Culture Media

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Definitions

Culture media

Microbiological culture media (plural) medium (single) is the medium that provide essential nutrients and minerals to support the growth of microorganisms in the laboratory.

Types of culture media

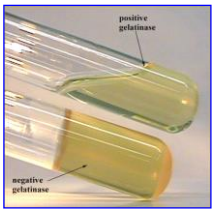
A. Based on physical properties

culture media

- **1- Liquid media** → Broth

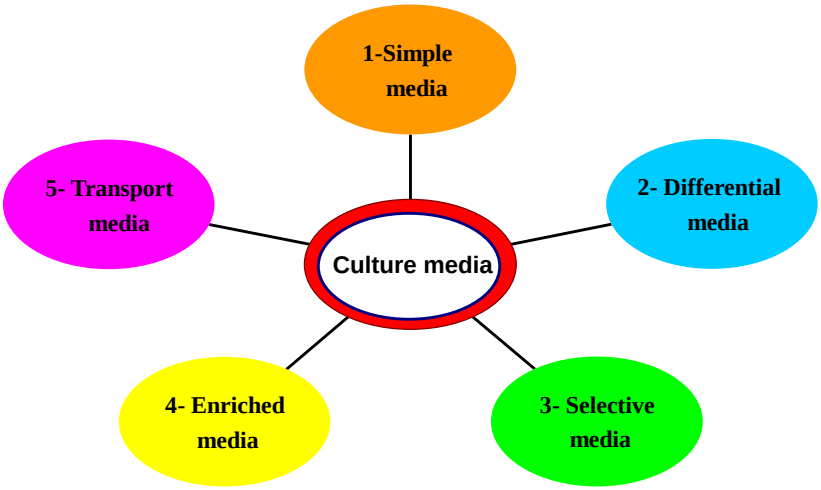
- **2- Solid media** → Agar

- **3- Semi solid media** → Gelatin



- **Solid media:** They contain solidifying agent such as agar-agar. The agar is added at 1.5-2.0% to make the medium solid. It solidify at 45-50 °C.
- **Semisolid media:** They have 0.2-0.5% agar concentration, and due to the reduced agar concentration, it appears as a soft, jelly-like substance.
- **Liquid media:** These media do not contain any solidifying agents such as agar-agar or gelatin. The liquid media are also called broth.

B. Based on nutritional composition



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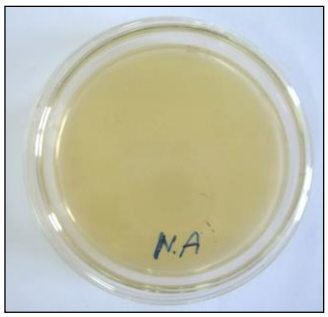
1- Simple media

Contains the essential nutrients as source of nitrogen and carbon such as:

Nutrient broth

Peptone water



Nutrient agar

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2- Differential media

Media that distinguish between different groups of bacteria and allow identification of microorganisms based on their biological characteristics. e.g. MacConkey agar, mannitol salt agar and blood agar.

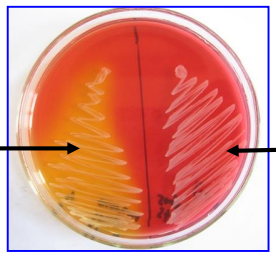
S. aureus *S. Saprophyticus*

- **Mannitol Salt agar (MSA)**
  (Selective and differential)
  It contains a high concentration 7.5–10% NaCl. It is selective for some Gram-positive bacteria such as *Staphylococcus* spp. that tolerate high salt concentrations.

3- Selective media

- It favors the growth of particular microorganism i.e. these media allow the growth of certain microbes while inhibiting the growth of others.
- It's an agar-based medium that is used to isolate microorganisms in labs. The inhibitory substance may be salt (NaCl), acid, a toxic chemical (crystal violet), an antibiotic (streptomycin).

