Culture Media in Microbiology Laboratory

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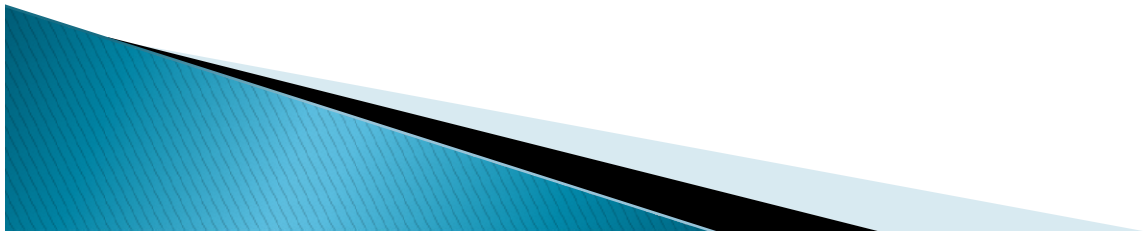
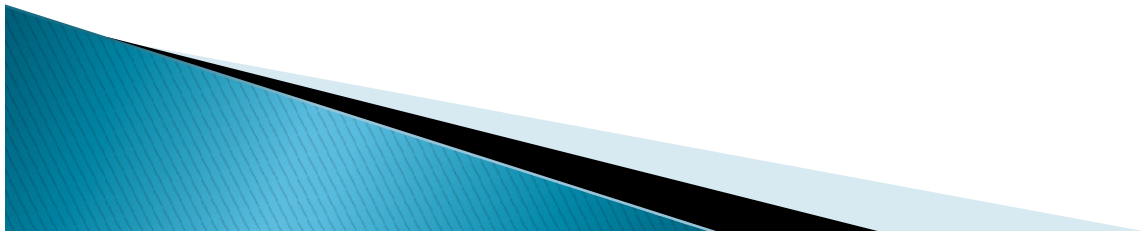


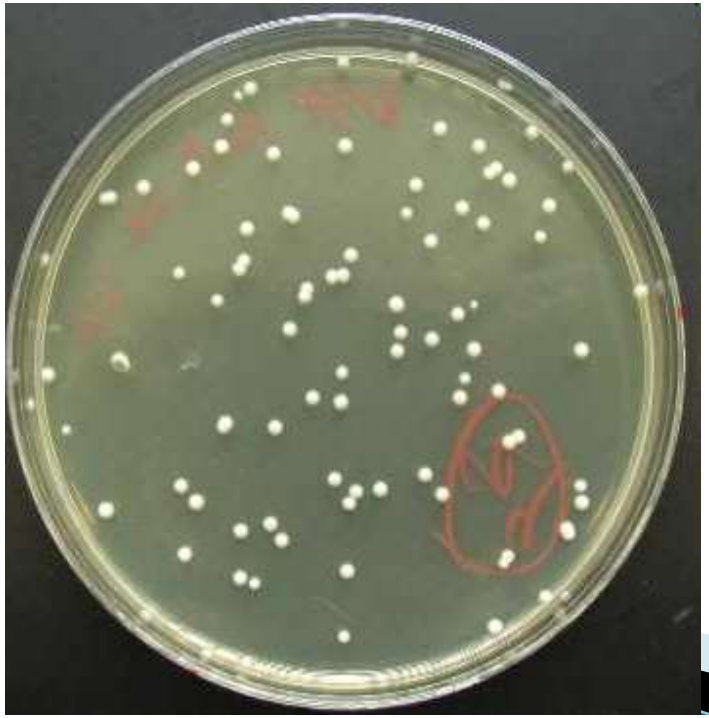
Learning Objectives

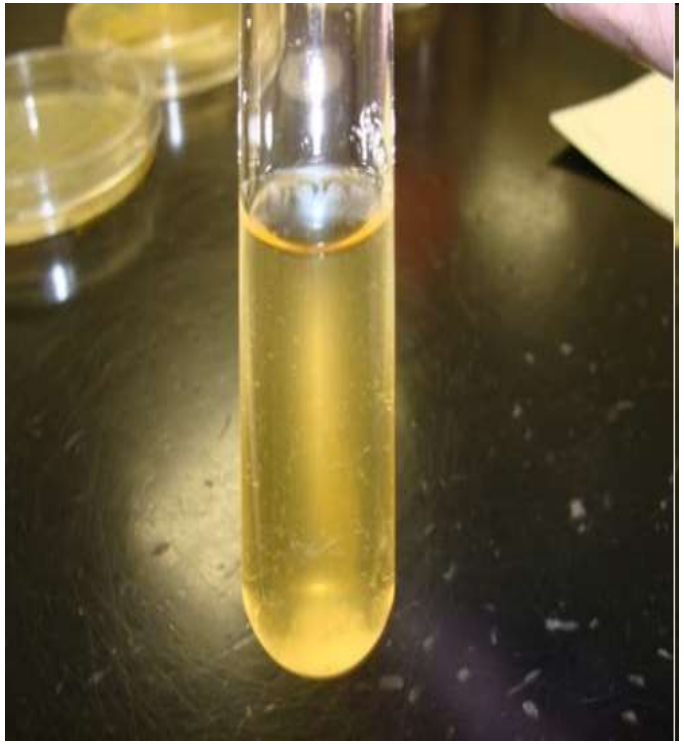
- Able to classify
- Know basic constituents & their role
- Should give examples
- Various sterilization techniques used for culture media preparation



What is culture media?

- Are substances which are used to grow bacteria in laboratory
- Difficult to identify by morphology alone





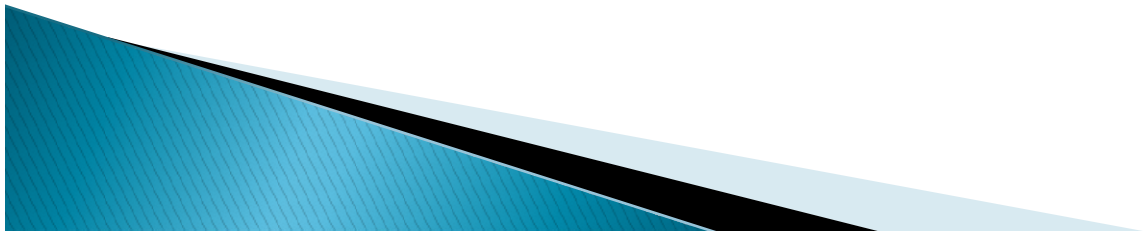
History

- Original culture media used by Louis Pasteur was liquid medium – Meat broth

- Disadvantages :
  - Bacteria doesn't exhibit specific characteristics
  - Difficult to isolate different bacteria in mixed population

