Cultivation of Bacteria

Cultivation/Culturing of Bacteria

- A microbial culture, is a method of multiplying microorganisms by letting them reproduce in predetermined culture media under controlled laboratory conditions.

- Microbial cultures are used to determine the type of organism, its abundance in the sample being tested, or both.

Purpose of culturing

- Isolation of bacteria.
- Properties of bacteria i.e. culturing bacteria is the initial step in studying its morphology and its identification.
- Maintenance of stock cultures.
- Estimate viable counts.
- To test for antibiotic sensitivity.
- To create antigens for laboratory use.
- Certain genetic studies and manipulations of the cells also need that bacteria to be cultured in vitro.
- Culturing on solid media is another convenient way of separating bacteria in mixture.

Culture Media

An artificial culture media must provide similar environmental and nutritional conditions that exist in the natural habitat of a bacterium.

A culture medium contains water, a source of carbon & energy, source of nitrogen, trace elements and some growth factors.

The pH of the medium must be set accordingly.

Uses:

- ✓ Enrich the number of bacteria.
- ✓ Select for certain bacteria and suppress others.
- ✓ Differentiate among different kinds of bacteria.

Pure culture

- In the laboratory bacteria are isolated and grown in pure culture in order to study the functions of a particular specie.
- A pure culture is a population of cells or growing in the absence of other species or types. A pure culture may originate from a single cell or single organism, in which case the cells are genetic clones of one another.

- Pure cultures are obtained by using variety of special techniques. All glassware, media and instruments must be sterilized i.e. aseptic techniques are used for obtaining pure cultures.
- Basic requirement for obtaining a pure culture are solid medium, a media container that can be maintained in an aseptic condition and a method to separate individual cell.
- A single bacterium, supplied with right nutrients, will multiply on the solid medium in a limited area to form a colony, which is a mass of cells all descended from the original one.

Agar

- Agar, a polysaccharide extracted from marine algae, is used to solidify a specific nutrient solution.
- Unlike other gelling agent, it is not easily degraded by many bacteria.
- It is not easily destroyed at higher temperatures, and therefore it can be sterilized by heating, the process which also liquefies it.
- Once solidified, agar medium will remain solid until

✓ The culture media is contained in a Petri dish, a two part, glass or plastic covered container.

Classification of Culture Media

- Bacterial culture media can be classified in at least three ways.

1. Consistency
2. Nutritional component
3. Functional use

Classification based on consistency

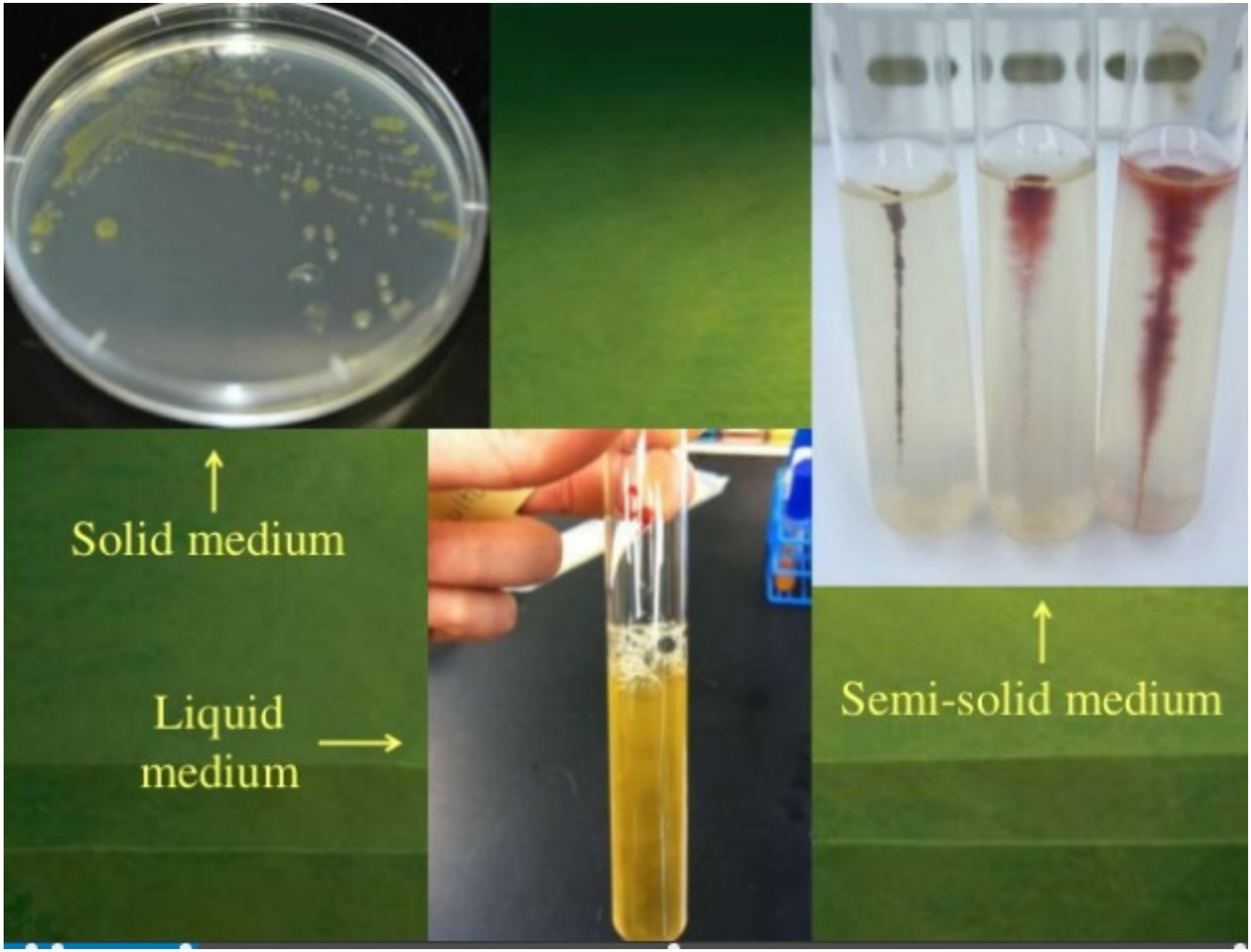
1. Liquid media.
2. Solid media.
3. Semi solid media.

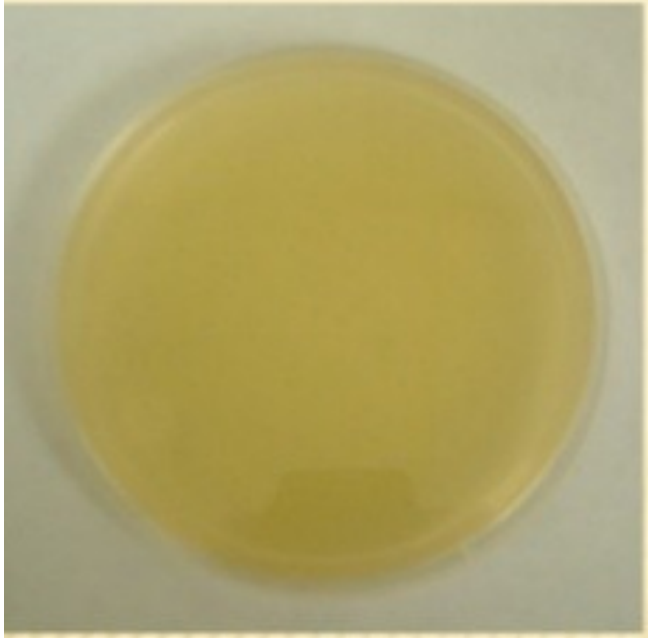
Classification based on consistency:

