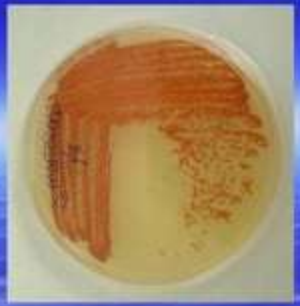

CULTURE MEDIA & CULTURE METHODS

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- What is culture?
- Bacteria have to be grown (cultured) for them to be identified.

CULTURE METHODS

- The indications for culture are:
  - To isolate bacteria in pure cultures.
  - To demonstrate their properties.
  - To obtain sufficient growth for the preparation of antigens.
  - To determine sensitivity to antibiotics.
  - To estimate viable counts.
  - Maintain stock cultures.

Colony – macroscopically visible collection of millions of bacteria originating from a single bacterial cell.

- Cooked cut potato by Robert Koch – earliest solid medium
- Gelatin – not satisfactory
  - liquefy at 24^{0}C

Agar

- Frau Hesse
- Used for preparing solid medium
- Obtained from seaweeds.
- No nutritive value
- Not affected by the growth of the bacteria.
- Melts at 98^{0}C & sets at 42^{0}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, for the isolation of pathogens from a mixture.
- Eg: Nutrient broth

Semi solid medium – 0.5% agar.

- Eg: Motility medium

Simple media / basal media

- Eg: NB, NA
- NB consists of peptone, meat extract, NaCl,
- NB + 2% agar = Nutrient agar

Complex media

- Media other than basal media.
- They have added ingredients.
- Provide special nutrients

Synthetic or defined media

- Media prepared from pure chemical substances and its exact composition is known
- 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

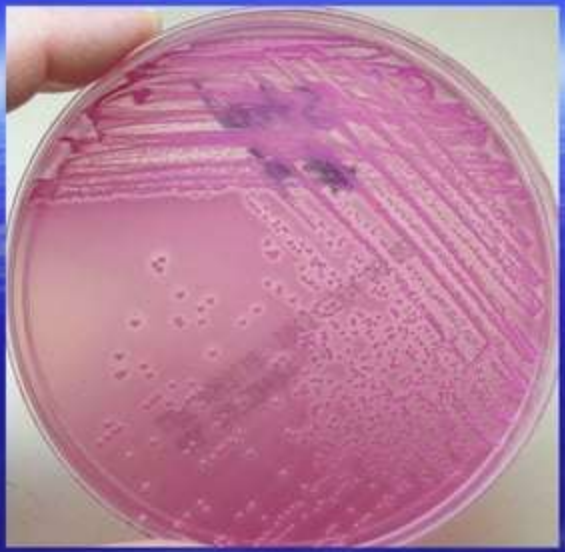
- Liquid media used to isolate pathogens from a mixed culture.
- Media is incorporated with inhibitory substances to suppress the unwanted organism.
- Eg:
  - Selenite F Broth – for the isolation of Salmonella, Shigella
  - Alkaline Peptone Water – for Vibrio cholerae

Selective media

- The inhibitory substance is added to a solid media.

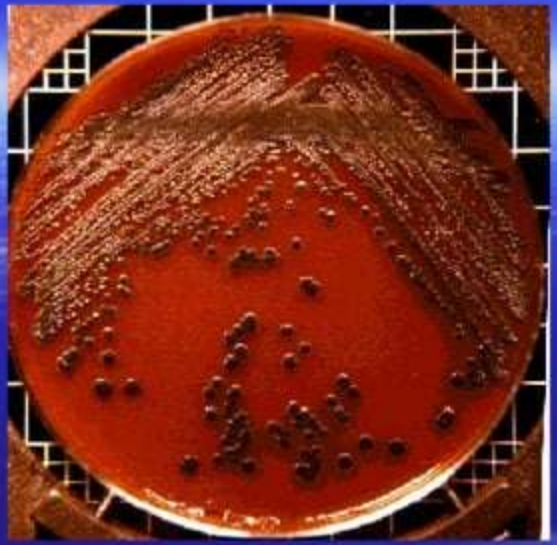
Eg:

- Mac Conkey's medium for gram negative bacteria
- TCBS – for V.cholerae
- LJ medium – M.tuberculosis
- Wilson and Blair medium – S.typhi
- Potassium tellurite medium – Diphtheria bacilli