History – solid media

- Cooked cut potato – The first solid medium used by Robert Koch
- Gelatin
  - Unsatisfactory – melts at 24 c
  - Also liquefied by many proteolytic bacteria
- Agar – Frau Hesse
- Petridish – Richard Petri

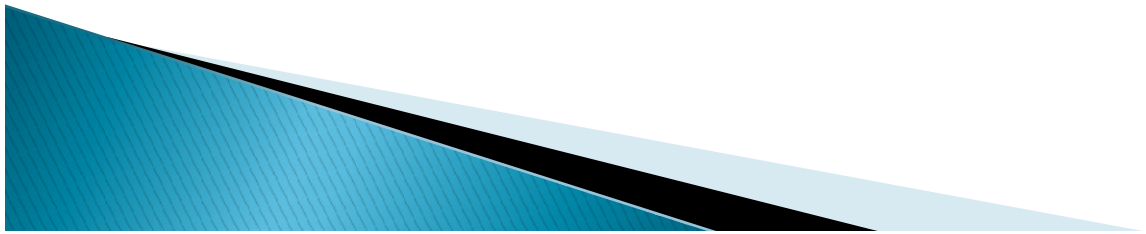


Agar

- Universally used solidifying agent
- Obtained from sea-weeds
- Made up of polysaccharide, also contains inorganic salts & protein like material
- No nutritive value, not affected by growth
- Melts at 98 c and usually sets at 42 c
- Available as powder or in long shreds

Peptone

- Universal ingredient of all culture media
- Complex mixture of partially digested protein
  - Constituents : proteoses, polypeptides & amino-acids
- Also contains
  - inorganic salts – PO4, K, Mg
  - Certain accessory growth factors –riboflavin
- Special brands
  - neopeptone, vegpeptone, meat extract – lab lemco



Basic requirements of culture media

- Nutrients
  - Energy source
  - Carbon source
  - Nitrogen source
- Mineral salts
  - Sulphates, phosphates, chlorides & carbonates of K, Mg & Ca.
- A suitable pH – 7.2 – 7.4
- Accessory growth factors
  - Tryptophan for Salmonella typhi
  - X & V factors for H. influenzae

Classification of Culture media

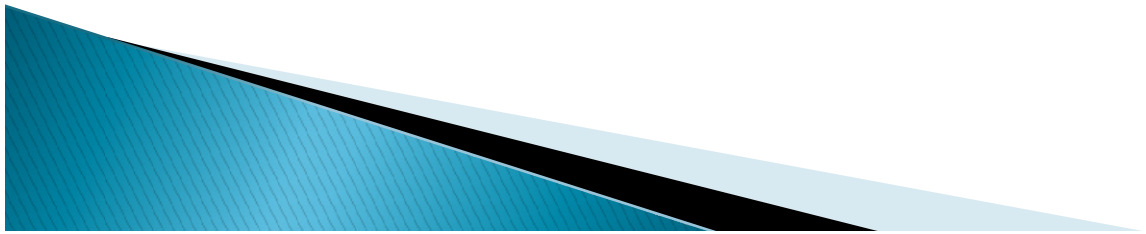
- Based on the consistency:

Liquid	Peptone water, Nutrient broth
Semisolid	Nutrient agar stabs
Solid	Blood agar, Serum agar

- Based on Oxygen requirement:
  - Aerobic media
  - Anaerobic media

Aerobic Media

- Simple media
- Complex media
  - Enriched media
  - Differential media
  - Enrichment media
  - Selective media
  - Transport media



Simple media

Consists of only basic necessities

- Liquid media
  - 1 %Peptone water

    1g peptone + 0.5gNacl + 100 ml water
  - Nutrient broth

    peptone water + 1% meat extract
- Solid media
  - Nutrient agar (nutrient broth + 2% Agar)

- Use: To grow non-fastidious microorganisms

Simple media

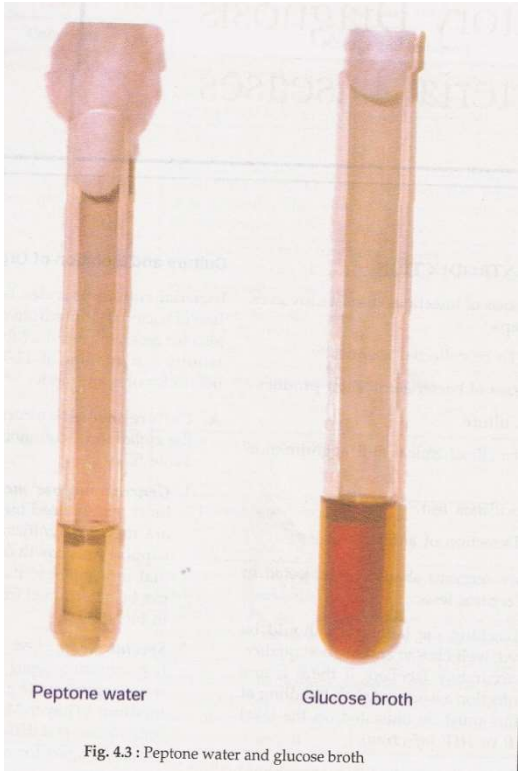
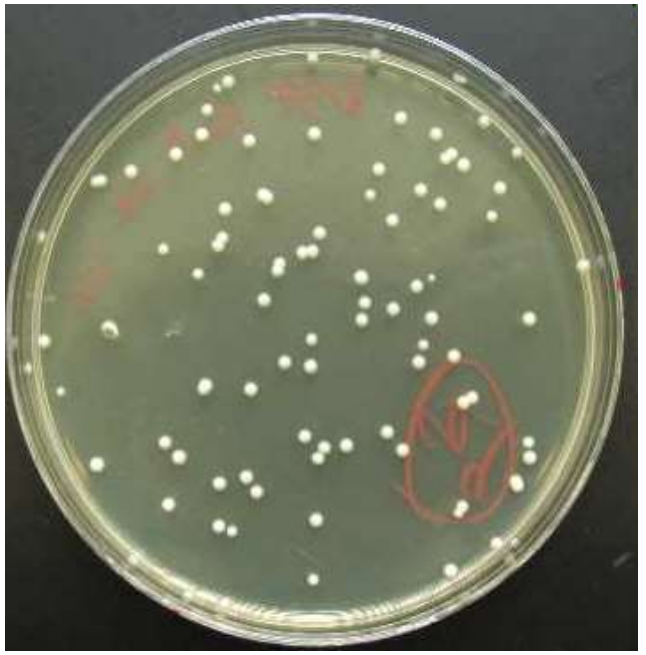
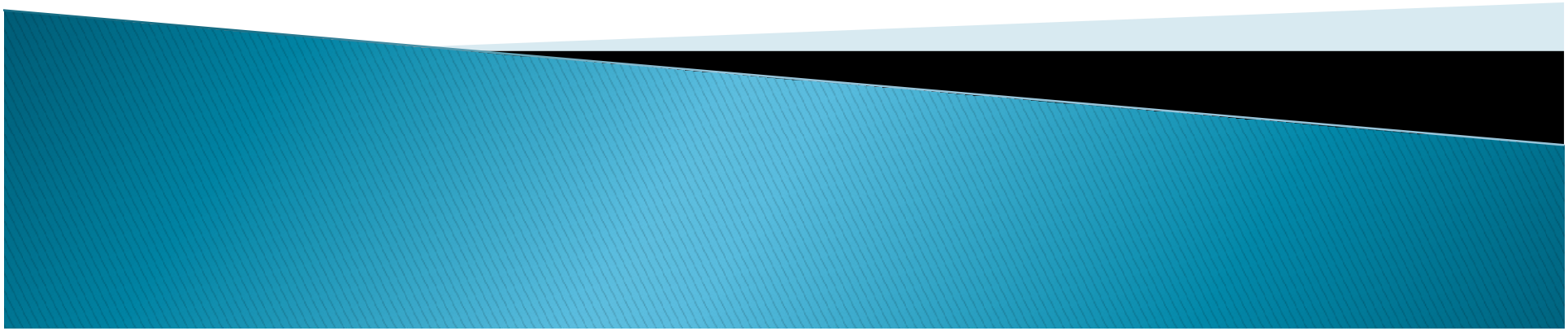


