CULTURE MEDIA

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Introduction

- **Culture media:** A nutrient material prepared for the growth of microorganisms in a laboratory.
- **Inoculum:** when microbes are introduced into a culture medium to initiate growth ,they are called inoculum.
- **Culture:** The microbes that grow and multiply in or on a culture medium are referred to as a culture.

- MO can be grown if req.nutrient provided
  - in optimum amt
  - at suitable pH
  - at suitable temp.
  - at suitable humidity
  - at suitable Oxygen conc.
  - optimum osmotic pressure

- Ideal culture media must contain
  - Water
  - Source of Carbon
  - Source of nitrogen
  - Mineral salt – Na, K, Ca, nitrate, sulphate, phosphate
  - Vitamin B
  - Specific sub. As per need of MO. Eg. Blood, Serum, Egg

COMMON CONTENTS OF CULTURE MEDIA

- **Water**: source of hydrogen and oxygen.
- **Electrolyte**: Sodium chloride or other.
- **Peptone**: complex mixture of partially digested proteins.
  - Obtained from lean meat or other protein material like heart muscle,casein or fibrin by digestion with proteolytic enzymes.
  - Contains proteoses,aminoacids,polypeptides,phosphates,minerals and other growth factors like nicotinic acid and riboflavin.

- **Meat extract:** contains protein degradation products,inorganic salts,carbohydrates and growth factors
- **Blood or serum:**for enriching culture media.
- **Agar:** prepared from see weed (red purple alagae).
- Not provide any nutrition.
- Melts at 98°C and solidifies at 42°C.

Classification

- Simple Media
  - Nutrient (Simple) agar
  - Nutrient (Simple) broth
  - Peptone water
- Enrichment media
  - Substances added in liquid media
    - Enhancing growth of wanted MO
    - Inhibiting growth of unwanted MO
      - Selenite F
      - Tetrathionate broth for Typhoid fever
- Enriched media
  - Substances added in solid media
    - Blood agar, Chocalate Agar
    - Loeffler serum slope
    - Dorset egg medium

- Selective media
  - Substances added in solid media
    - Enhancing growth of wanted MO
    - Inhibiting growth of unwanted MO
    - Eg. TCBS media – for cholera bacilli
      - Mac conkey agar
      - Wilson & blair bismuth sulphite agar – for typhoid bacilli
      - LJ media – For TB bacilli
      - Saboraud's Dextrose agar – For Fungus
      - Potassium tellurite
- Differential & Indicator media
  - pH indicator added – to differentiate group of bacteria
  - Eg. Mac conkey media
    - Sugar media
    - Wilson –blair media

- Solid media
  - 2.5 – 3 % agar
- Semisolid media
  - 0.4 – 0.5 % agar
- Liquid media
  - Called broth
    - Eg. Glucose broth
    - Hartley's broth & Brain heart infusion broth – for blood culture
    - Glycerol saline

- Transport media :
  - Semisolid or liquid media
  - Non nutritive media
  - To be used when immediate processing is not possible after collection of specimen
  - Inhibit overgrowth of commensal & ensure survival of possible pathogen
  - Prepared in screw cap bottle
  - Swab to be plunged in depth of medium
  - Viral transport media – contain Antibiotic & antifungal to inhibit growth of commensal
  - For cholera bacilli – VR medium, Alkline peptone water
  - For stool specimen – Carry blair medium, Glycerol saline.
    - 1:5 to 1:10 dilution of stool to be added
  - For urethral, vaginal & cervical swab – Amie's transport medium
    Stuart's transport media
- Aerobic media
  - Majority of media
- Anaerobic media
  - Thioglycollate broth
  - RCM broth

Container of media

- Petri dish – 9 or 10 cm in diameter for most solid media
- Screw cap bottle / cotton plug test tube
  - For Tuberculous & fungal media
  - For transport media
  - For liquid media

Preparation of culture media

- Dissolve ingredient in DDW as per req.
- Boil it till it mix properly.
- Autoclave it at 121^0 c at 15 lbs for 15 min
- Allow it to cool down upto 55^0 c.
- Pour it in pre sterilised container
- Keep sample of sterilised load in incubator at 37^0 c to check sterility.
- Keep remaining media in freeze.

- Heat labile ingredient (eg. Sugar) are added to basal medium after autoclaving with precaution.
- Egg & Serum base media to be inspissated at 75^0 c – 85^0 c , as protein is coagulated at this temp.
- pH of medium is adjusted by adding buffer.
- Generally pH of common media is 7.2-7.4.

- 20 – 25 ml of media to be poured in to 9 cm petri dish which approximate 4 mm thickness of media.
- Test tube or bottle should be filled maximum up to 80 % of their capacity.
- To prepare slant, container to be kept in suitable slope position.

