producers= colonies w/out black centers (example: *Shigella*)
 - **Carbohydrate source:** lactose
 - LF= red or pink-red (normal coliforms)
 - NLF= colorless



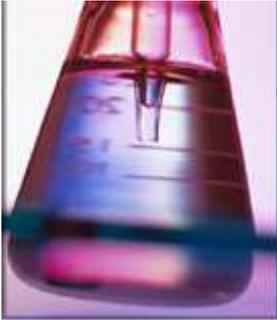


- Xylose Lysine Deoxycholate (XLD)
 - **Indicator:** phenol red
 - **Inhibitors:** sodium deoxycholate
 - **Sulfur source:** same as SSA
 - **Carbohydrate source:** xylose
 - All are xylose fermenters **except *Shigella***
 - **Lysine decarboxylation**
 - After xylose fermentation, the colony will first turn yellow, then if the organism is (+) for lysine decarboxylation, the colony will turn red



...con't. XLD

- Typical organisms
 - *Salmonella*= red colonies with black centers
 - *Citrobacter* and *Proteus*= yellow with black centers



Preparation of Culture Media

- Materials: petri dishes
test tubes (Wassermann Kahn)
glass rods
Erlenmeyer flask
beaker
hot plate
- Plated Media using NA or TSA/Soyabean Casein Digest Agar
Steps: 1. weighing
2. dissolving
3. plugging
4. sterilizing
5. dispensing
6. formation
- Computation: 2 NA plates/member X 6 members=12 NA plates/group
12 plates X 20 ml/plate= 240 ml/plate

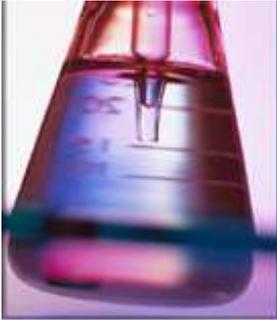
$$28\text{g}:1000\text{ ml} = X\text{g}:270\text{ ml}$$

12/30/13



Dr. Shyamal Kr Paul, Culture media

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Preparation of Culture Media

- Tubed Media using NB

- Steps:
- | | |
|---------------|----------------|
| 1. weighing | 4. plugging |
| 2. dissolving | 5. sterilizing |
| 3. dispensing | 6. formation |

Computation: for liquid=2ml/Kahn
slant= 5ml/Loeffler's
butt-slant= 3ml/Kahn
butt= 3ml/Kahn

for NB: 2ml X 3 members= 6ml/group

13g: 1000ml = Xg: 10 ml





Preparation of Culture Media