Fig. 4.3 : Peptone water and glucose broth



Complex media

Enriched media



Enriched media

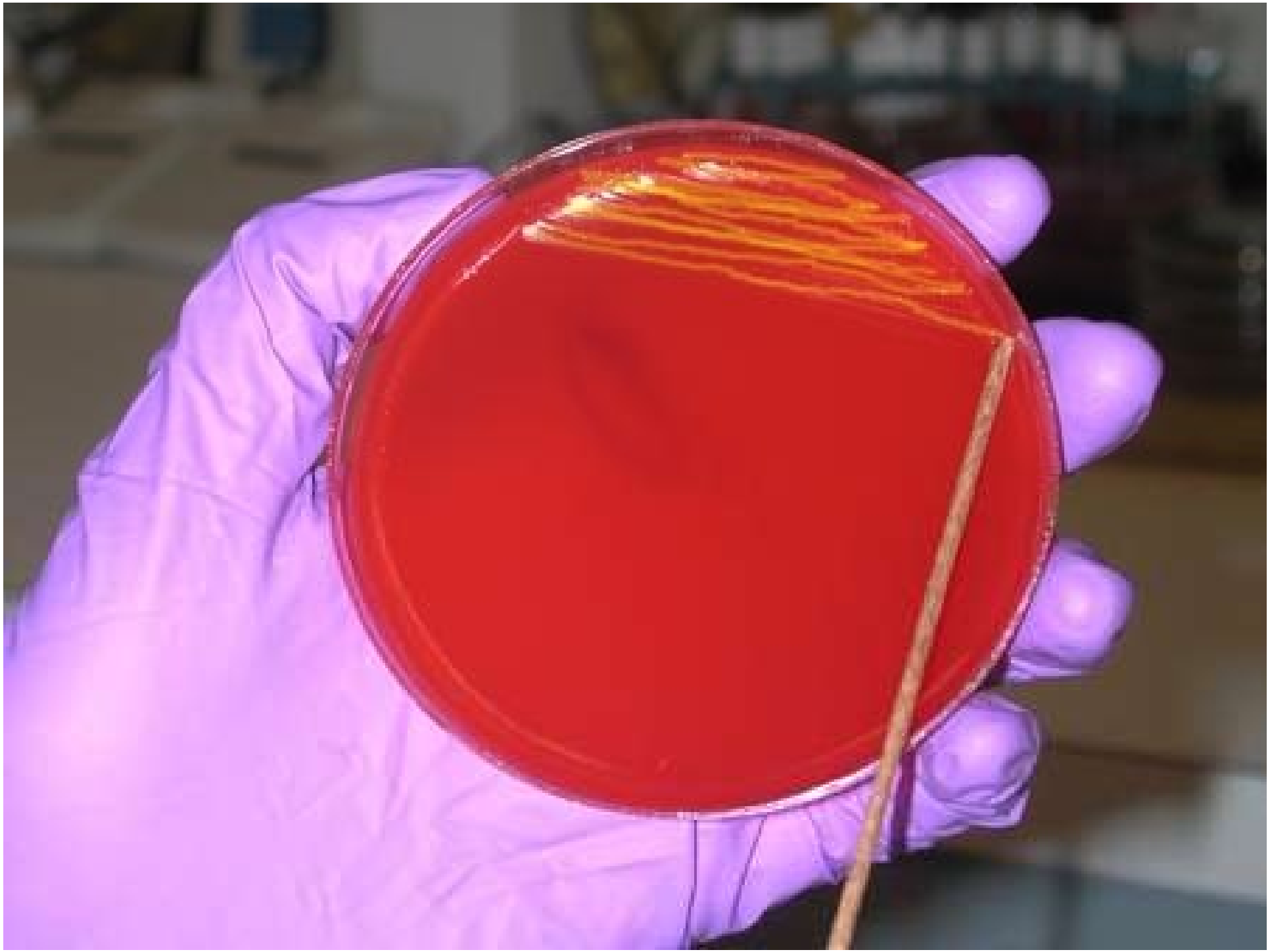
- Basal media + Blood/serum/egg
- Used to grow bacteria which are more exacting in their nutritional needs
- Examples
  - Blood agar
  - Chocolate agar
  - Loeffler's serum slope
  - Dorset's egg medium

Blood agar

- Nutrient agar + 5 to 10% sheep blood
- Cool the sterile nutrient agar to 45^0 c
- Add the blood aseptically with constant shaking
- Mix the blood with molten nutrient agar
- Pour in to the Petri dishes & allow to set