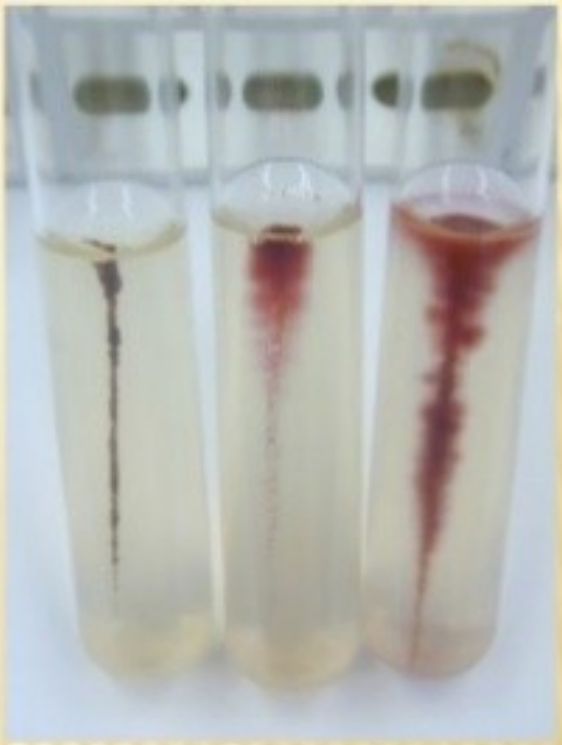
A. **Liquid media:** These are available for use in test-tubes, bottles or flasks. Liquid media are sometimes referred as “broths” (e.g nutrient broth). In liquid medium, bacteria grow uniformly producing general turbidity. No agar is added. Mostly used for inoculums preparation.

B. **Solid media:** An agar plate is a Petri dish that contains a growth medium (typically agar plus nutrients) used to culture microorganisms. 2% of agar is added. Agar is the most commonly used solidifying agent. Colony morphology, pigmentation, hemolysis can be appreciated. Examples include Nutrient agar and Blood agar.

C. **Semi-solid media:** Such media are fairly soft and are useful in demonstrating bacterial motility and separating motile from non-motile strains. Examples of Semi-solid media (Hugh & Leifson’s oxidation fermentation). 0.5% agar is added.


Solid medium
Liquid medium
Semi-solid medium





Classification based on Nutritional Components

1. Simple media.
2. Complex media.
3. Synthetic or chemically defined media.

Classification based on Nutritional Components

1. **Simple media:** Simple media such as peptone water, nutrient agar can support most non-fastidious bacteria. It is also called as basal media. Eg: NB, NA. Nutrient Broth consists of peptone, yeast extract and NaCl. When 2% of agar is added to Nutrient Broth it forms Nutrient agar.

2. **Complex media**. Media other than basal media are called complex media. They have special ingredients in them for the growth of microorganisms. These special ingredients like yeast extracts or casein hydrolysate, which consists of a mixture of many chemicals in an unknown proportion.

3. **Synthetic media/Chemically defined media**: Specially prepared media for research purposes where the composition of every component is well known. It is prepared from pure chemical substances. Eg: peptone water (1% peptone + 0.5% NaCl in water).

3.Classification based on Functional Use or Application

1. Enriched media.
2. Selective media.
3. Differential media.
4. Transport media.
5. Indicator media.
6. Anaerobic media.

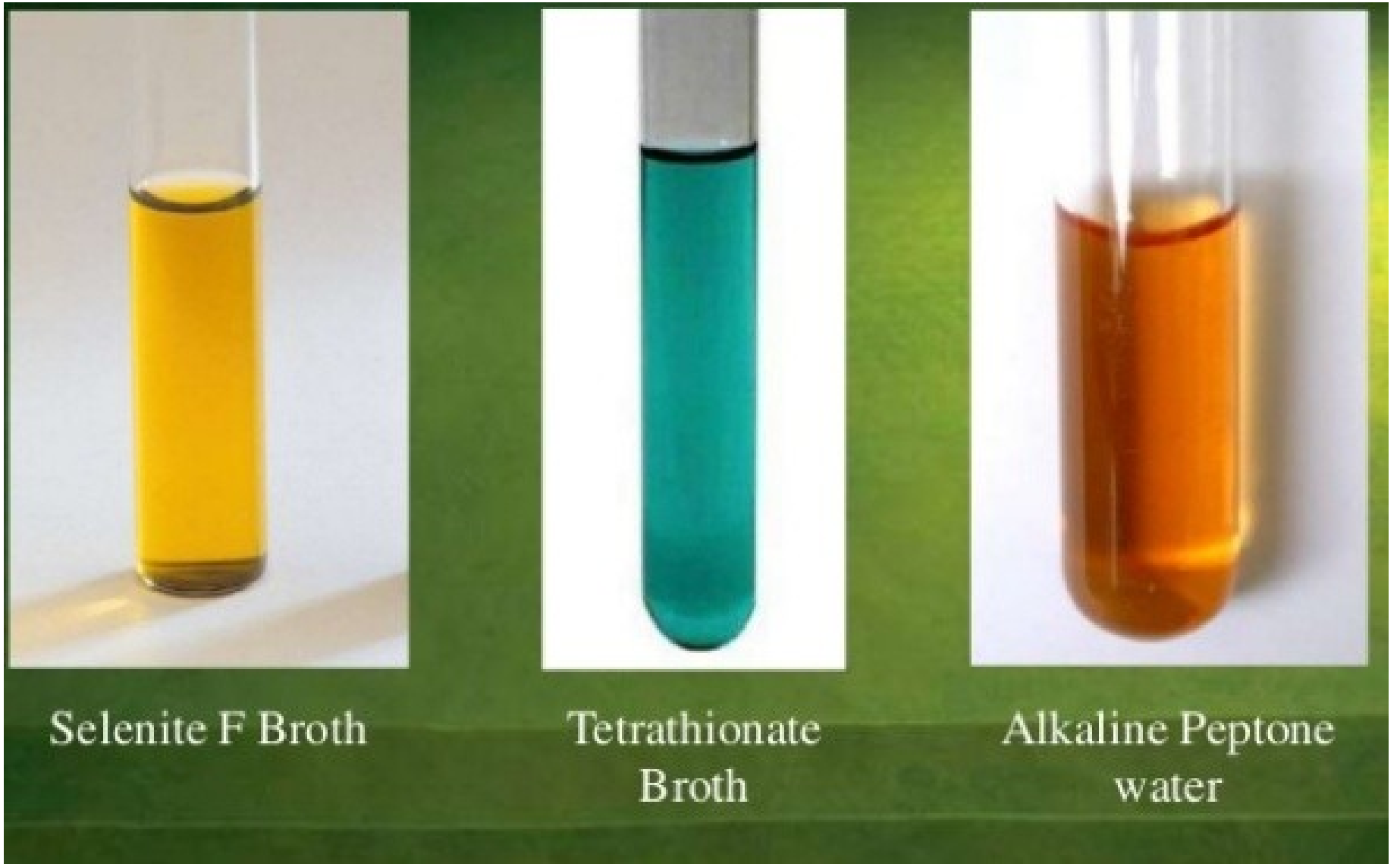
Classification based on Functional Use or Application

1. Enriched media :

- Addition of extra nutrients in the form blood, serum, egg yolk etc to basal medium makes them enriched media.
- Media used to isolate pathogens from a mixed culture.
- Stimulate growth of desired bacterium and inhibit growth of unwanted bacterium
- Media is incorporated with inhibitory substances to suppress the unwanted organism, thus increase in numbers of desired bacteria.