Mac Conkey's medium

TCBS

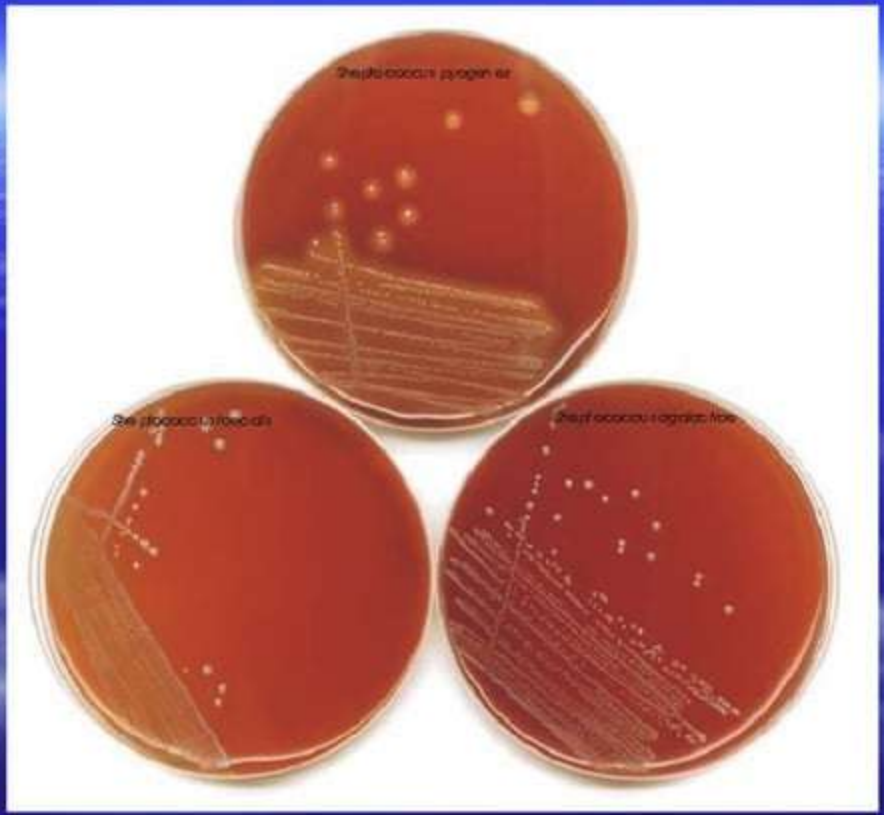


Potassium Tellurite media

LJ media

Indicator media

- These media contain an indicator which changes its colour when a bacterium grows in them.
- Eg:
  - Blood agar
  - Mac Conkey's medium
  - Christensen's urease medium



Urease medium

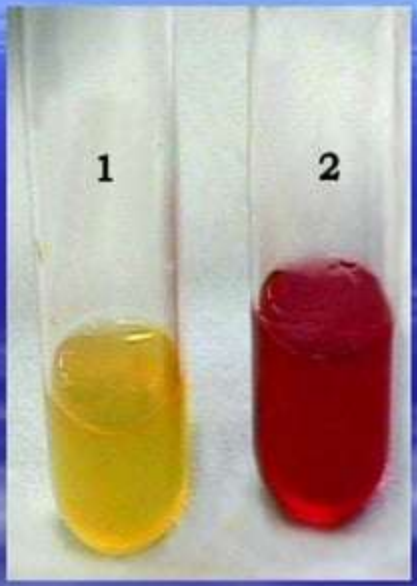
Differential media

- A media which has 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.

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

Sugar media

- Media containing any fermentable substance.
- Eg: glucose, arabinose, lactose, starch etc.
- Media consists of 1% of the sugar in peptone water.
- Contain a small tube (Durham's tube) for the detection of gas by the bacteria.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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
  - Buffered glycerol saline – enteric bacilli