➢ Use: To cultivate the fastidious organisms
Pneumococci, streptococci

Blood agar

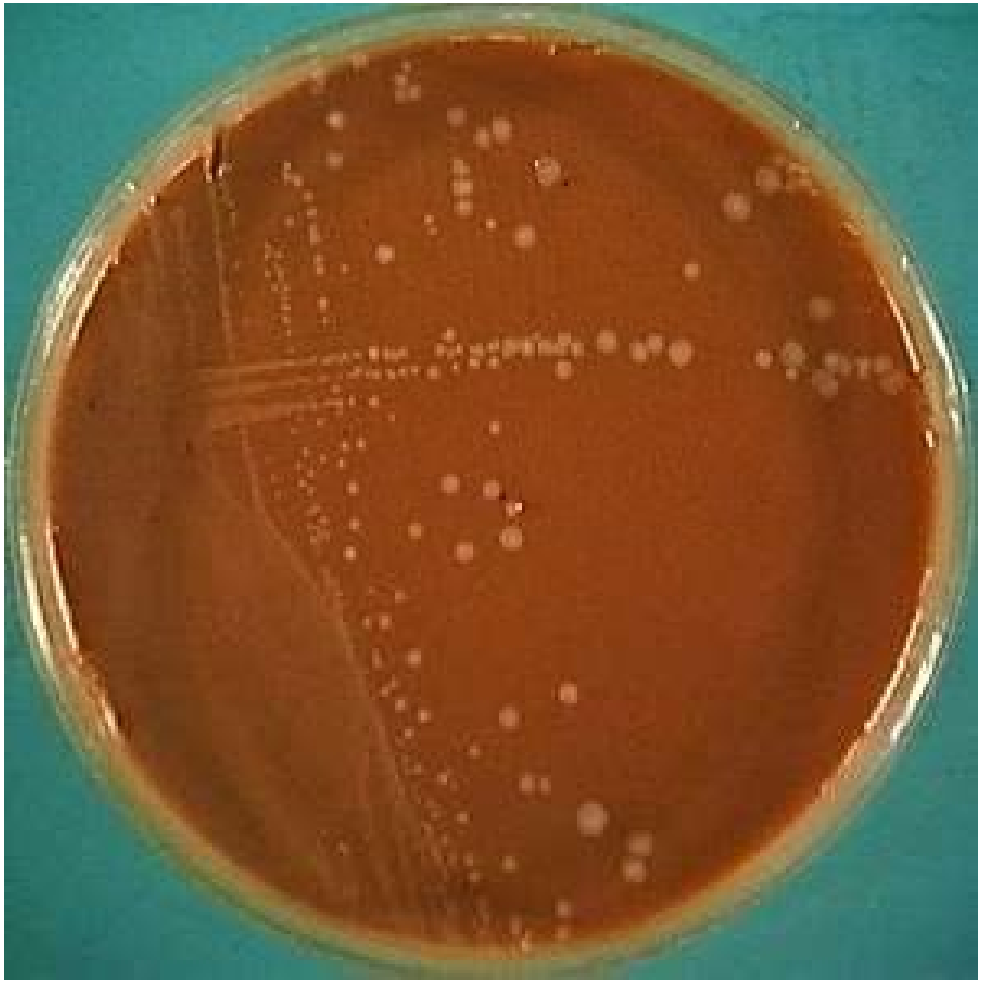


Chocolate agar

- Also called as – Heated blood agar
- Heat nutrient agar to about 75^0 c
- Add blood to the molten nutrient agar and allow to remain at 75^0c
- Gently mix till it is chocolate brown in color
- Pour in Petri dishes/ test tubes for slopes

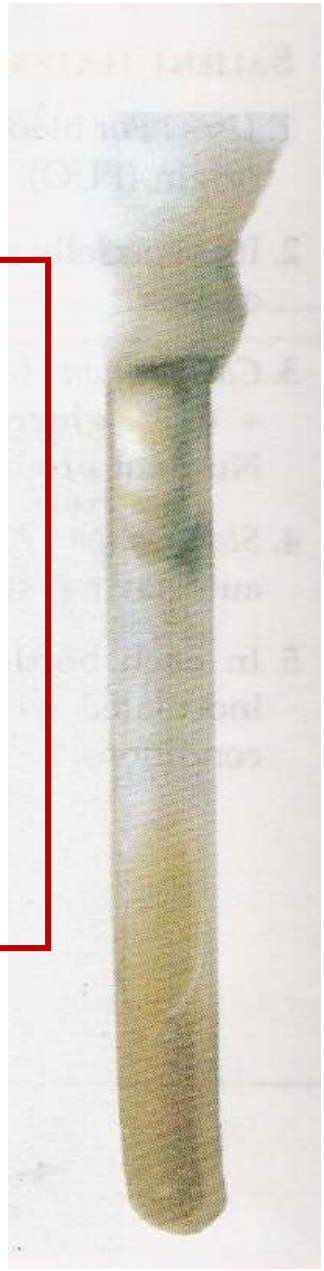
- Use: To culture H. influenzae & N.meningitidis

Chocolate agar



Loeffler's serum slope

- N. broth 100ml+Serum300ml+1gmGlucose
- Method of preparation :
  - Glucose broth – sterilize by steaming
  - Add serum aseptically
  - Distribute in small screw-capped bottles
  - Sterilize by inspissations, at 82°c for 1hr x 3 days
- Use: To cultivate C. diphtheriae



Dorset's egg medium

- Glucose broth + coagulated hens egg
- Sterilize by inspissation

- Use : to grow C.diphtheriae, M.tuberculosis

Enrichment media

- When certain substances are added to a liquid medium which enhance the growth of the pathogenic organisms and suppress the unwanted bacteria

  - Selenite-F broth – Salmonella typhi
  - Alkaline peptone water – Vibrio cholerae

- Use

  To prevent the non-pathogenic or commensal bacteria from overgrowing the pathogenic bacteria