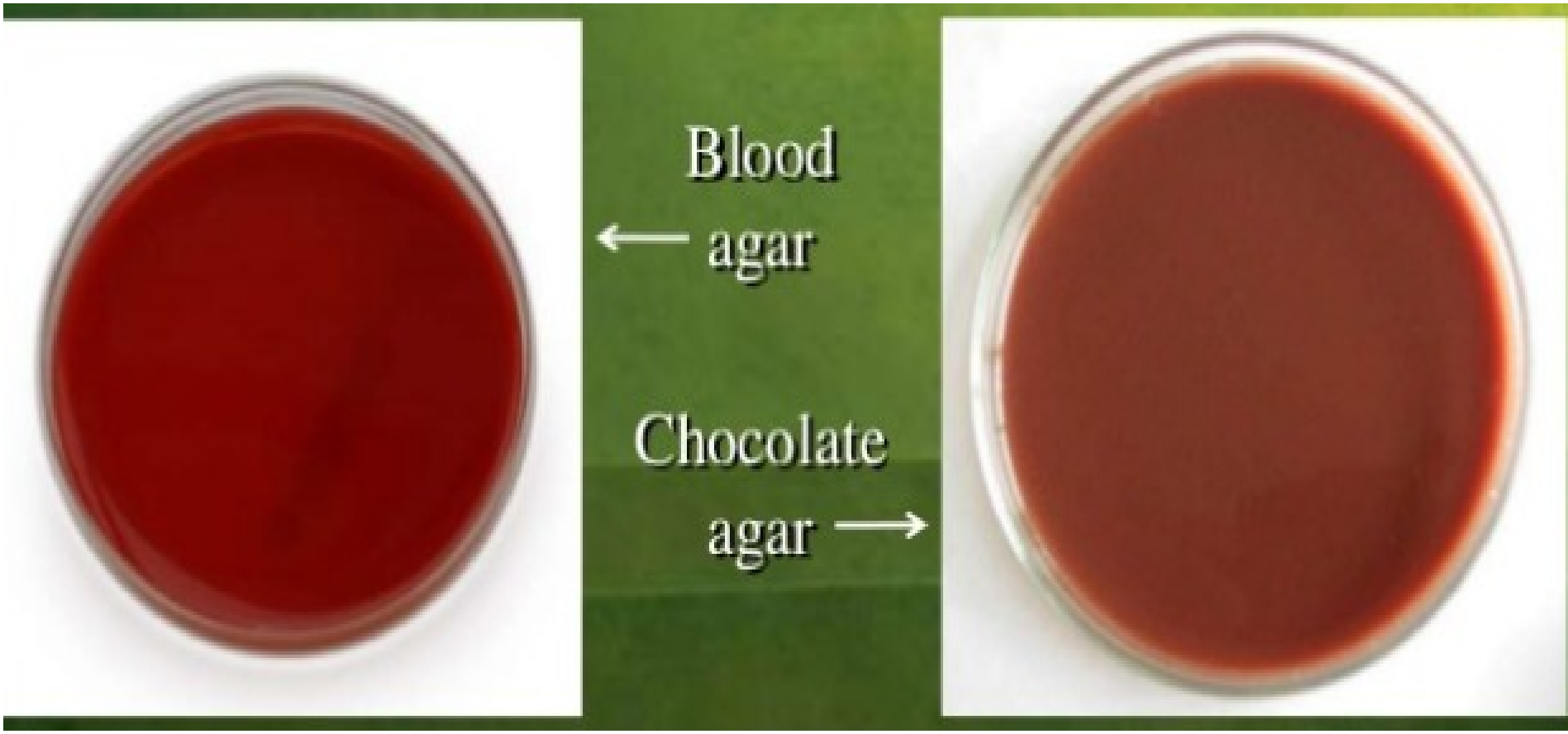
Examples of Enriched media: Chocolate agar Blood agar.

- Selenite F Broth – for the isolation of Salmonella, Shigella.
- Tetrathionate Broth – inhibit coliforms .
- Alkaline Peptone Water – for Vibrio cholerae.


Selenite F Broth
Tetrathionate Broth
Alkaline Peptone water

- **Chocolate Agar** • Chocolate agar - is a non-selective, enriched growth medium used for growing fastidious bacteria, such as Haemophilus influenzae .

- **Blood Agar** • Blood agar plate (BAP) Contains mammalian blood (usually sheep or horse), typically at a concentration of 5– 10%. BAP are enriched, differential media used to isolate fastidious organisms and detect hemolytic activity.


Blood
agar
Chocolate
agar

2. Selective media:

- The inhibitory substance is added to a solid media thus causing an increase in number of colonies of desired bacterium.
- Selective media and enrichment media are designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria.
- Any agar media can be made selective by addition of certain inhibitory agents that don't affect the pathogen. To make a medium selective include addition of antibiotics, dyes, chemicals, alteration of pH or a combination of these.

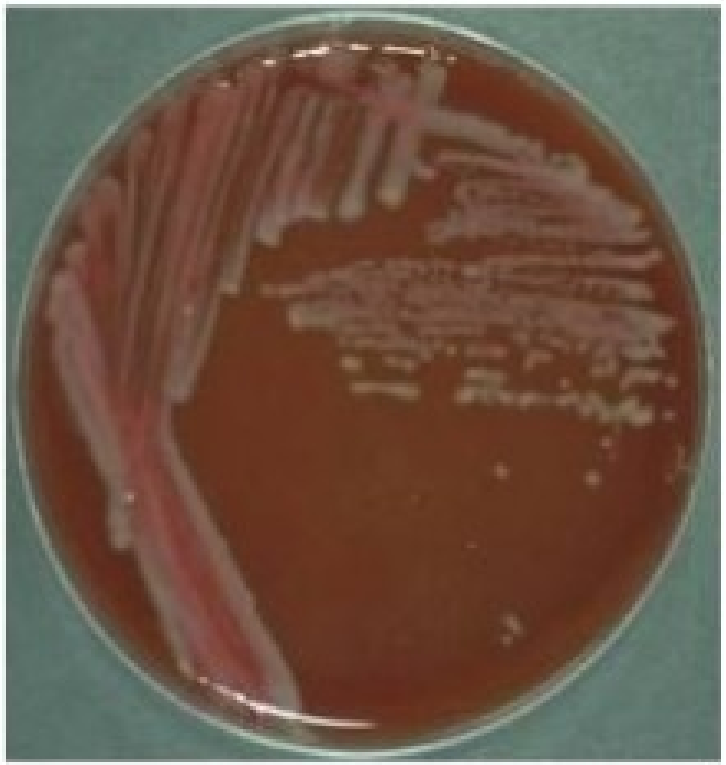
Examples of Selective media :

- Thayer Martin Medium selective for Neisseria gonorrhoeae.

- EMB agar is selective for gram-negative bacteria. The dye methylene blue in the medium inhibits the growth of gram-positive bacteria; small amounts of this dye effectively inhibit the growth of most gram-positive bacteria.

- Campylobacter Agar (CAMPY) is used for the selective isolation of Campylobacter jejuni.