S. aureus *S. Saprophyticus*



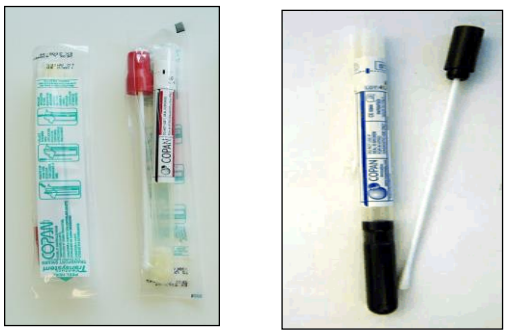
4- Enriched media

Certain types of bacteria need special nutrients in order to growth, simple media can be enriched by adding serum, vitamins, yeast and salt e.g.

- **Blood agar**
- **Heated Blood agar (Chocolate agar)**
- **Brain heart infusion agar**
- **Tissue and body fluid's extract**
- **Serum agar**

5- Transport media

Simple media used for transport samples from different regions to the lab. e.g. Stuart transport medium.



Transport Swab

Preparation of culture media

1- measure the amount of dehydrated medium that you need

2- Dehydrated medium is dissolved in a measured amount of distilled water and pH adjusted.

3- Sterilize the medium using the autoclave

4- Cool after autoclaving

5- Flaming for flask opening and pour in to Petri dishes

6- Flam the medium surface and leave it to cool

7- Put in special bags and keep it in refrigerator

Formula:

Amount of media powder to weigh out (g) =

Amount of medium you want to prepare (L) x Concentration of medium (g/L)

Example: You want to make 500 ml (0.5 L) of a medium, and the instructions on the bottle say to make a concentration of 40 g/L.

Culture Methods

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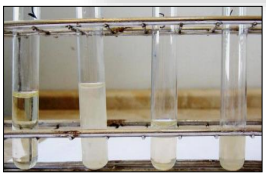

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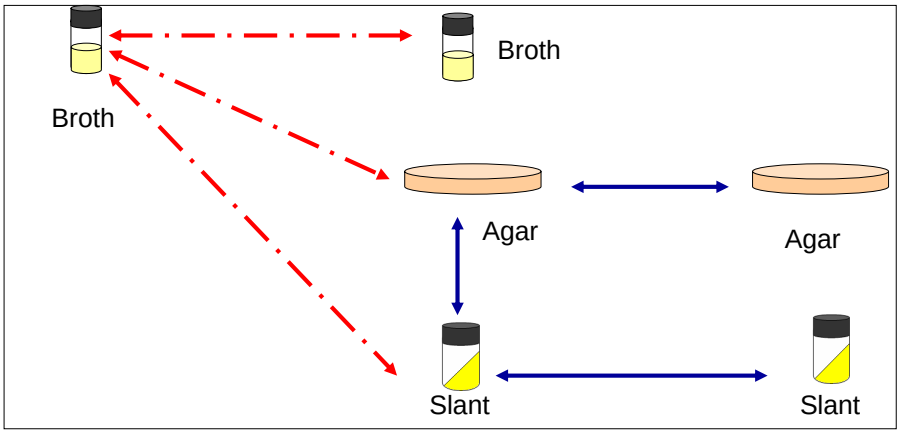
Culture: It is growing of microorganisms on a culture medium.

Pure culture: It is growing of a single bacterial species on a culture medium, to be able to study its cultural, morphological, and physiological characteristics.

Mixed culture: It contains two or more different bacteria.

Colony: It is a large number of bacterial cells on solid medium, which is visible to the naked eye.

Subculture: It is transferring of microorganisms from one culture medium to another by using specific procedures.

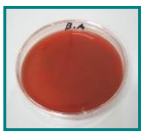


Types of bacterial culture methods

Bacteria can be cultured in 3 main different methods

A. Bacterial cultured in liquid media

B. Bacterial cultured on solid media



C. Bacterial cultured in semisolid media

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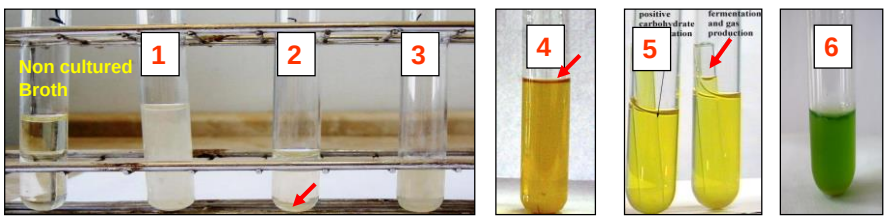
A. Bacterial growth in liquid media (growth phenomena)