Enrichment media

1 % Alkaline peptone water

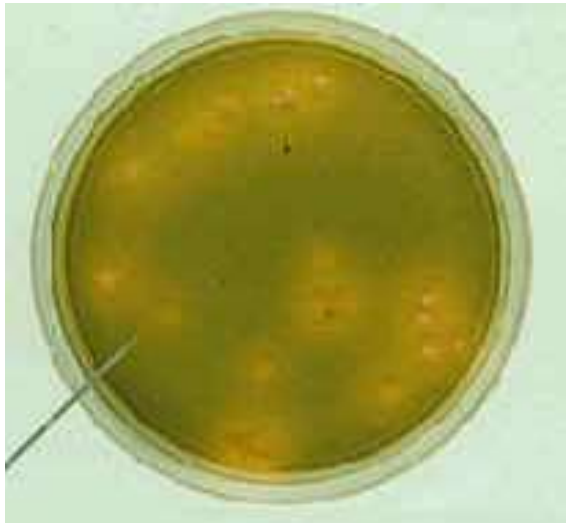
Selenite F/Tetrathoionate broth

Selective media

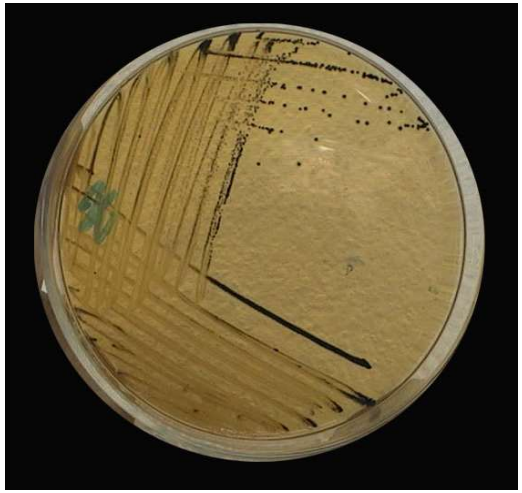
- Serve the same purpose as Enrichment media but are solid in consistency
  - Wilson & Blair's medium
  - S.S. agar
  - Lowenstein Jensen's medium
  - T.C.B.S. agar

- Use: To cultivate
  - Salmonella & Shigella
  - Mycobacteria
  - Vibrio

Selective media



T.C.B.S. Agar



S.S. agar

L.J. Medium

Lowenstein–Jensen's medium

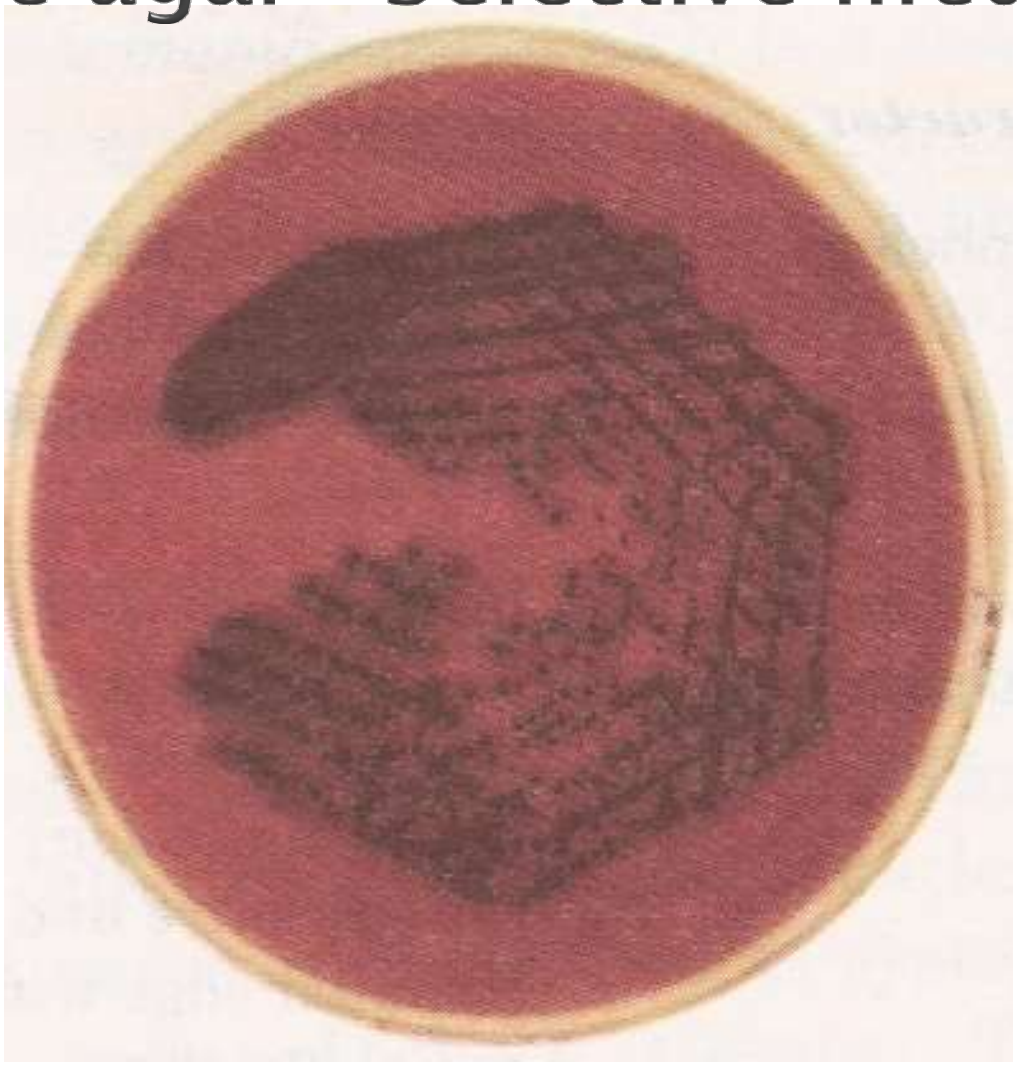
- Mineral salt solution – 600ml
  Malachite green solution – 20ml
  Beaten egg – 1000ml

- Mix the above
- Distribute in Mc Cartney bottles
- Sterilize by Inspissation

- Use: To cultivate Mycobacteria

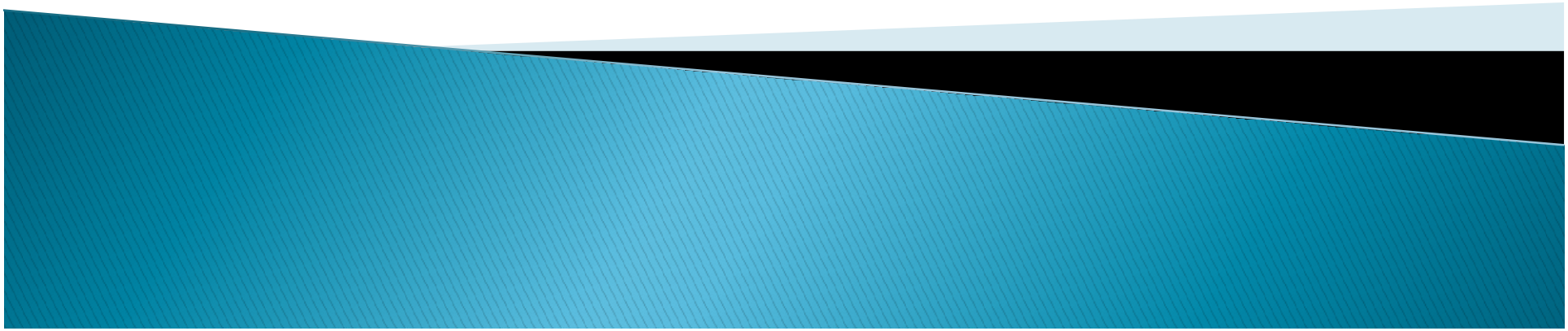
Mycobacterium tuberculosis on
Lowenstein-Jensen (LJ) medium