EMB agar



Campylobacter agar

3. Differential Media

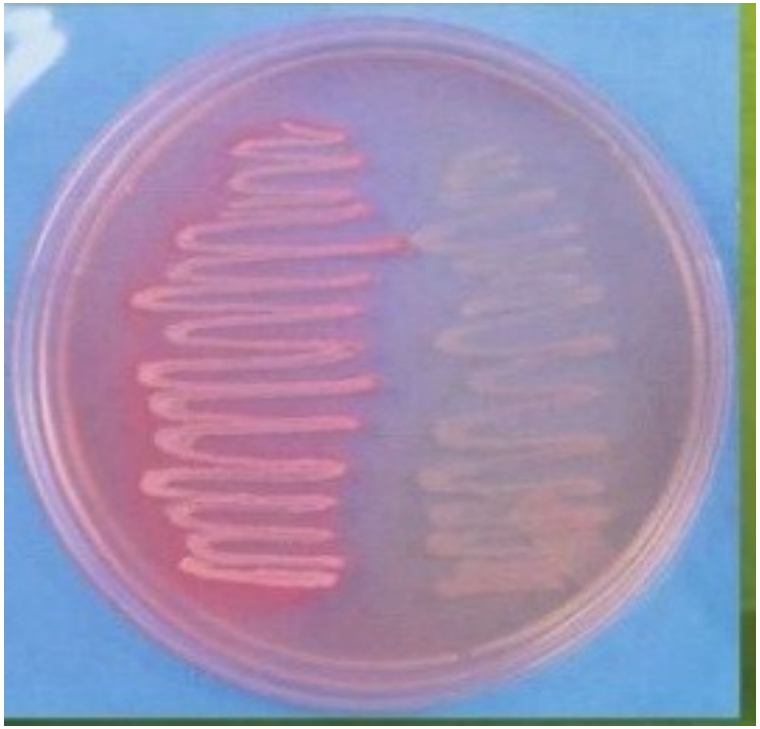
- Certain media are designed in such a way that different bacteria can be recognized on the basis of their colony color. Various approaches include incorporation of dyes, metabolic substrates etc, so that those bacteria that utilize them appear as differently colored colonies. Substances incorporated in it enable it to distinguish between bacteria.

Example of differential media: MacConkey's agar, CLED agar, XLD agar etc.

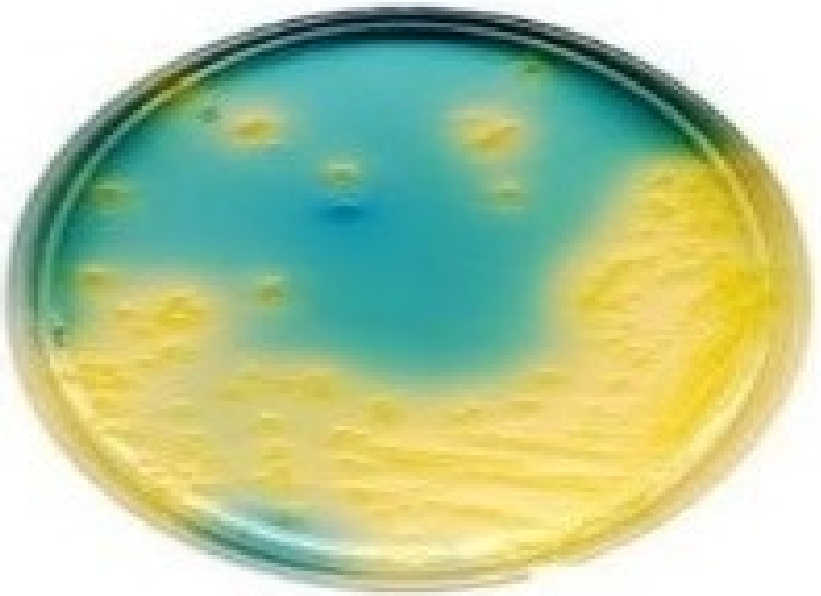
- XYLOSE LYSINE DEOXYCHOLATE AGAR • XLD is used as a selective and differential medium for the recovery of Salmonella and Shigella species.

- CYSTEINE LACTOSE ELECTROLYTE DIFFECIENT AGAR • C.L.E.D. Agar is a non selective solid medium for cultivation of pathogens from urine specimens. Lack of salts (electrolytes) inhibits swarming of Proteus spp.

- MacConkey Agar culture medium designed to grow Gram-negative bacteria and differentiate them for lactose fermentation. It contains bile salts (to inhibit most Gram-positive bacteria), crystal violet dye (which also inhibits certain Gram-positive bacteria). Lactose fermenters – Pink colonies and Non lactose fermenters – colourless colonies.

