CULTURE MEDIA & CULTURE METHODS

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Need for Culture media:

- Bacteria: mixed population in nature
- By appropriate procedures they have to be grown separately (isolated) on culture media and obtained as pure culture for study
- Medium → Nutrients → support growth

Liquid medium:

- Diffused growth
- No characteristics for identification
- Difficult to isolate
- Earliest liquid medium: urine or meat broth used by Louis Pasteur

Solid medium:

- Distinct colony morphology
- Characteristics → easy to identify
- ***Colony*** – macroscopically visible collection of millions of bacteria originating from a single bacterial cell

- Earliest solid medium:
  Cooked cut potato by Robert Koch
- Gelatin - not satisfactory
  - liquefy at 24^{o}C

Agar

- Universally used for preparing solid medium
- Obtained from seaweed: *Gelidium* →
- No nutritive value
- Not affected by the growth of the bacteria.
- Melts at 98°C & sets at 42°C
- 2% agar is employed in solid medium

Types of culture media

I. Based on their consistency

a) Solid medium

b) Liquid medium

c) Semi solid medium

II. Based on the constituents/ ingredients

a) Simple medium

b) Complex medium

c) Synthetic or defined medium

d) Special media

Special media

- Enriched media
- Enrichment media
- Selective media
- Indicator media
- Differential media
- Sugar media
- Transport media
- Media for biochemical reactions

III.Based on Oxygen requirement

- Aerobic media
- Anaerobic media

Solid media – contains 2% agar

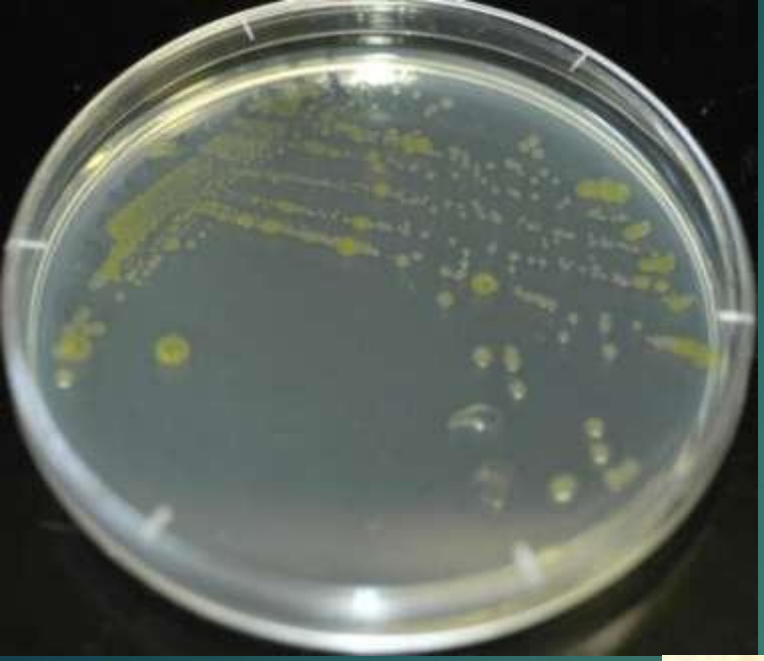
- Colony morphology, pigmentation, hemolysis can be appreciated.
- Eg: Nutrient agar, Blood agar

Liquid media – no agar.

- For inoculum preparation, Blood culture, continuous culture.
- Eg: Nutrient broth

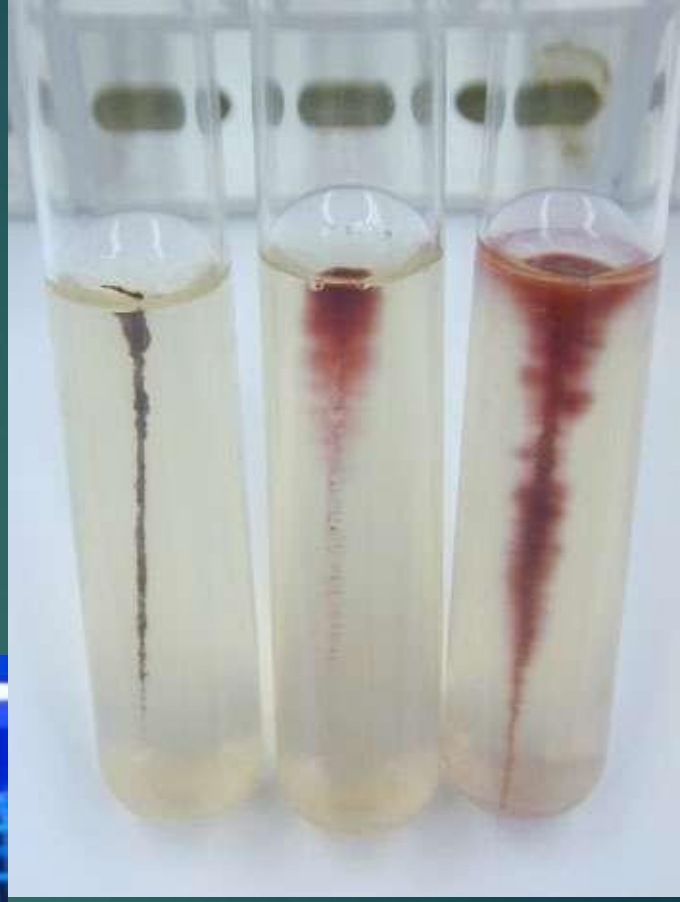
Semi solid medium – 0.5% agar.