Tellurite agar- Selective medium



C. diphtheriae on TBA

Differential media



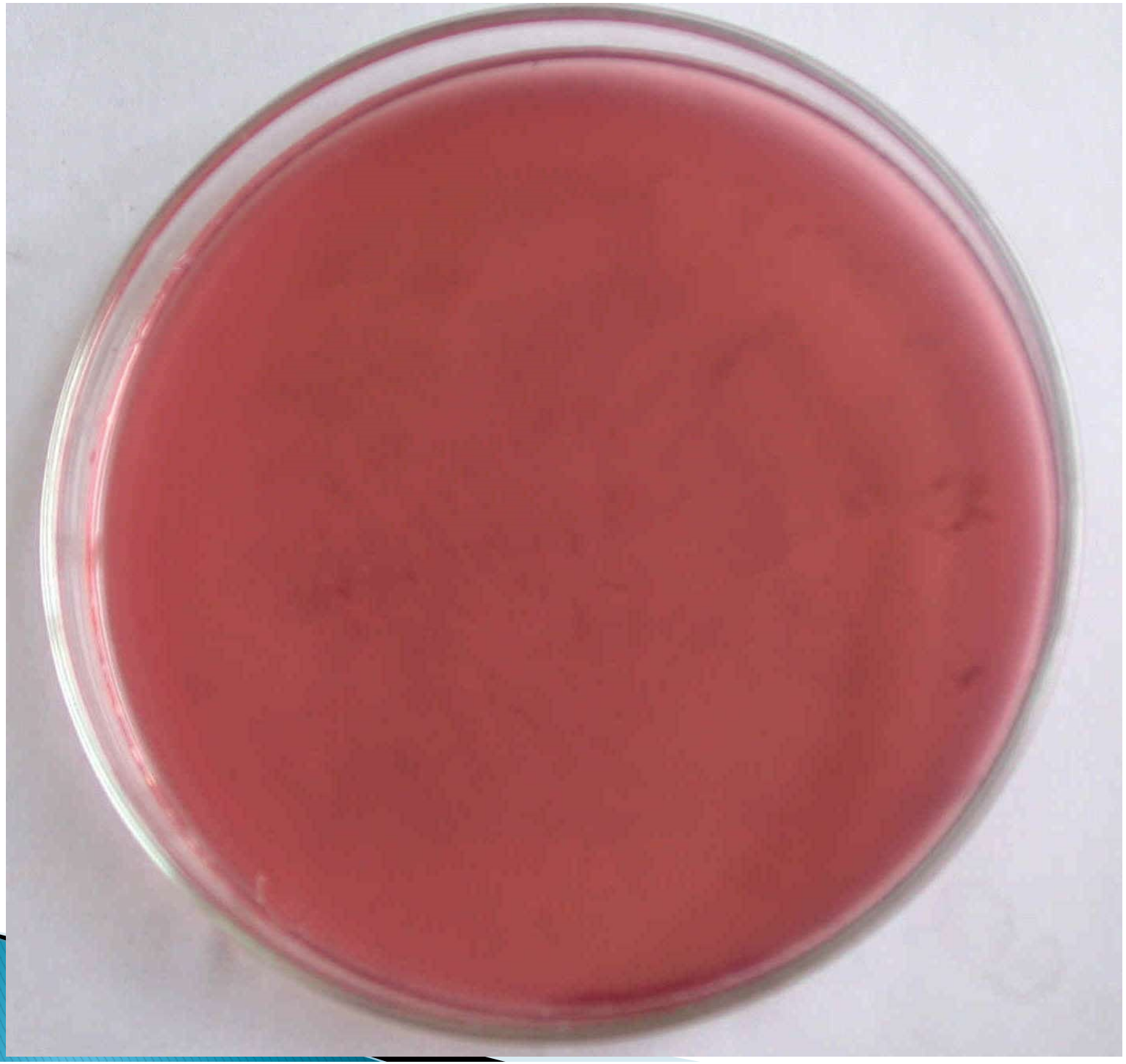
Mac Conkey's agar

- Contains :
  - N.agar
  - Bile salts
  - Lactose
  - Neutral red
- Bacteria fermenting lactose produce acid and this changes the color of the indicator and the colonies turn pink
- Use: To differentiate
  - Lactose fermenters (E. coli, Klebsiella)
  - Non-lactose fermenters (Salmonella,Shigella)

Preparation of Mac Conkey's agar

Ingredients:

- Peptone – 2 gms
- Lactose – 1 gm
- Sodium taurocholate – 0.5 gm
- Agar – 2 gms
- Neutral red – 2% soln. in 50% ethanol – 0.35 ml
- Distilled water – 100 ml



Transport media

- Are used in case of delicate organisms whenever there is a delay in the transportation of the specimen to the lab
- To maintain viability of them & to prevent the multiplication of non-pathogenic bacteria
  - Stuart's medium – Gonococci
  - Cary-Blair's medium – V. cholerae
  - V-R medium – V. cholerae

Blood culture media

- Brain-heart infusion – General purpose
- Glucose broth – Streptococci
- Bile broth – Salmonella
- Castaneda Biphasic – Brucella

Preparation of BHI/BPM

- Brain-heart infusion agar:
  Brain heart infusion dehydrated powder- 5.2gm
  Agar powder - 1gm
  Distilled water -
  100ml
- Mix the ingredients and dissolve by heating
- Cool the mixture and adjust the pH to 7.2 to 7.4
- Distribute into the required bottles and autoclave at 121c at 15lbs/in^2 pressure for 15 minutes

Brain-heart infusion broth

- Brain-heart infusion broth- 3.7 gms
- Sodium polyanethol sulphonate – 0.25mg
- Distilled water – 100ml
- Mix the ingredients and dissolve by heating and rest of the procedure is same as the previous one

BHI/BPM

- Dissolve BHIA as mentioned previously and distribute 20 ml in 100 ml flat bottles and 10 ml in 50 ml bottles with perforated screw cap and rubber liner
- Perforation is sealed with adhesive tape
- Autoclave along with separately prepared BHIB
- Place the bottles in flat positions till solidification
- Pour 50 ml of BHI broth to 100 ml bottles and 10 ml of the broth to 50 ml bottles
- Incubate at 37 c for 48 hours and at room temp for another 48 hours before use