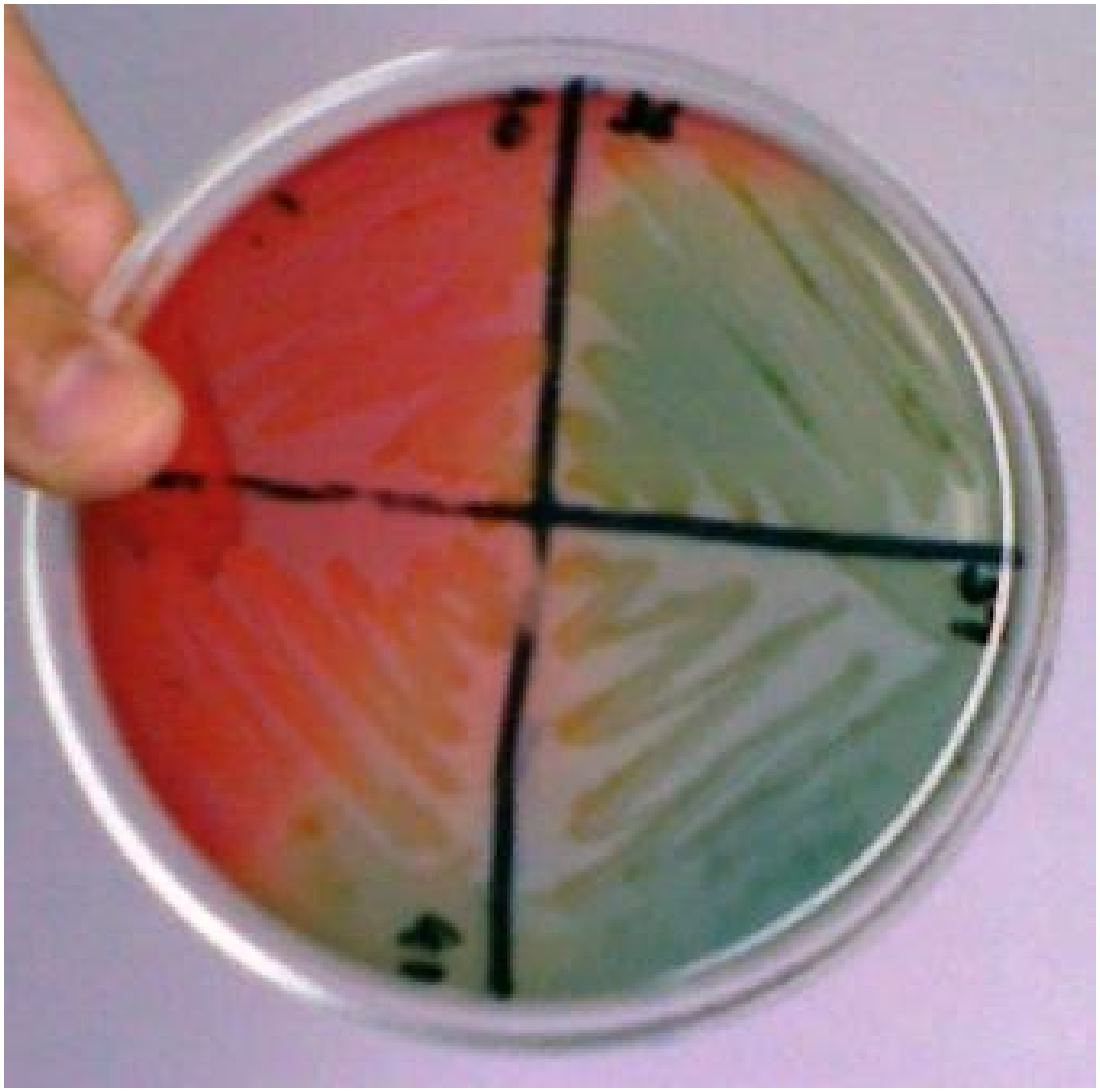


Mac’Conkey Agar



C.L.E.D Agar

Growth of pathogenic *E.coli* on CLED agar



4. Transport Media

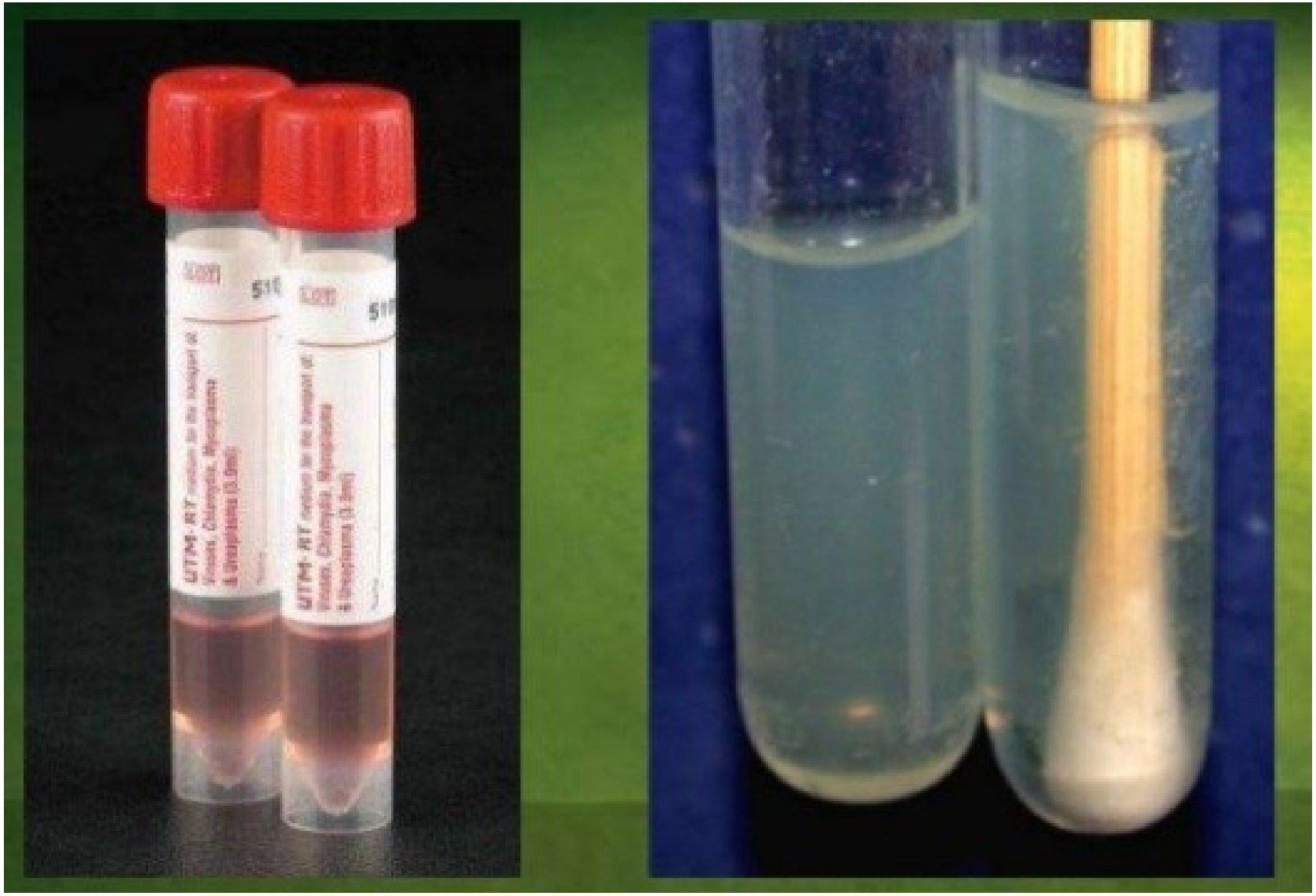
- Clinical specimens must be transported to the laboratory immediately after collection to prevent overgrowth of contaminating organisms or commensals. Delicate organisms may not survive the time taken for transporting the specimen without a transport media. This can be achieved by using transport media.

Transport media should fulfill the following criteria:

- Temporary storage of specimens being transported to the laboratory for cultivation.
- Maintain the viability of all organisms in the specimen without altering their concentration.
- Contain only buffers and salt.
- Lack of carbon, nitrogen, and organic growth factors so as to prevent microbial multiplication.
- Transport media used in the isolation of anaerobes must be free of molecular oxygen.

Example of Transport media:

- Cary Blair medium for campylobacter species.
- Alkaline peptone water medium for vibrio cholerae.
- Stuart's medium – non nutrient soft agar gel containing a reducing agent & charcoal used for Gonnococci.
- Buffered glycerol saline for enteric bacilli.

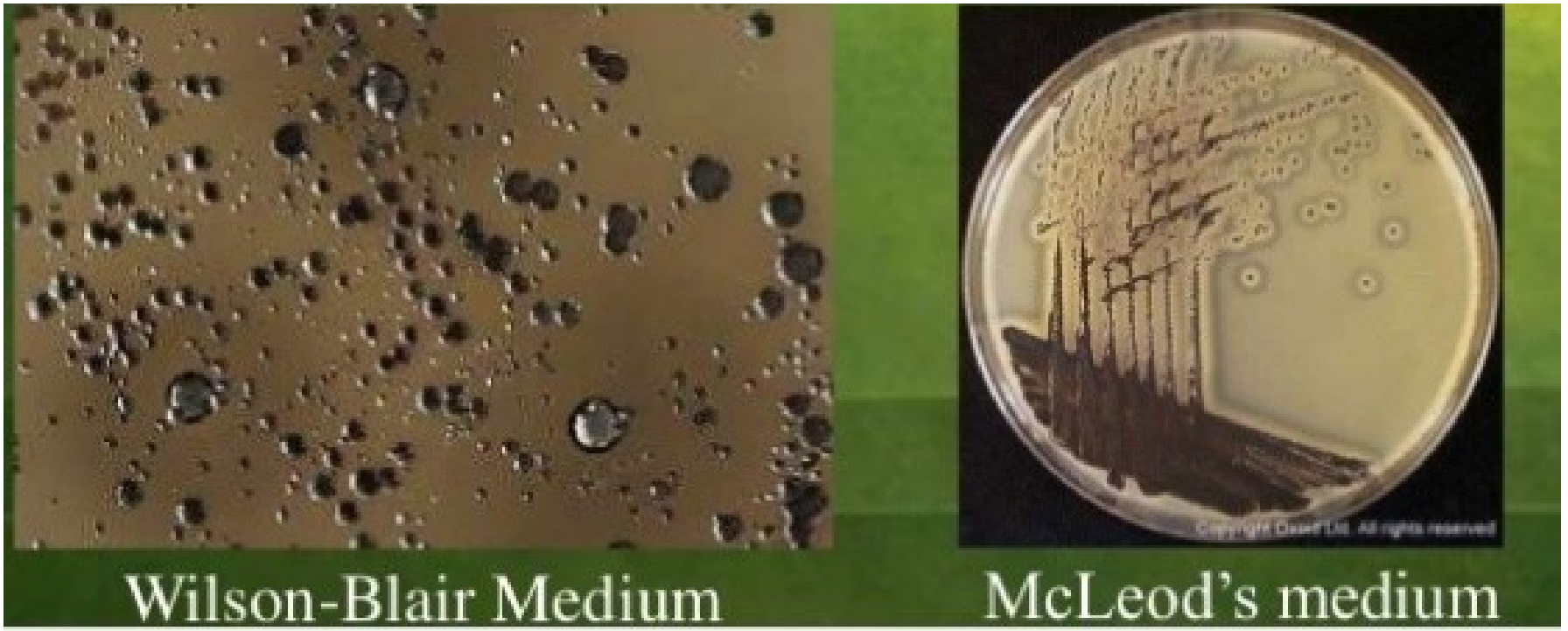


5. Indicator Media

- Contains an indicator which changes its color when a bacterium grows in them.