- Eg: Motility medium



Solid medium

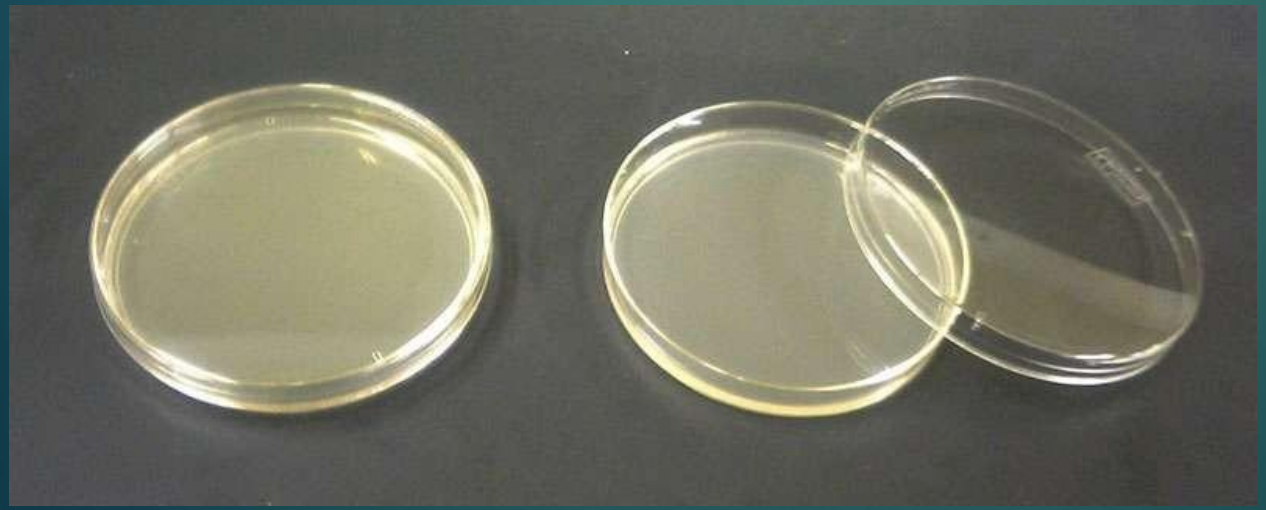
Liquid medium

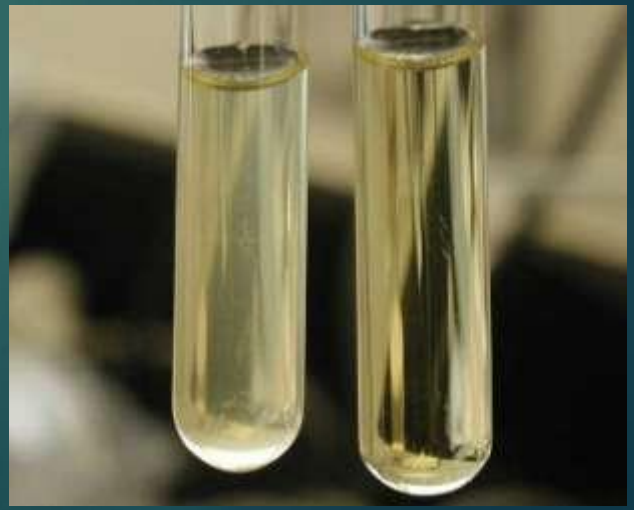


Semi-solid medium

Simple media / basal media:

- Most common in routine diagnostic laboratories
  Eg: Nutrient Broth, Nutrient Agar
- NB consists of peptone, meat extract, NaCl, water
- NB + 0.5% Glucose = Glucose Broth
- NB + 2% agar = Nutrient agar
- Agar conc. Reduced (0.2 - 0.5%) = Semi-solid medium





Complex media

- Media other than basal media.
- They have added complex ingredients such as yeast extract or casein hydrolysate, which consist of a mixture of many chemical species in unknown proportions
- Provide special nutrients

Synthetic or defined media

- Media prepared from pure chemical substances
- exact composition is known
- Used for special studies, eg. metabolic requirements
- Eg: peptone water- (1% peptone + 0.5% NaCl in water)

Enriched media

- Substances like blood, serum, egg are added to the basal medium.
- Used to grow bacteria that are exacting in their nutritional needs.
- Eg: Blood agar, Chocolate agar

Blood agar ←

Chocolate agar →

Enrichment media

- Liquid media used to isolate pathogens from a mixed culture.
- Stimulate growth of desired bacterium
  Inhibit growth of unwanted bacterium
- Media is incorporated with inhibitory substances to suppress the unwanted organism → increase in numbers of desired bacteria
- Eg:
  Selenite F Broth – for the isolation of *Salmonella*, *Shigella*
  Tetrathionate Broth – inhibit coliforms
  Alkaline Peptone Water – for *Vibrio cholerae*