BD BACTEC
PEDS PLUS
1-3 mL

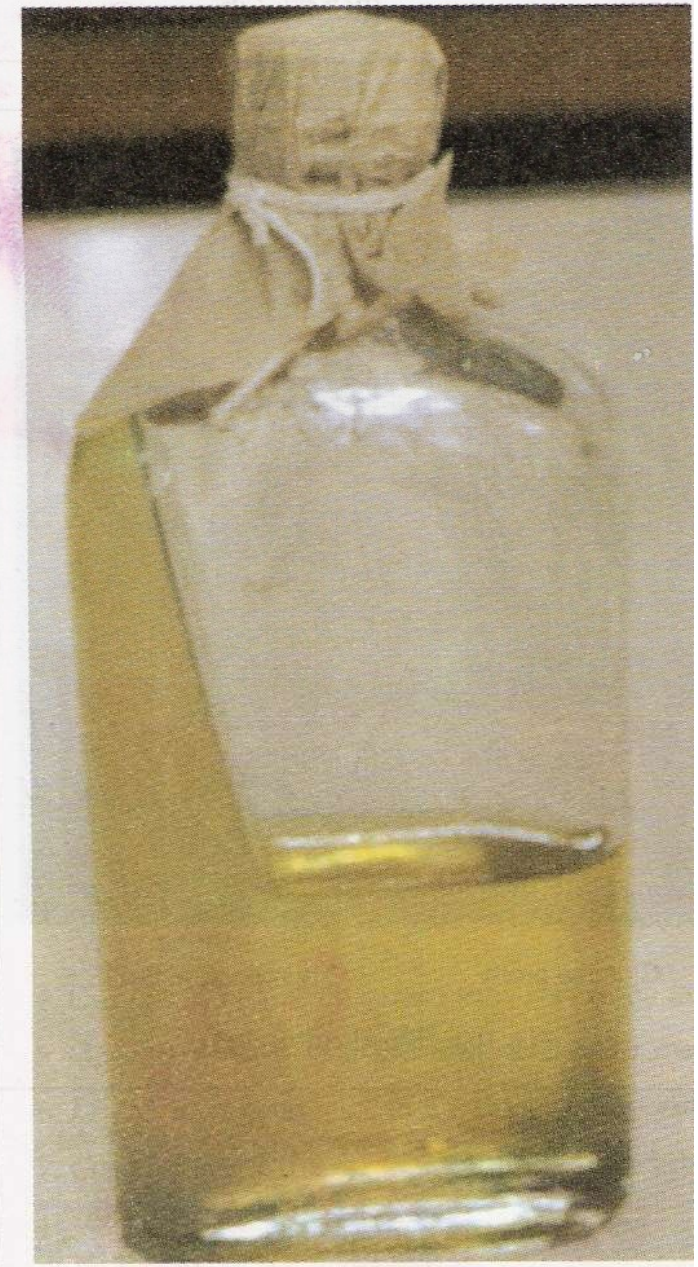
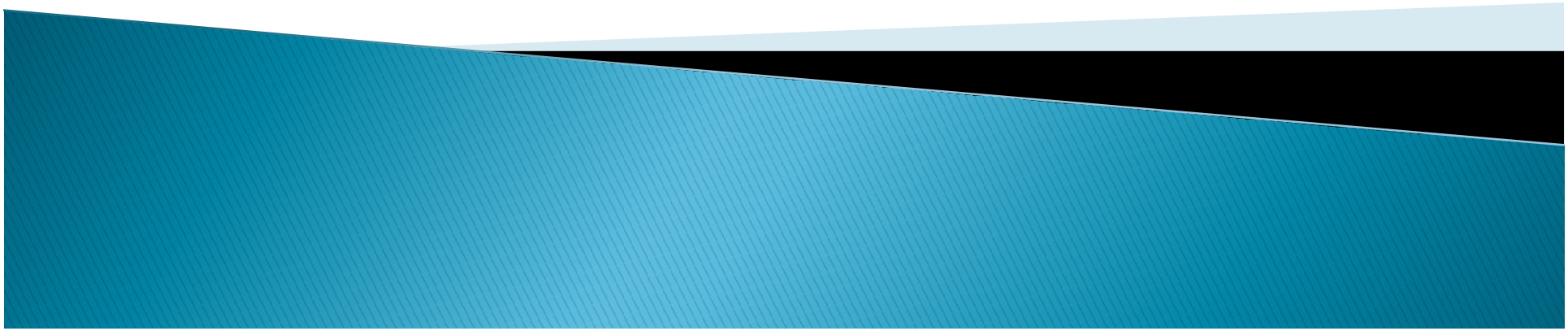


Fig. 10.9A : Castaneda medium

Fig. 10.8 : Blood culture set

Anaerobic culture media

Robertson's cooked meat medium



RCM

- Contains :
  - Peptone infusion broth
  - Meat particles of fresh bullock heart
- Use :
  - To culture anaerobic organism

Anaerobic media

Robertson's Cooked Meat medium

Fresh bullock heart	– 500 gms
Distilled	– 500 ml
NaoH 1 mol/lit	– 1.5ml

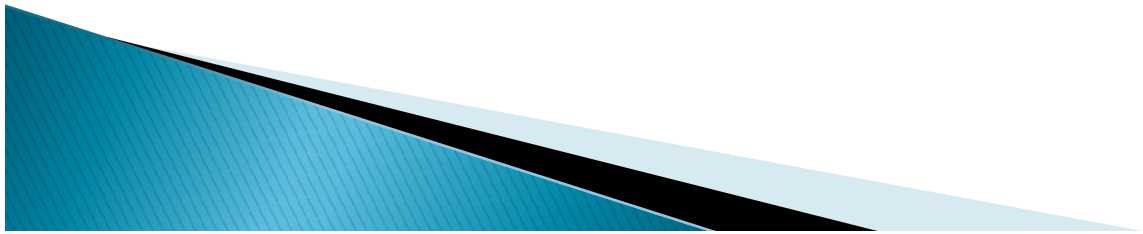
- Mince the heart, place in alkaline boiling water and simmer for 20 min to neutralize the Lactic acid
- Drain off the liquid through a muslin filter, press the minced meat in a cloth while hot and dry by spreading it on a cloth
- Distribute in bottles

Anaerobic media

Peptone Infusion Broth

Liquid filter from cooked meat – 500 ml
Peptone – 2.5 gms
Sodium chloride – 1.25 gms