Anaerobic media

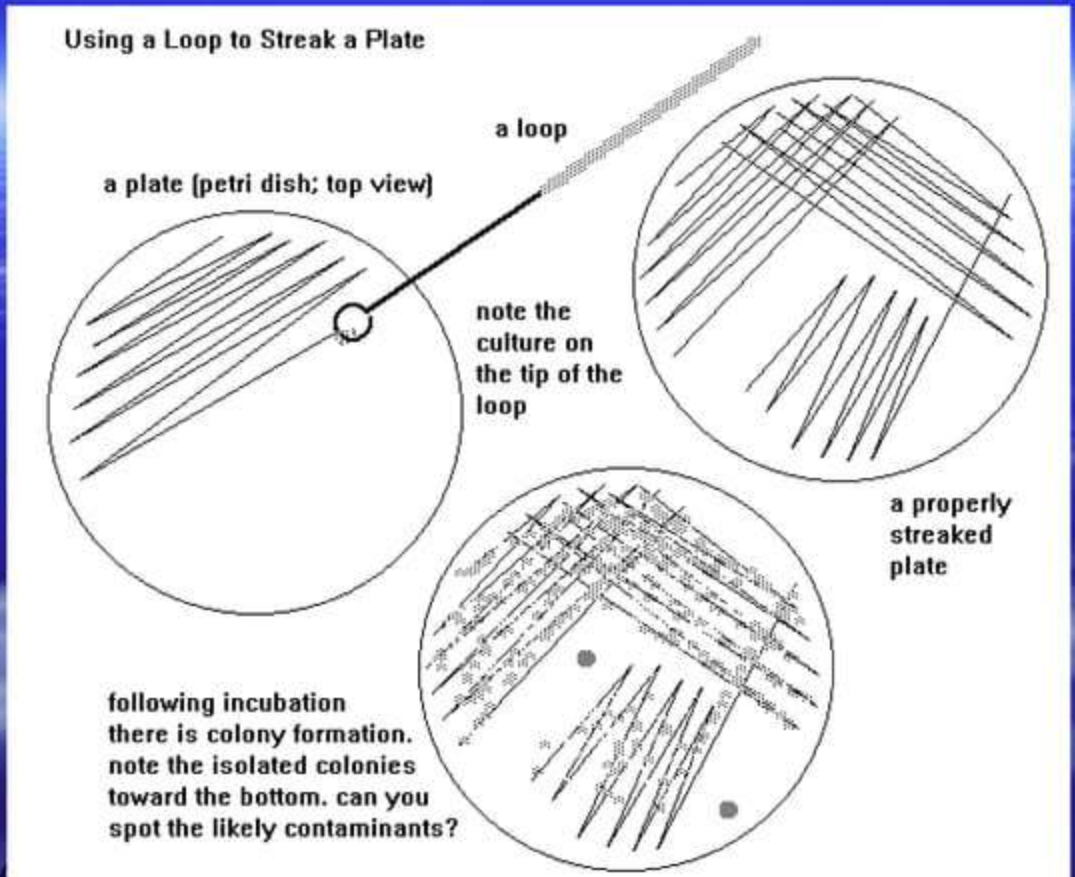
- These media are used to grow anaerobic organisms.
- Eg: Robertson's cooked meat medium, Thioglycolate medium.

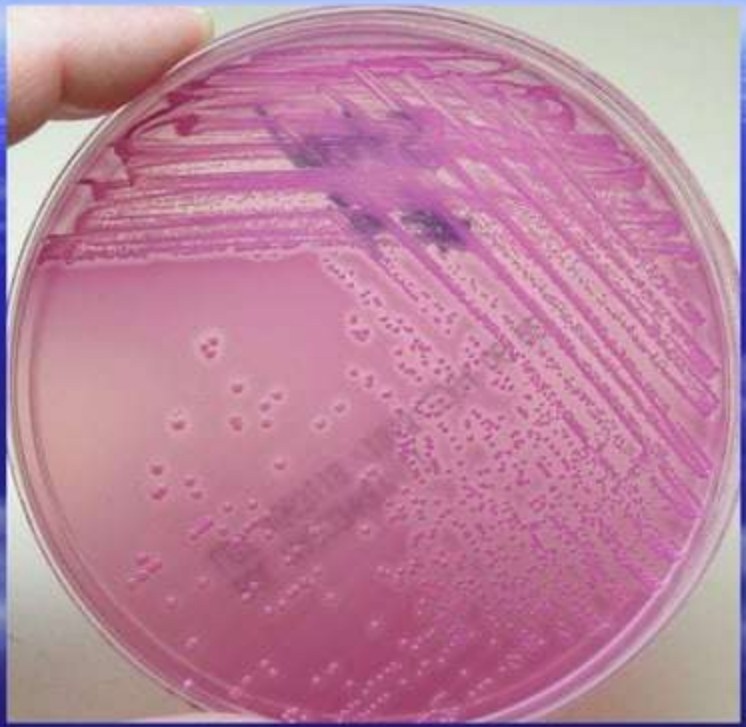
Culture methods include:

- 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
a loop
a plate (petri dish; top view)
note the culture on the tip of the loop
a properly streaked plate
following incubation there is colony formation. note the isolated colonies toward the bottom. can you spot the likely contaminants?



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 stoke cultures.

Gelatin liquefaction

Oxidation – Fermentation medium

POUR PLATE CULTURE

- Agar medium is melted (15 ml) and cooled to 45°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°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.

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 desiccator.

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.

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 – in the envelope
- 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

THANK YOU