- Eg: Wilson-Blair medium – S. typhi forms black colonies.
- McLeod's medium (Potassium tellurite)– Diphtheria bacilli.
- Urease media: Urea → CO_2 + NH_3.

  NH_3 → Medium turns pink


Wilson-Blair Medium
McLeod's medium


Urease medium

6. Anaerobic media

- Anaerobic bacteria need special media for growth because they need low oxygen content, reduced oxidation –reduction potential and extra nutrients.
- Media for anaerobes may have to be supplemented with nutrients like hemin and vitamin K.
- Boiling the medium serves to expel any dissolved oxygen.

Example of Anaerobic media:

- Thioglycollate medium.

Thioglycollate medium

Culture Methods

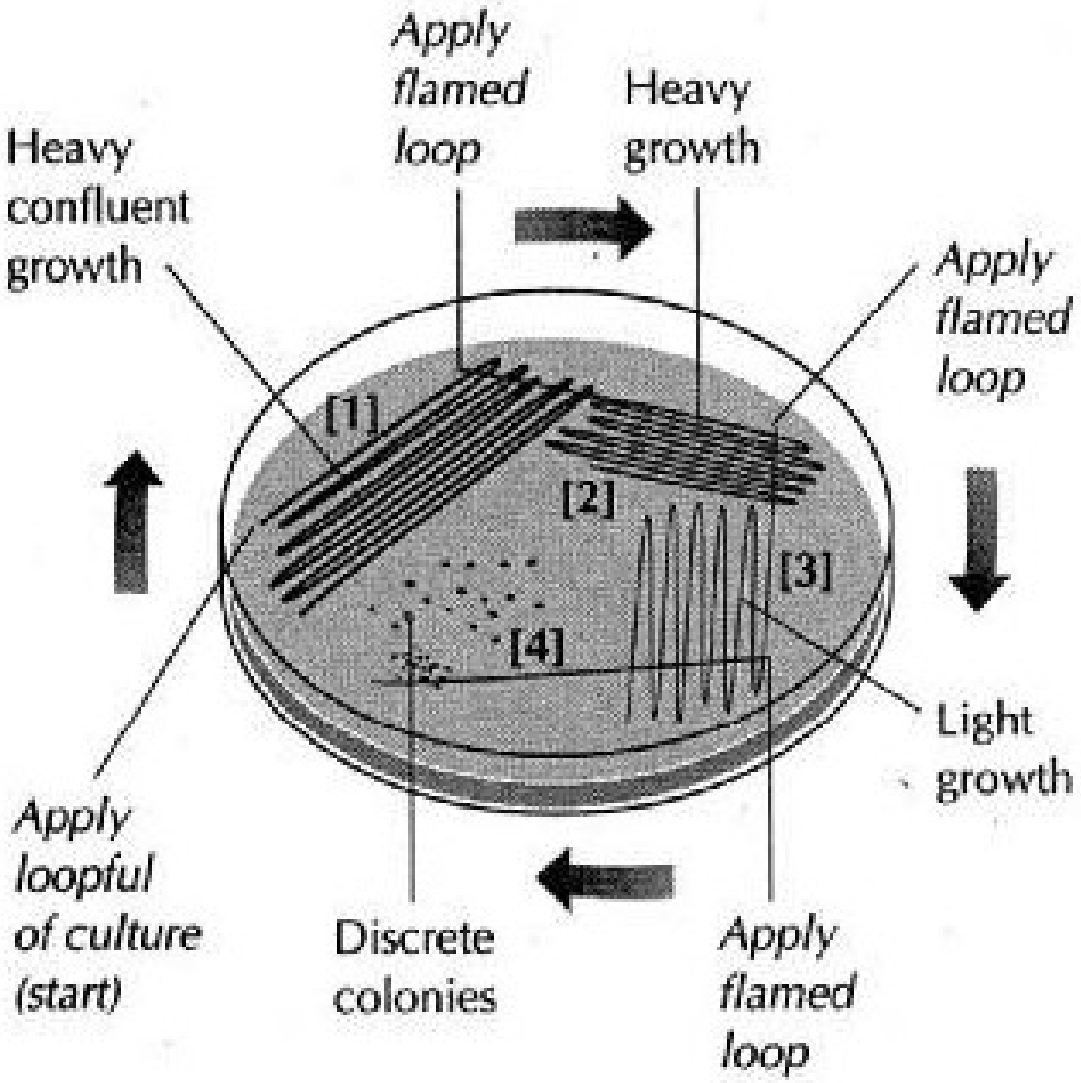
Culture Methods

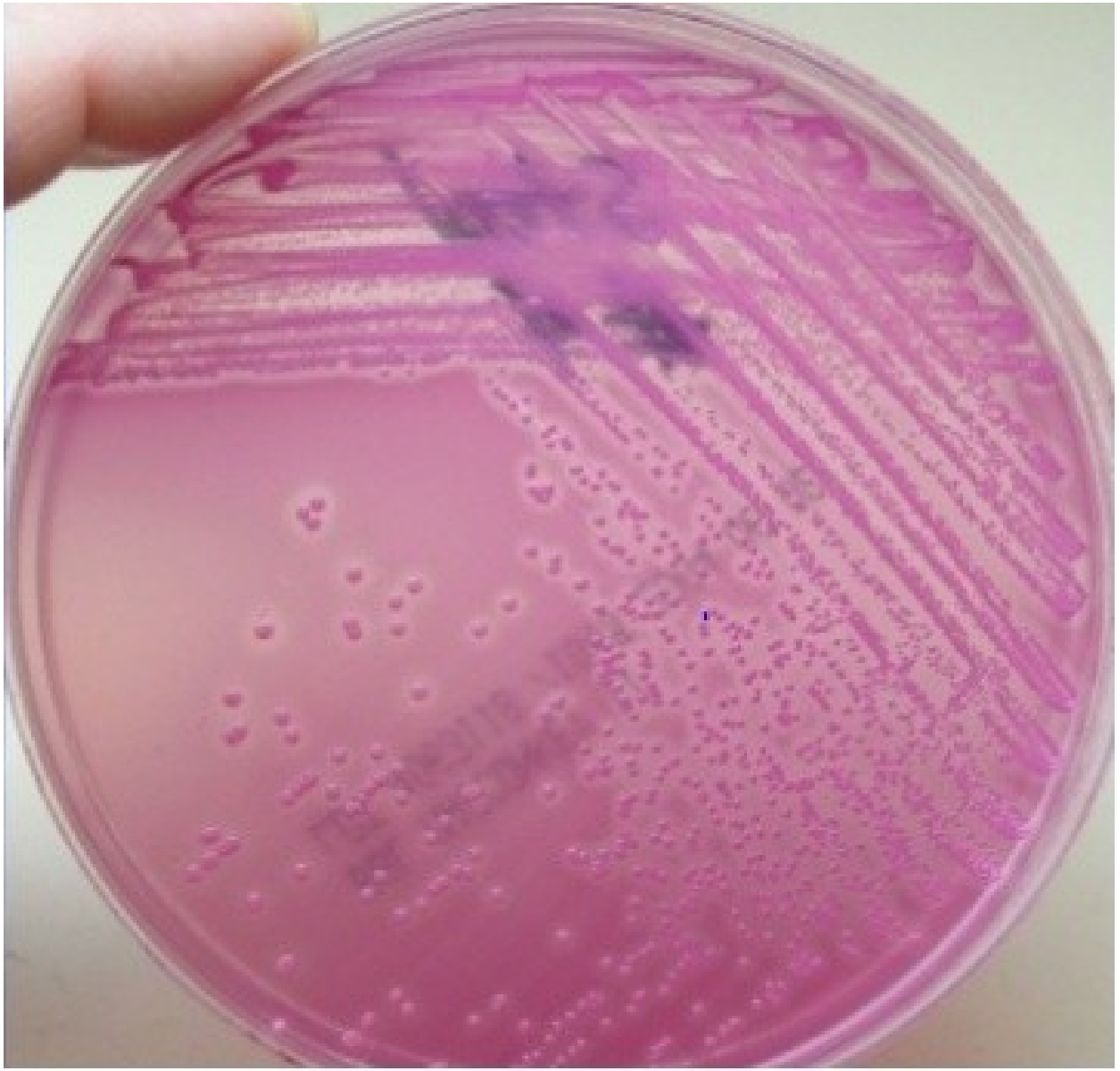
- Streak culture
- Lawn culture
- Stroke culture
- Stab culture
- Pour plate method