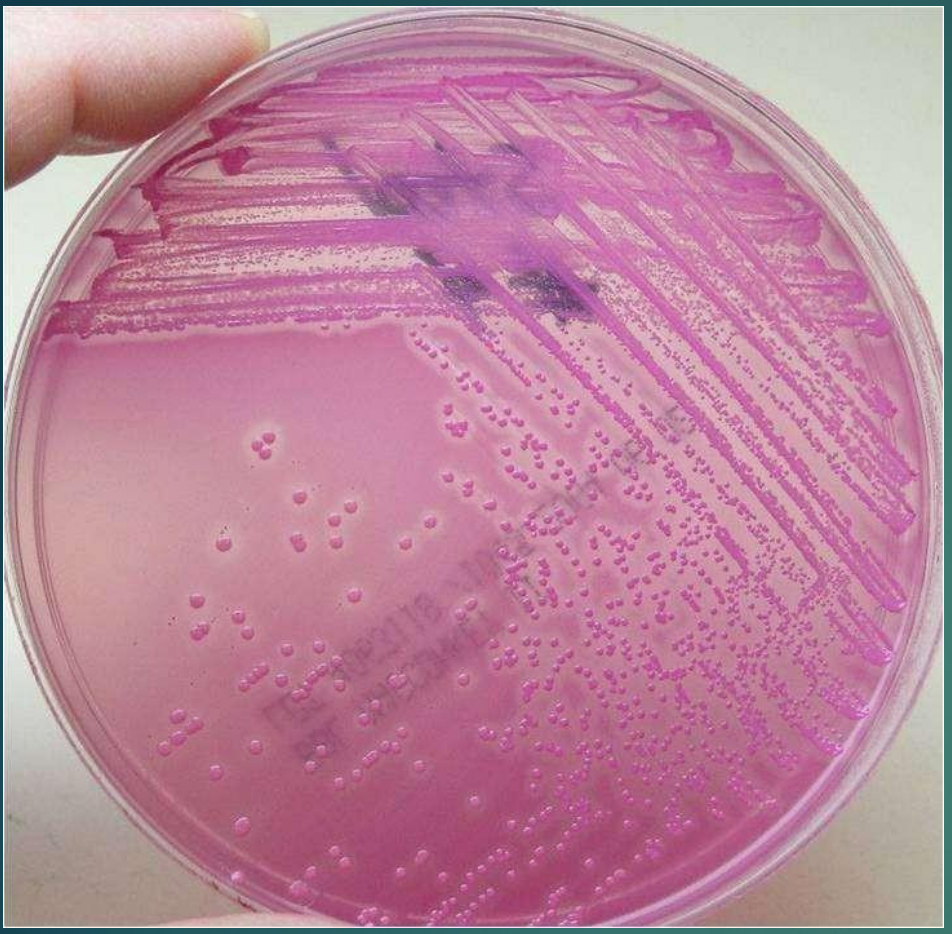
Selenite F Broth

Tetrathionate Broth

Alkaline Peptone water

Selective media

- The inhibitory substance is added to a solid media
- Increase in number of colonies of desired bacterium
- Eg:
- Desoxycholate citrate medium for dysentery bacilli
- Mac Conkey's medium for gram negative bacteria
- TCBS – for *V. cholerae*
- LJ medium – *M. tuberculosis*



Mac Conkey’s medium

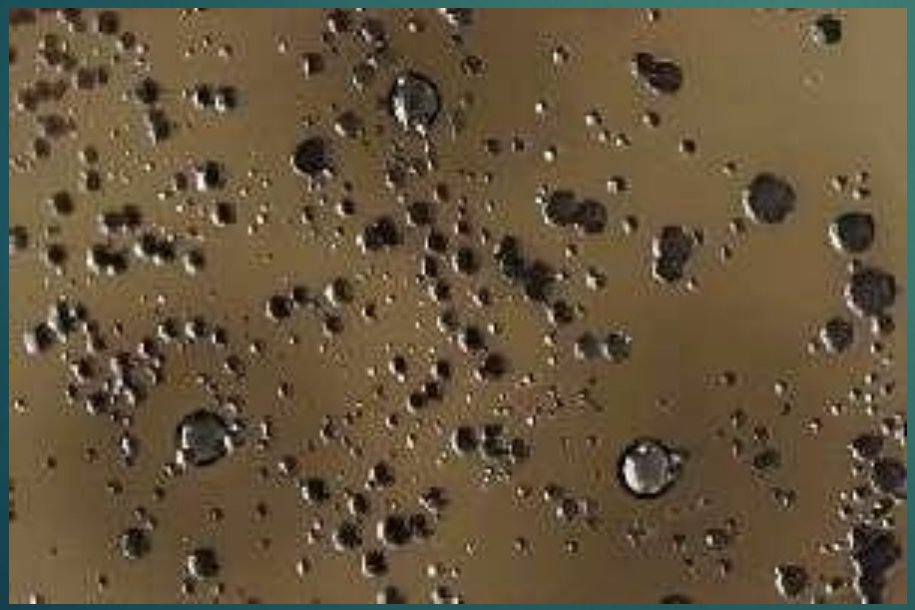
Thiosulfate citrate bile salts sucrose (TCBS) agar

Deoxycholate citrate agar

LJ media

Indicator media

- contain an indicator which changes its colour when a bacterium grows in them
- Eg:
  Wilson-Blair medium – *S. typhi* forms black colonies
  McLeod's medium (Potassium tellurite)– Diphtheria bacilli



Wilson-Blair Medium

McLeod's medium

Urease producing bacteria

↓

Urease

↓

$Urea \rightarrow CO_2 + NH_3$

↓

$NH_3 \rightarrow$ Medium turns pink

Urease medium

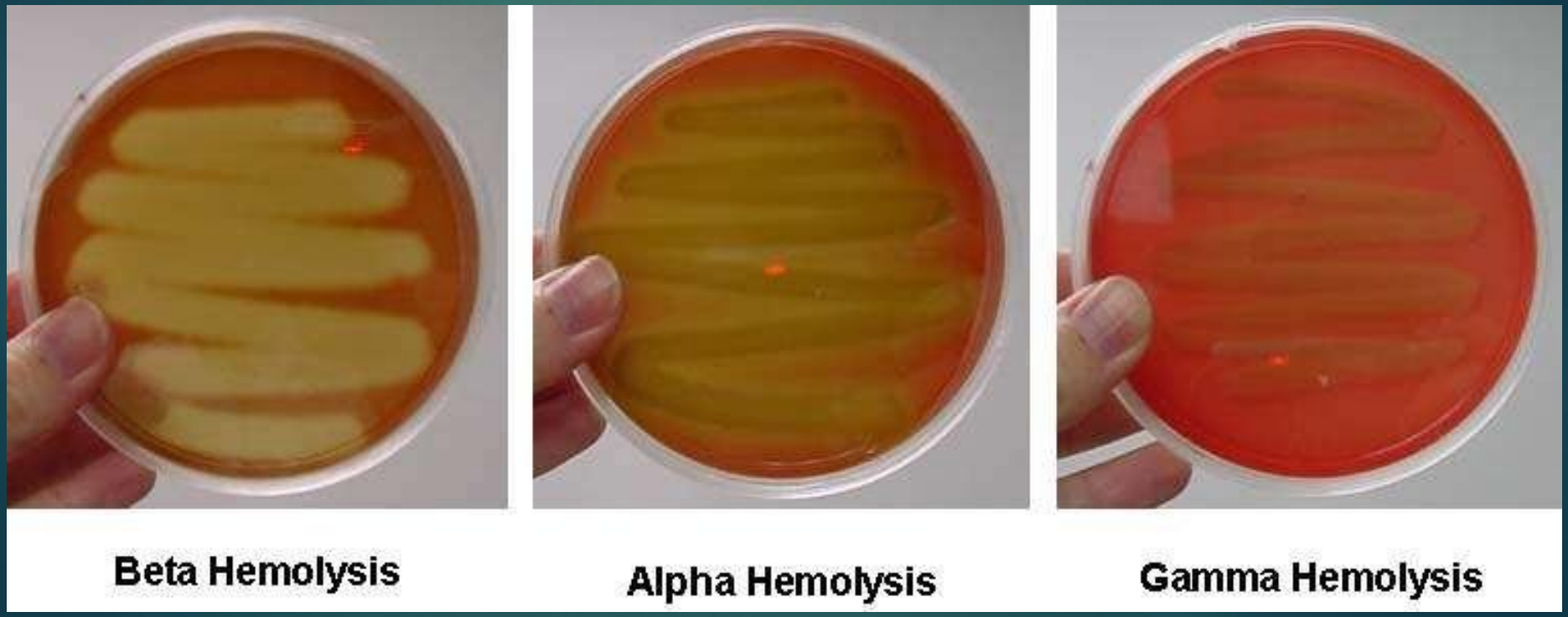
Blood agar:

shows three types of Hemolysis

α Hemolysis

β Hemolysis

γ Hemolysis

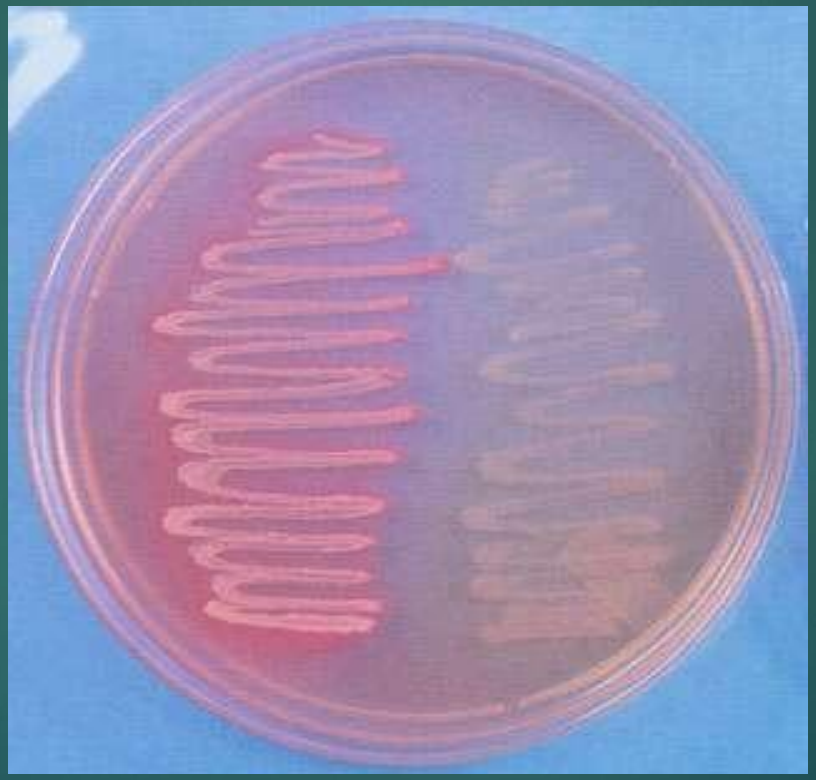


Differential media

- Substances incorporated in it enabling it to distinguish between bacteria.
- Eg: Mac Conkey's medium
  - Peptone
  - Lactose
  - Agar
  - Neutral red
  - Taurocholate
- Distinguish between lactose fermenters & non lactose fermenters.

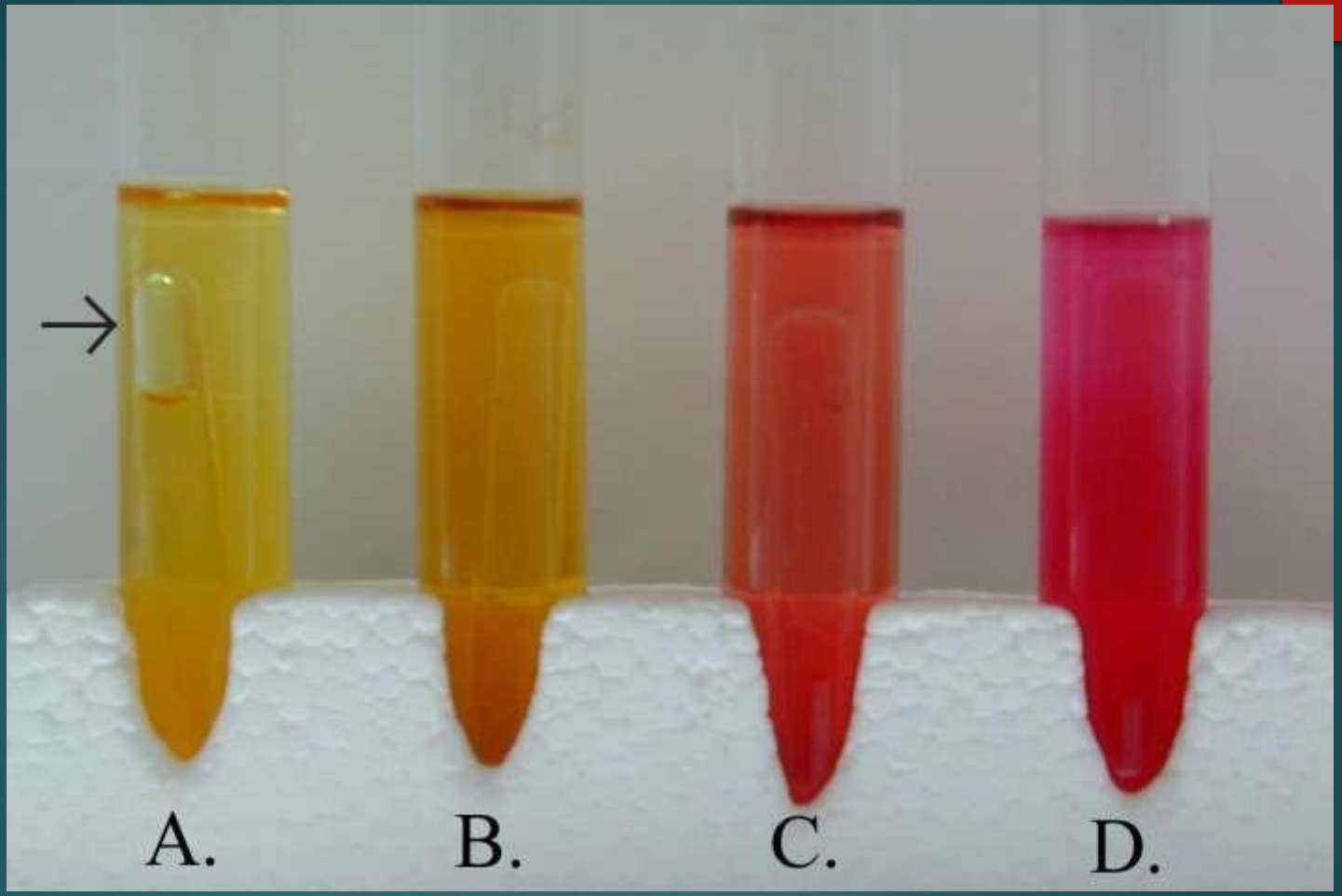
MacConkey agar:

- Lactose fermenters – **Pink** colonies
- Non lactose fermenters – colourless colonies



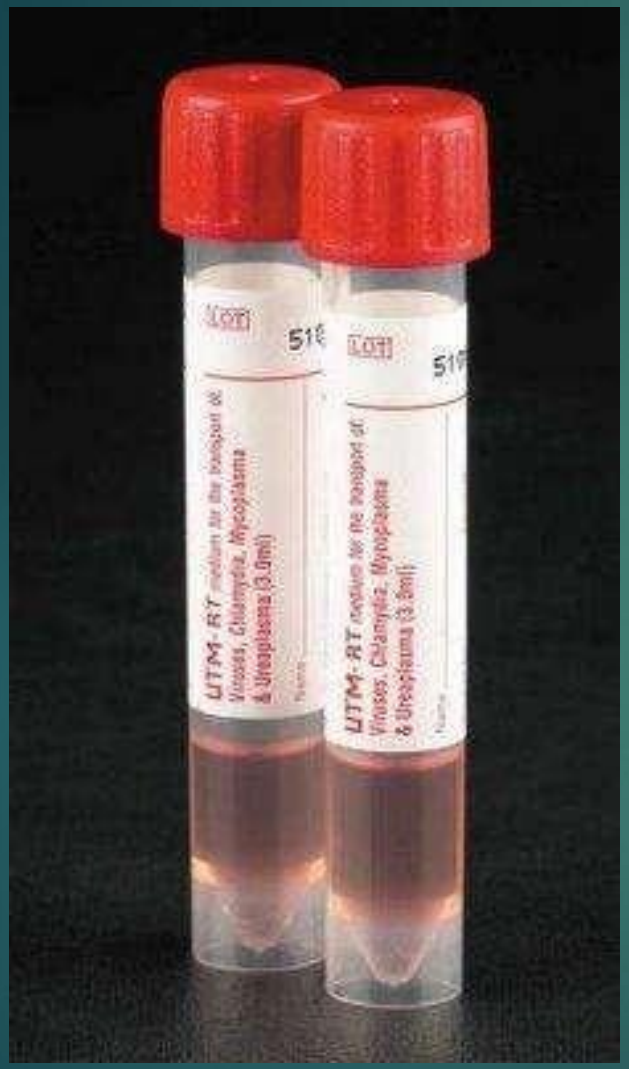
Sugar media

- Media containing any fermentable substance
- Eg: glucose, arabinose, lactose, starch etc.
- Media consists:
  1% of the sugar in peptone water + Indicator
- Contain a small tube (Durham’s tube) for the detection of gas by the bacteria


A.
B.
C.
D.

Transport media

- Media used for transporting the samples.
- Delicate organisms may not survive the time taken for transporting the specimen without a transport media.
- Eg:
  - Stuart's medium – non nutrient soft agar gel containing a reducing agent & charcoal
    - used for *Gonnococci*
  - Buffered glycerol saline – enteric bacilli


LOT
UTM-RT
Viruses, Chlamydia, Mycoplasma
& Ureaplasma (3.0ml)

Anaerobic media

- These media are used to grow anaerobic organisms.
- Eg: Robertson’s cooked meat medium, Thioglycolate medium.

CULTURE METHODS

- Culture methods employed depend on the purpose for which they are intended.
- **Purposes:**
  - To isolate bacteria in pure cultures.
  - To demonstrate their properties.
  - To obtain sufficient growth for the preparation of antigens and for other tests.
  - For bacteriophage & bacteriocin susceptibility.
  - To determine sensitivity to antibiotics.
  - To estimate viable counts.
  - Maintain stock cultures.

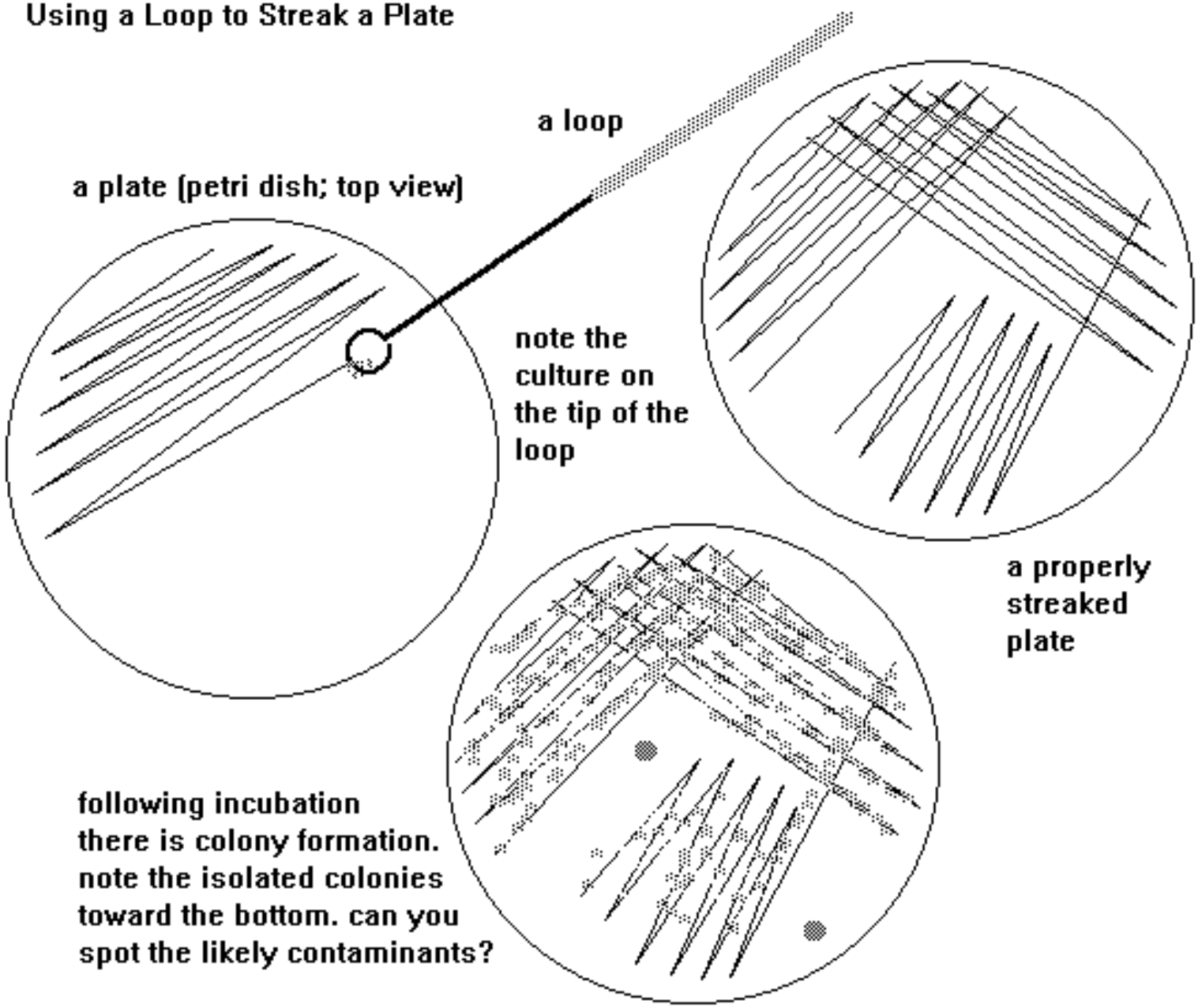
Culture methods include:

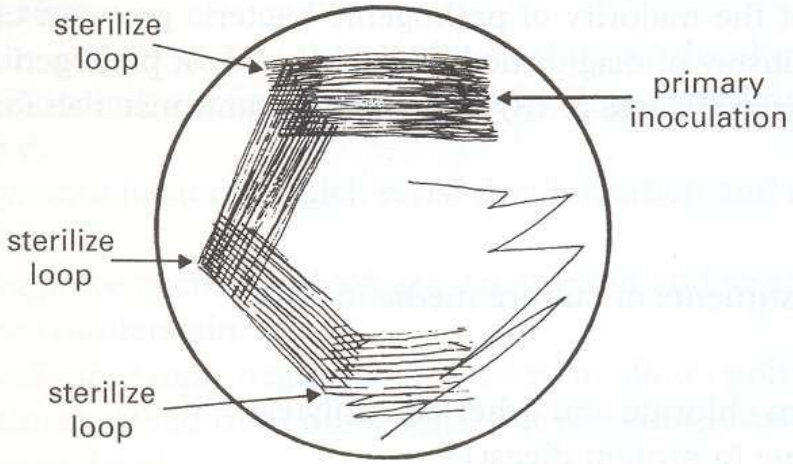
- Streak culture
- Lawn culture
- Stroke culture
- Stab culture
- Pour plate method
- Liquid culture
- Anaerobic culture methods

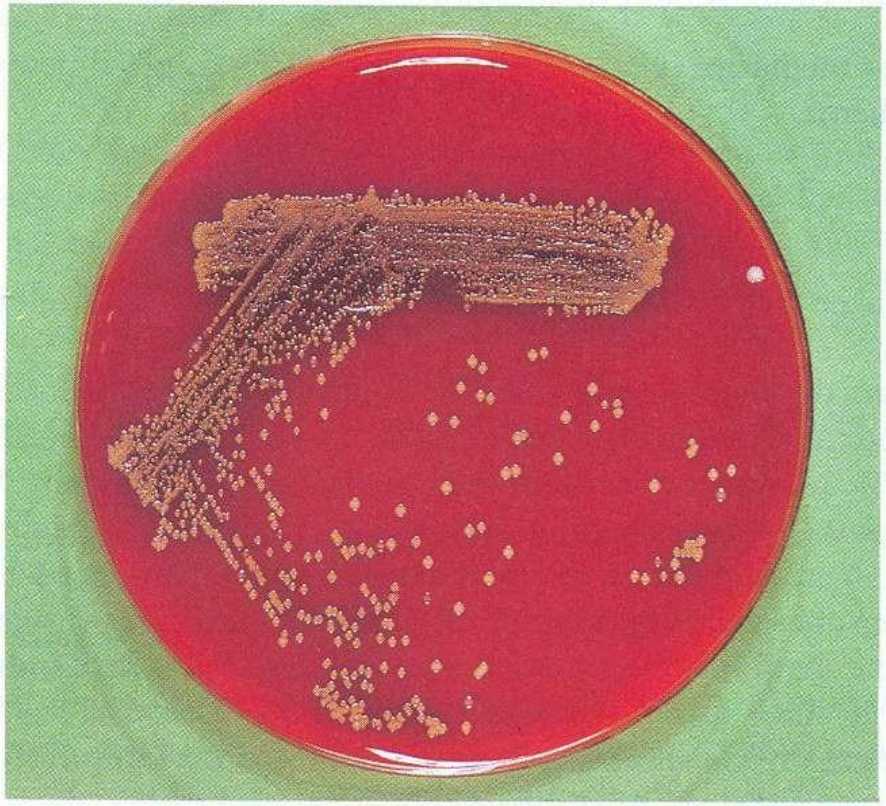
STREAK CULTURE

- Used for the isolation of bacteria in pure culture from clinical specimens.
- Platinum wire or Nichrome wire is used.
- One loopful 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.