1. **Turbidity:** Most bacteria produce turbidity as a result of growth in liquid media like *Escherichia coli*.
2. **Sediment formation:** *Staphylococcus* spp.
3. **Slime:** *Klebsiella pneumoniae*
4. **Pellicle formation:** *Bacillus* spp.
5. **Gas:** *Escherichia coli*
6. **Exopigmentation:** *Pseudomonas aeruginosa*



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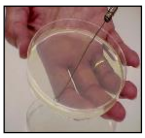
B. Bacterial growth on solid media



1. **Streak – plate method**

2. **Spreading – plate method**

3. **Agar – slop method**

Methods used for pure culture techniques

A method used for preservation of bacterial stocks and performing biochemical tests

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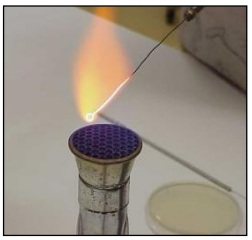
1. Streak - Plate Method

- The streak plate method is the most common method to get pure cultures of bacteria.
- It involves dilution of the bacteria so that the individual bacterial species can be selected from the others.
- An inoculating loop is sterilized first, and then a loop with bacteria either from solid culture (colony) or liquid broth is streaked over a nutrient plate.



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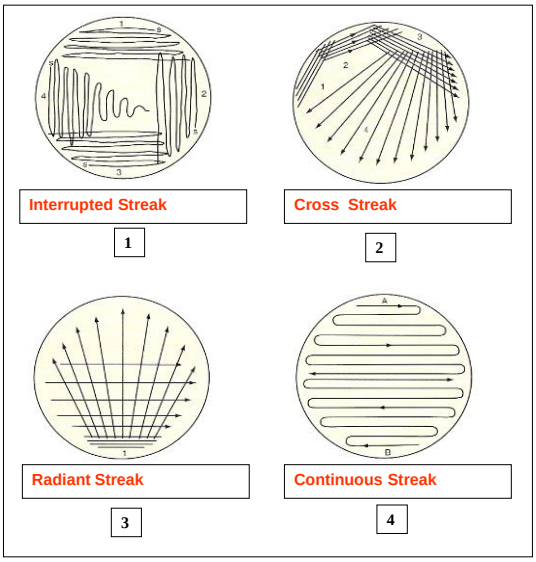
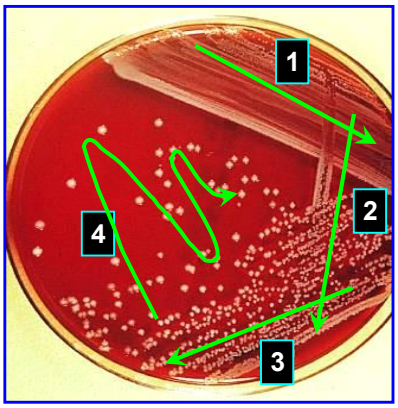
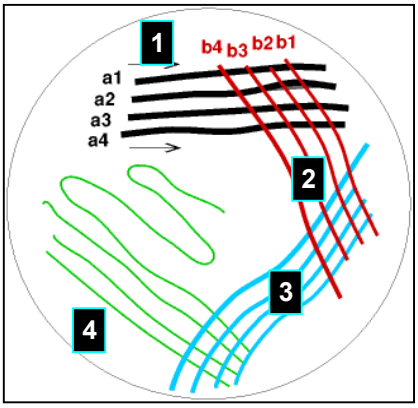
Streak Patterns

1. **Interrupted Streak**
2. **Cross Streak**
3. **Radiant Streak**
4. **Continuous Streak**