Streak culture

Used for the isolation of bacteria in pure culture from clinical specimens.

- Platinum wire is used.
- One loop full of the specimen is transferred onto the surface of a well dried plate.
- Spread over a small area at the periphery.
- The inoculum is then distributed thinly over the plate by streaking it with a loop in a series of parallel lines in different segments of the plate.
- On incubation, separated colonies are obtained over the last series of streaks.


Apply flamed loop
Heavy growth
Heavy confluent growth
Apply flamed loop
[1]
[2]
[3]
[4]
Light growth
Apply loopful of culture (start)
Discrete colonies
Apply flamed loop



Lawn Culture

- Provides a uniform surface growth of the bacterium.
- Lawn cultures are prepared by flooding the surface of the plate with a liquid suspension of the bacterium
- Uses

– For bacteriophage typing.

– Antibiotic sensitivity testing.

– In the preparation of bacterial antigens and vaccines.

Antibiotic sensitivity testing

Stroke Culture

- Stroke culture is made in tubes containing agar slope / slant.

Uses:

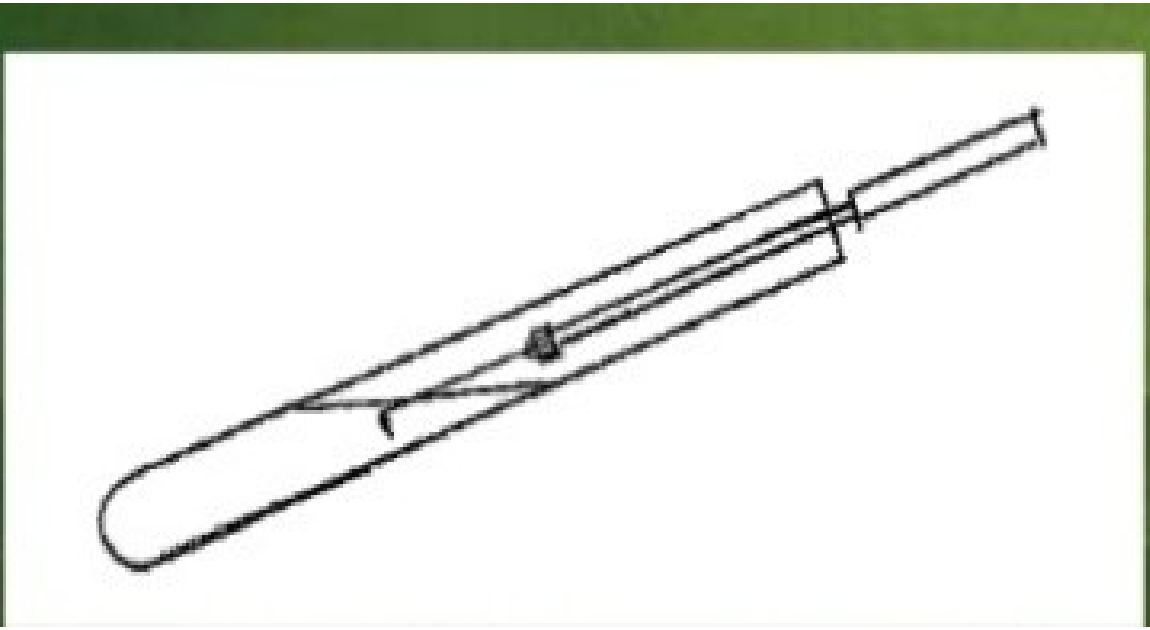
Provides a pure growth of bacterium for slide agglutination and other diagnostic tests.

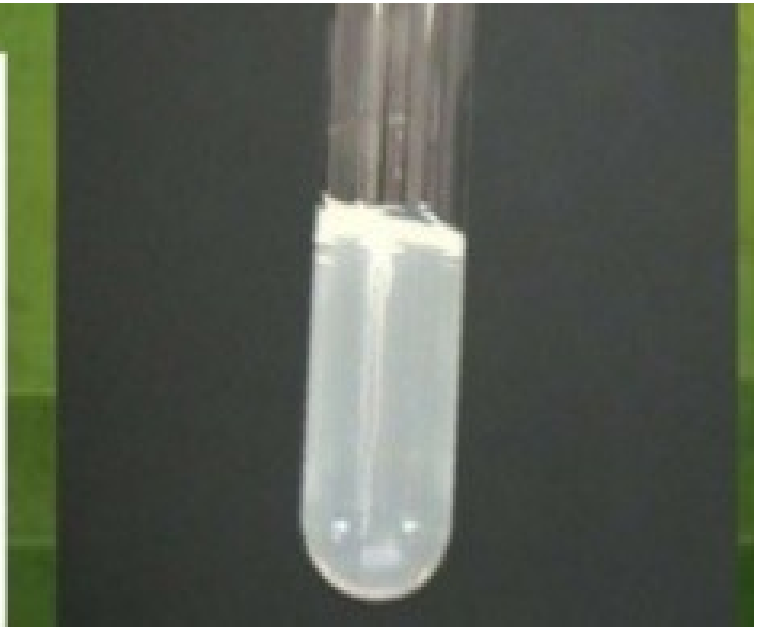
Stab Culture

Prepared by puncturing a suitable medium – gelatin or glucose agar with a long, straight, charged wire.

Uses

- Demonstration of gelatin liquefaction.
- Oxygen requirements of the bacterium under study.
- Maintenance of stock cultures.