- Steam at 100c for 20 min, add 1 ml pure Hcl and filter
- Adjust the pH to 8.2, steam at 100^0c for 30 min and adjust the ph to 7.2



Preparation of complete medium

- Place meat in 1 ounce bottles to the depth of 2.5 cms and cover it with 15 ml of broth
- Autoclave at 121^0 c for 20 min
- After sterilization, adjust the pH to 7.5

- Use: To cultivate the anaerobic bacteria

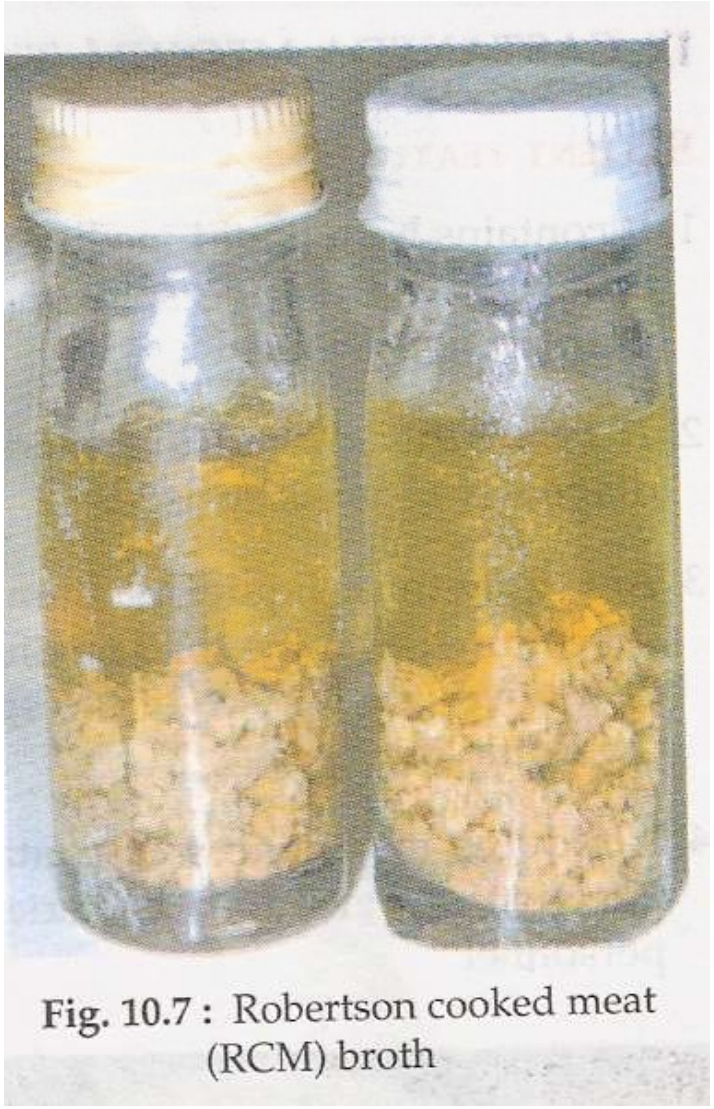


Fig. 10.7 : Robertson cooked meat (RCM) broth

Sabauroud's Dextrose Agar

Dextrose	– 4 gm%
Neopeptone	– 1 gm%
Agar	– 1.5 gm%
Distilled water	– 100 ml
pH	– 5.4

Use: For the cultivation of Fungi

Growth of fungus on SDA

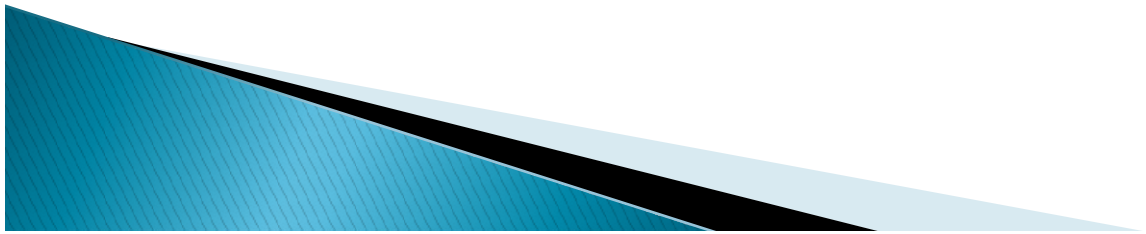
SDA

Carbohydrate media

- Peptone water – 100 ml, Desired sugar 1 gm% and Andrade's indicator – 0.005% soln(1ml)
- Dissolve the desired carbohydrate in peptone water and steam for 30 min or sterilize by filtration.
- Distribute into sterile test tube containing inverted Durham's tubes to detect gas production and steam for 30 min
- Use: To test the fermenting ability of an organism

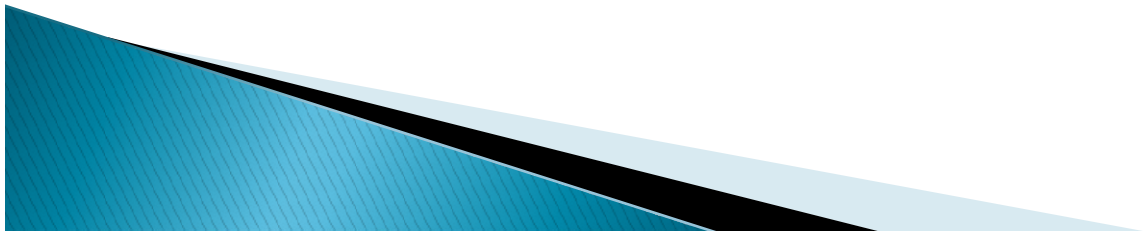
Sterilization of culture media

- Autoclave – all media
- Tyndallisation
  - Heat-labile substances like sugar solutions
- Inspissation
  - Protein containing media
  - Egg or serum containing media



Storage of culture media

- Prepared media in individual screw capped bottles can be stored for weeks at room temp
- Poured plates deteriorate quickly and often contaminated, hence cold storage is necessary
- For smaller labs domestic refrigerators & for larger labs insulated cold room(4–5^{o}c)
- Deep freeze refrigerators for preservation of sera, antibiotics & amino acids (–10 to – 40^{0}c)



Media for preservation and storage of cultures

- Nutrient agar slopes
- Semi solid nutrient agar
- Blood agar or Blood agar slopes in screw capped bottles

References

- Mackie & Mc Cartney
  Practical Medical Microbiology, 13th ed
- Text Book of Microbiology
  Ananthanarayan & Paniker, 7th edition
- Standard Operative procedure manual for Microbiology laboratories.
  Ministry of Health & Family welfare, Govt. of India

Thank you