Cross Streak





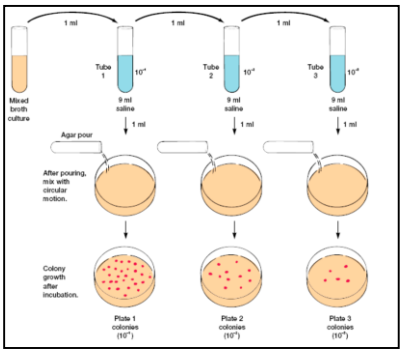
2. Pour - Plate Method

1. The original sample is diluted several times to decrease or dilute the population sufficiently.

2. A volume of 1 ml of each dilution is then dispensed into the bottom of a Petri plate.

3. Agar is then poured into to each plate and left to solidify.

4. The surface colonies are circular and large, subsurface colonies are lens shaped and much smaller.

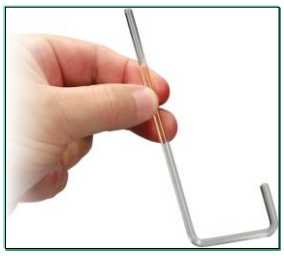


3. Spreading - Plate Method

1. Pipette 0.1 ml of the diluted broth onto the surface of a plate of nutrient agar.

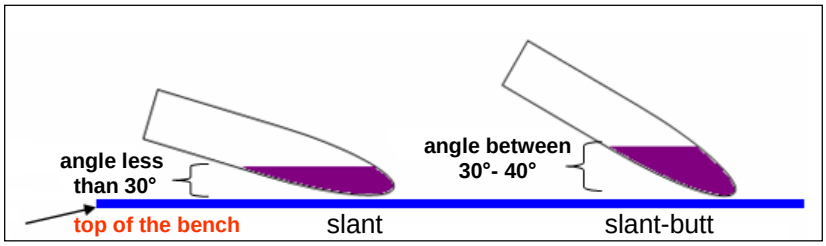
2. Spread the inoculum over the surface of the agar with a bent glass rod (L shape).

3. Incubate the plate, inverted, at 37° C for 24 hours.

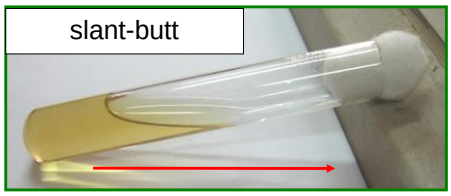


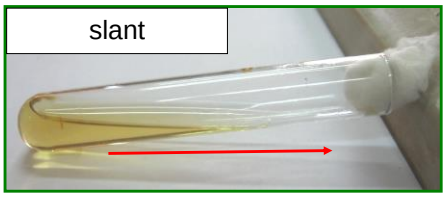
4. Agar - Slop Method

- The test tubes are held at a slant (angle less than 30°) and are allowed to solidify on an angle, called a **slant**.
- The test tubes are held at a slant (angle between 30°- 40°) and are allowed to solidify on an angle, called a **slant - butt.**



- A slant increases the surface area for organism growth.
- This method is used for preservation of bacterial stocks and performing biochemical tests.

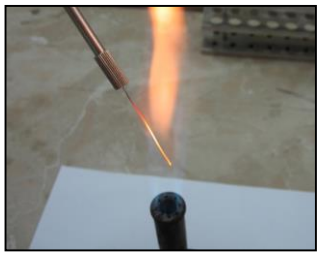




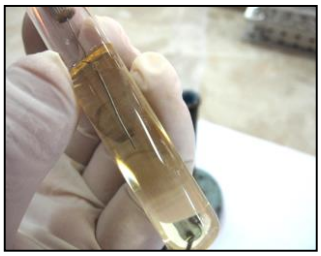
C. Growth in Semisolid Media

- These media are used for:
- Motility test, to Determine whether certain bacteria are motile .
- Gelatin hydrolysis test, as certain bacteria have the ability to hydrolyze Gelatin.

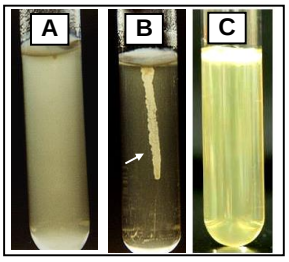
Motility test



Flame the inoculating needle.



Make a deep stab inoculation with tested bacteria into the semi solid medium



A- Motile bacteria.
B- Non motile bacteria.
C- Uninoculated medium.