Using a Loop to Streak a Plate




sterilize loop
primary inoculation
sterilize loop
sterilize loop



LAWN CULTURE

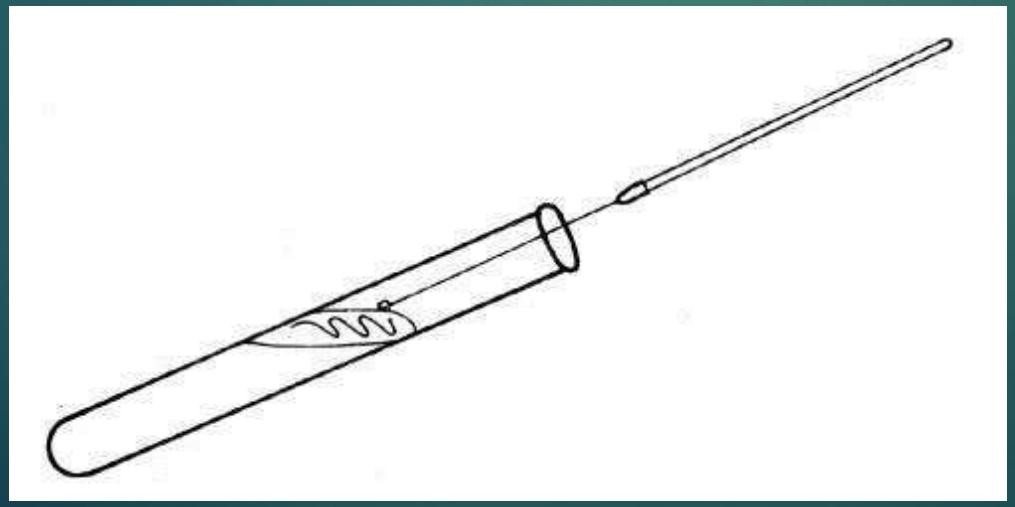
- Provides a uniform surface growth of the bacterium.
- Uses
  - For bacteriophage typing.
  - Antibiotic sensitivity testing.
  - In the preparation of bacterial antigens and vaccines.
- Lawn cultures are prepared by flooding the surface of the plate with a liquid suspension of the bacterium.

Antibiotic sensitivity testing

STROKE CULTURE

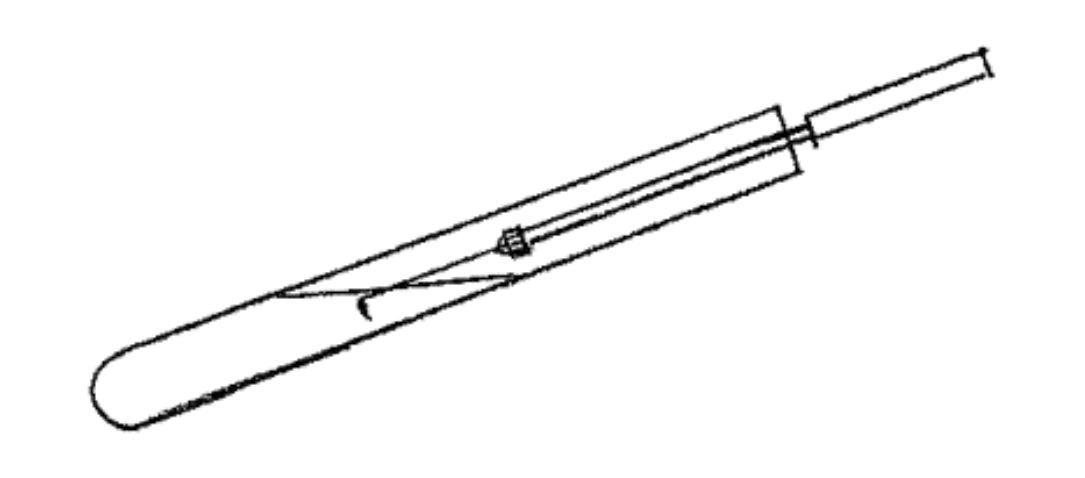
- Stroke culture is made in tubes containing agar slope / slant.

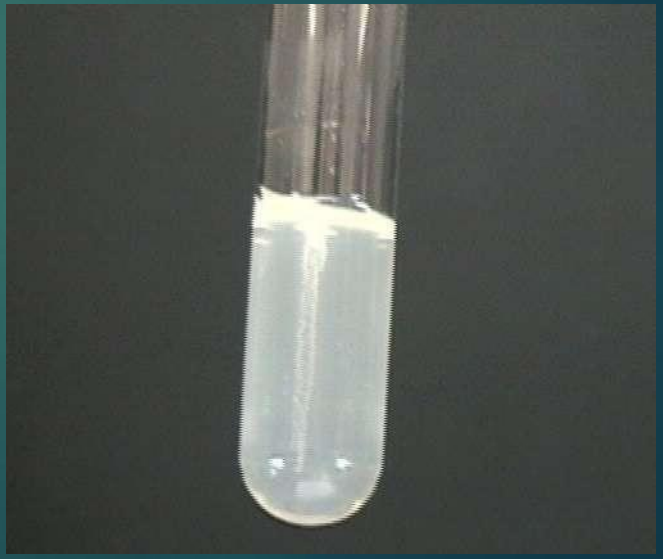
- Uses
  - Provide a pure growth of bacterium for slide agglutination and other diagnostic tests.