Pour Plate Culture

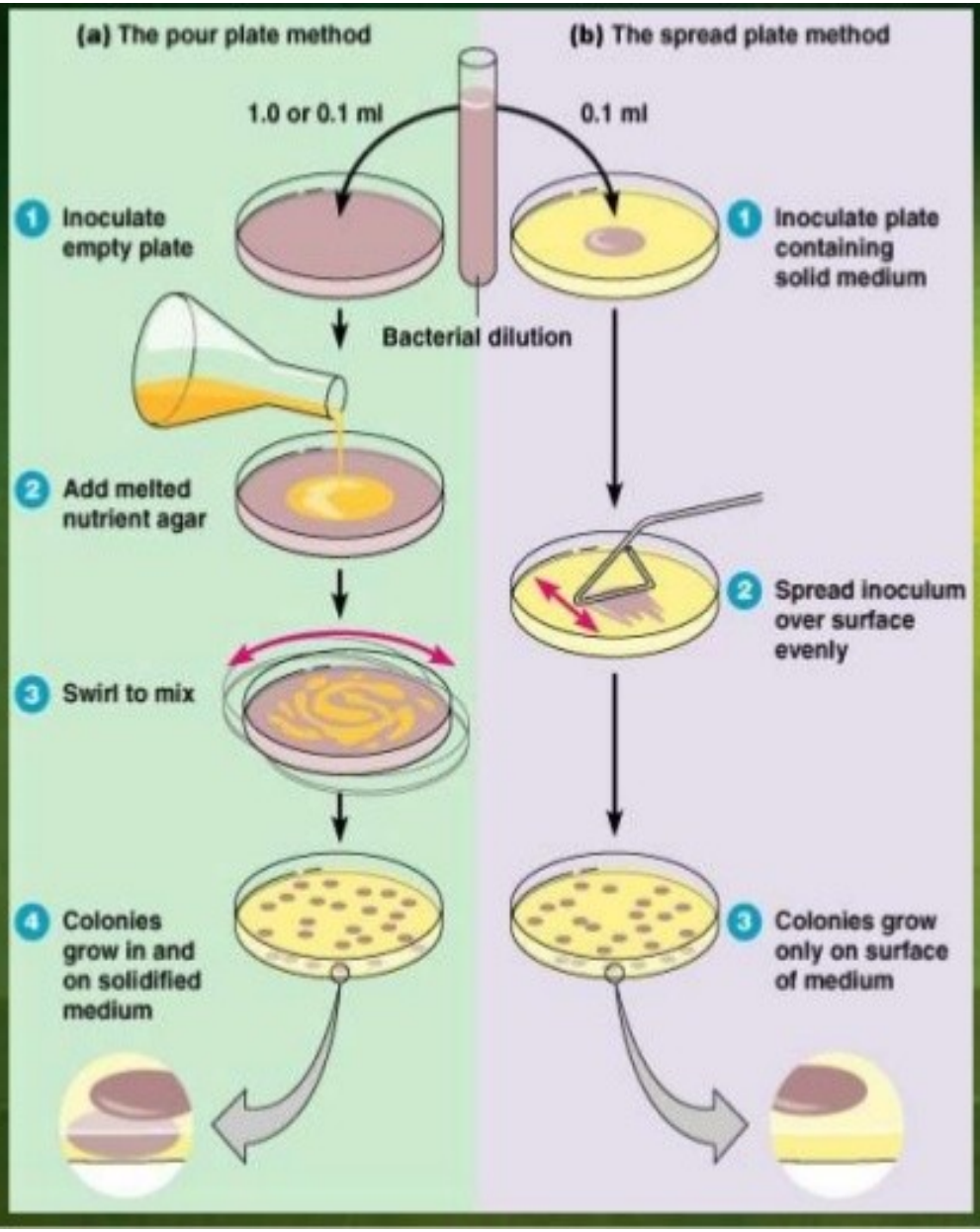
- 1 ml of the innoculum is added to the molten agar.
- Mix well and pour to a sterile Petri dish.
- Allow it to set.
-

  depth of the medium.

Uses:

– Gives an estimate of the viable bacterial count in a suspension.

– For the quantitative urine cultures.


(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate
1 Inoculate plate containing solid medium
Bacterial dilution
2 Add melted nutrient agar
2 Spread inoculum over surface evenly
3 Swirl to mix
4 Colonies grow in and on solidified medium
3 Colonies grow only on surface of medium

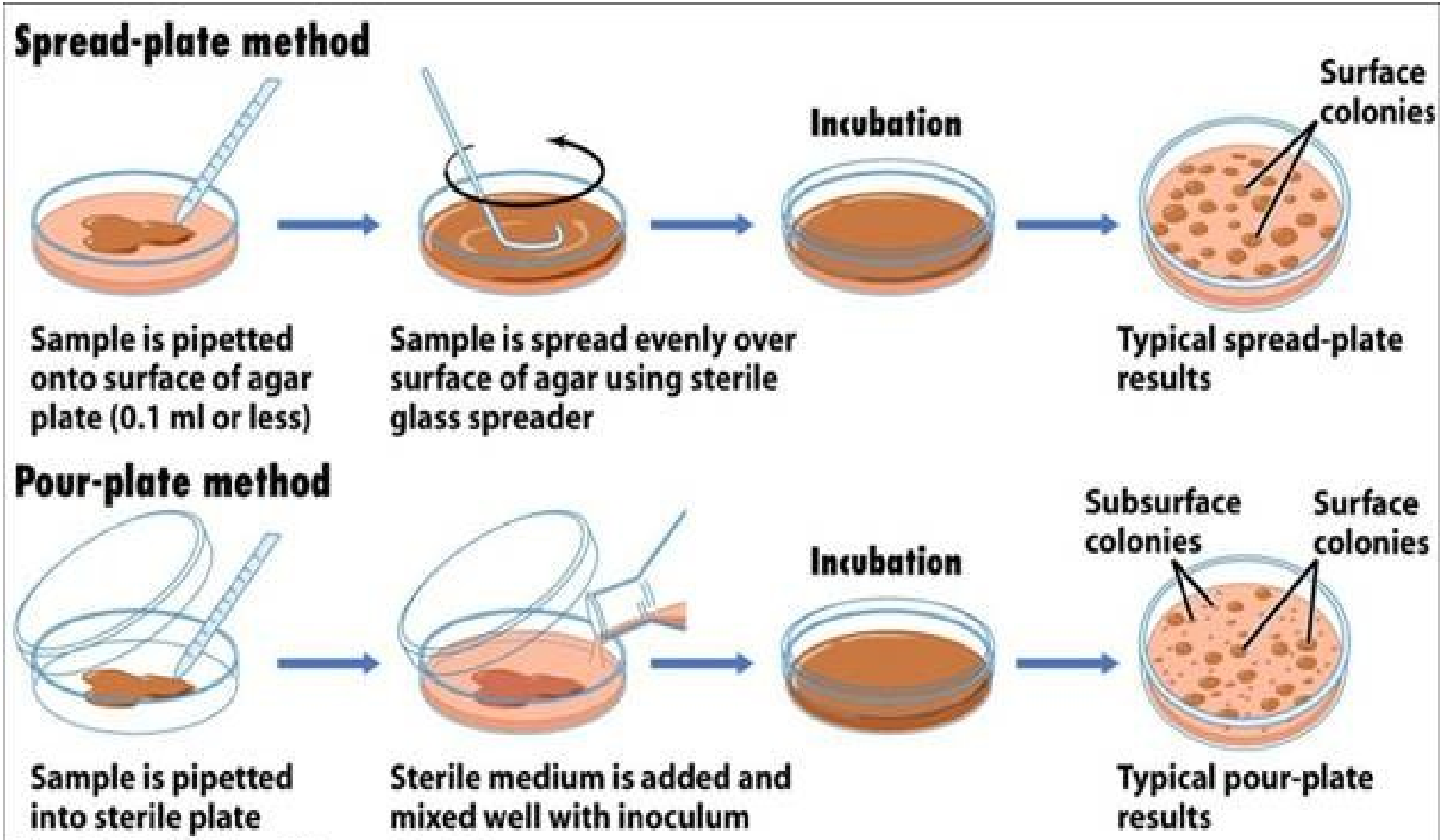


Figure 6-10 Brock Biology of Microorganisms 11/e