STAB CULTURE

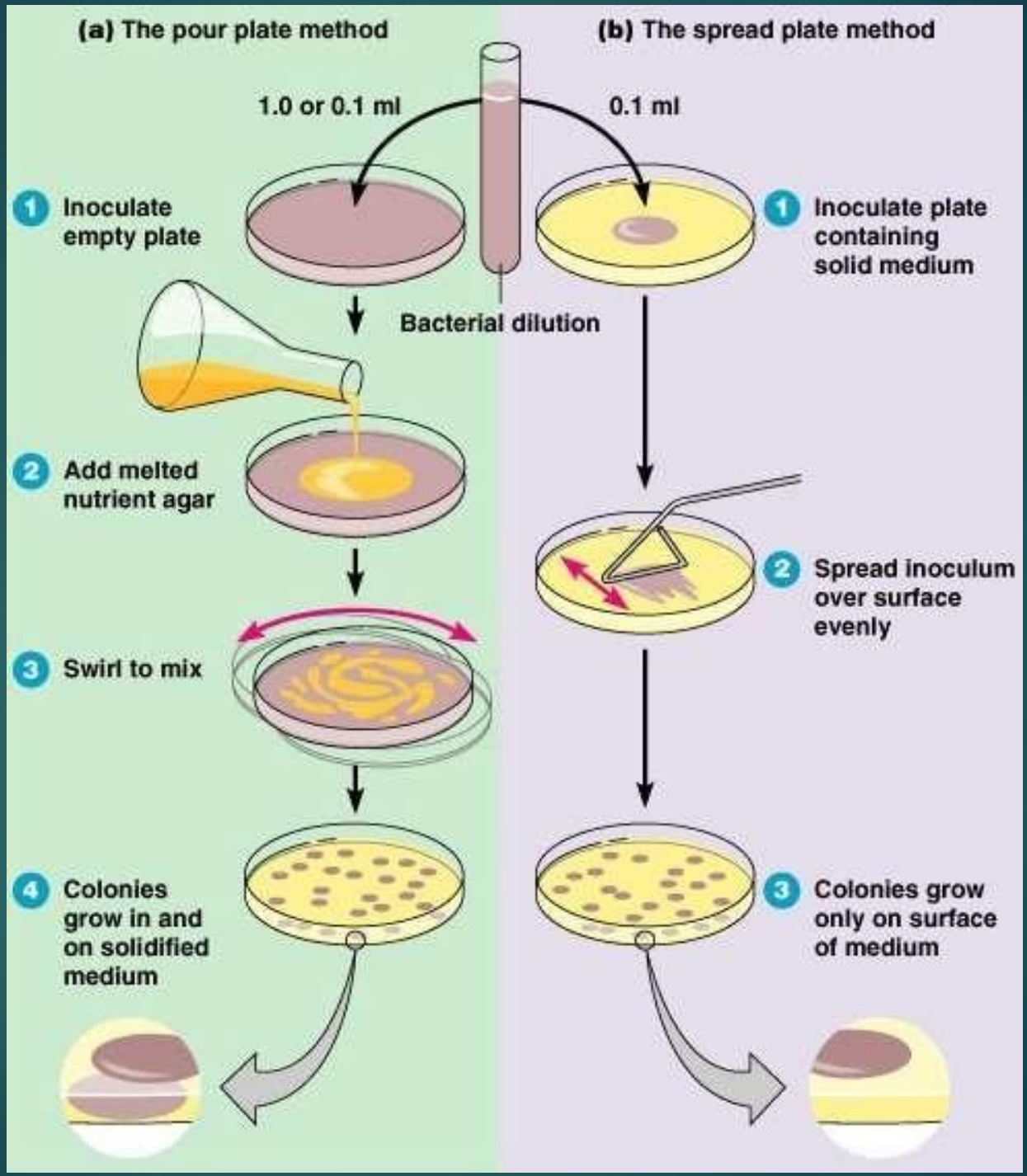
- Prepared by puncturing a suitable medium – gelatin or glucose agar with a long, straight, charged wire.
- Uses
  - Demonstration of gelatin liquefaction.
  - Oxygen requirements of the bacterium under study.
  - Maintenance of stock cultures.





POUR PLATE CULTURE

- Agar medium is melted (15 ml) and cooled to 45^{o}C.
- 1 ml of the inoculum is added to the molten agar.
- Mix well and pour to a sterile petri dish.
- Allow it to set.
- Incubate at 37^{o}C, colonies will be distributed throughout the 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

LIQUID CULTURES

- Liquid cultures are inoculated by touching with a charged loop or by adding the inoculum with pipettes or syringes.
- Uses
  - Blood culture
  - Sterility tests
  - Continuous culture methods
- Disadvantage
  - It does not provide a pure culture from mixed inocula.

Blood culture bottles

ANAEROBIC CULTURE METHODS

- Anaerobic bacteria differ in their requirement and sensitivity to oxygen.
- *Cl. tetani* is a strict anaerobe - grows at an oxygen tension < 2 mm Hg.

Methods:

- Production of vacuum
- Displacement of oxygen with other gases
- Chemical method
- Biological method
- Reduction of medium

Production of vacuum:

- Incubate the cultures in a vacuum desiccators.

Displacement of oxygen with other gases

- Displacement of oxygen with hydrogen, nitrogen, helium or CO_2.
- Eg: Candle jar

Chemical method

- Alkaline pyrogallol absorbs oxygen.
- Chromium and Sulphuric acid

McIntosh – Fildes' anaerobic jar

- Consists of a metal jar or glass jar with a metal lid which can be clamped air tight.
- The lid has 2 tubes – gas inlet and gas outlet
- The lid has two terminals – connected to electrical supply.
- Under the lid – small grooved porcelain spool, wrapped with a layer of palladinised asbestos.

Working:

- Inoculated plates are placed inside the jar and the lid clamped air tight.
- The outlet tube is connected to a vacuum pump and the air inside is evacuated.
- The outlet tap is then closed and the inlet tube is connected to a hydrogen supply.
- After the jar is filled with hydrogen, the electric terminals are connected to a current supply, so that the palladinised asbestos is heated.
- Act as a catalyst for the combination of hydrogen with residual oxygen.

Gaspak

- Commercially available disposable envelope.
- Contains chemicals which generate H_2 and CO_2 on addition of water.
- Cold catalyst – permits combination of Hydrogen & Oxygen
- Indicator is used – reduced methylene blue.
  - Colourless – anaerobically
  - Blue colour – on exposure to oxygen


methylene blue
indicator strip
anaerobe
jar
GasPak
BBL
GasPak Plus

Biological method

- Absorption of oxygen by incubation with aerobic bacteria, germinating seeds or chopped vegetables.

Reduction of oxygen

- By using reducing agents – 1% glucose, 0.1% Thioglycolate

Bibliography:

Ananthnarayan and Paniker's Textbook of Microbiology

Methods of preservation of microbial cultures:

Preservation in soil:
Soil borne bacteria and fungi can be stored in their natural habitat i.e.; soil .About 5gms of soil sample is autoclaved at 15lb pressure for 30 minutes and inoculated with 1ml of aqueous suspension of cells/spores. ·The microorganisms are allowed to grow for 10 days and the soil culture thus obtained is stored in refrigerator.

Cold Room Storage:
• Live cultures on a culture medium can be successfully stored in refrigerator or cold rooms. When the temperature is maintained at 4 degree centigrade.
Storage in silica gel: Both bacteria and yeasts can be stored in silica gel at low temperature for 1 to 2 years.

Cryopreservation:

• Cryopreservation (i.e., freezing in liquid nitrogen at -196°C or in the gas phase above the liquid nitrogen at -150°C) helps survival of pure cultures for long storage times. Most species can remain viable under these conditions for 10 to 30 years without undergoing change in their characteristics.

Lyophilization or freeze-drying:

• Freeze-drying is a process where water and other solvents are removed from a frozen product via sublimation. Freeze-dried products are hygroscopic and must be protected from moisture during storage.

• Under these conditions, the microbial cells are dehydrated and their metabolic activities are stopped; as a result, the microbes go into dormant state and retain viability for years. Many species of bacteria remain unchanged in their characteristics for more than 30 years.

THANK YOU