- Checklist before using prepared media
  - Sterility of the media is OK
  - Media are free from contamination on nacked eye examination.
  - Not dehydrated
  - Solid media should be used within 7 – 10 days of preparation.

Simple media

- Nutrient broth contains peptone water and 1% meat extract.
- When glucose is added to nutrient broth – called glucose broth.
- When 2-3% agar is added to nutrient broth ,it becomes nutrient agar.

Enriched media

- Nutrients like blood, serum or egg is added to basal medium.
- **Blood agar** : nutrient agar- Cool to around 50° C. Add sheep blood (with sodium citrate),
- 60 ml /1litre of nutrient agar and mix. Pour in plates quickly before agar solidifies.
- Let the media solidify. Store in refrigerator.
- **Use**: To differentiate hemolytic from non hemolytic colonies.

- For the growth of staphylococci,streptococci,pneumococci,neisseria group.

- **Chocolate agar**: For chocolate agar preparation warm agar blood mixture over bunsen flame till colour of mixture turns brown. Pour in plates.

-**Uses**: for growth of Haemophilus,hemolytic streptococci, pneumococci and neisseria group.

- **Loeffler's serum slope:**Serum is added to enrich the medium.

- Content: N.B.,glucose,serum.
- Uses: for growth of C.dipthriae.

- **Dorset egg medium:** Egg for enriching the medium.

- Content:N.B.,egg white and yellow.
- Uses: for growth of Mycobacterium tuberculosis(both human and bovine varities).

Enrichment media

- Some substances are incorporated in the liquid medium which have stimulating effect on the bacteria to be grown or inhibits its competitors.
- This results in an absolute increase in the number of wanted bacteria related to other.
- **Blood culture bottle(Glucose broth):** contains N.B. and glucose.

-Uses: glucose is for enriching the medium. Used for growth of pyogenic organisms,subacute bacterial endocarditis.

- **Selenite –F–broth:** Selenite inhibits growth of coliforms and allow the growth of salmonella species.
- **Tetrathionate broth:** tetrahtionate has similar action as that of selenite.
- **Alkaline peptone water:** pH 8.2

– Used to grow vibrio cholerae.

Selective media

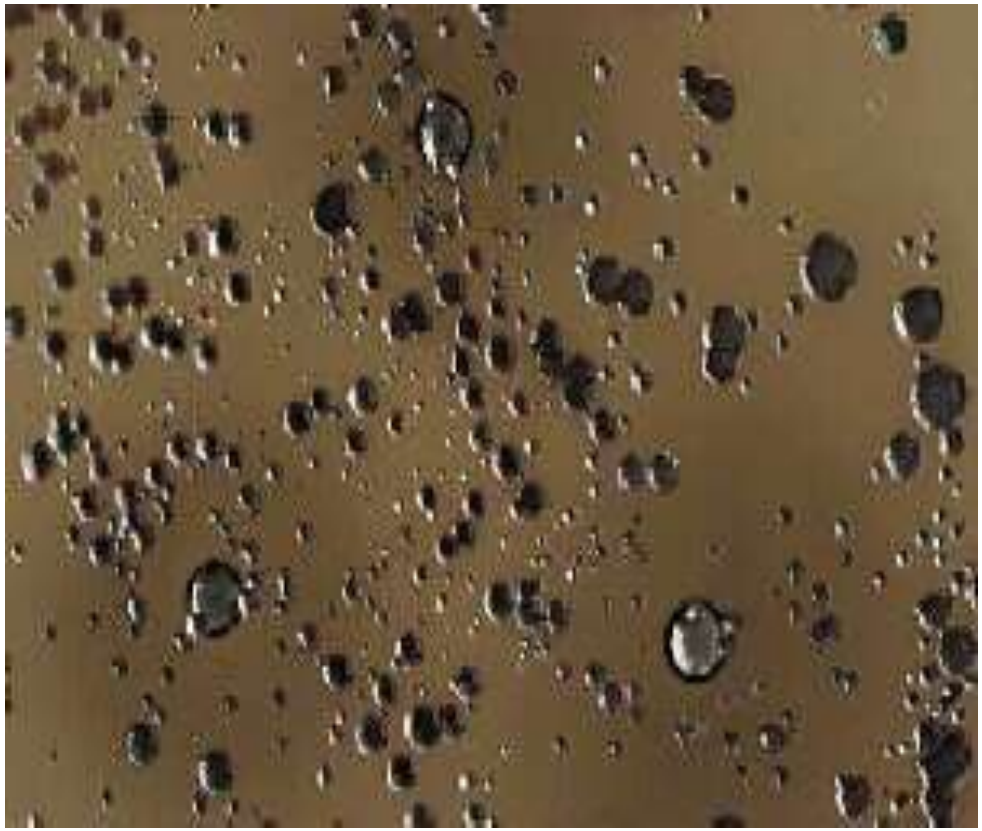
- Selective media contain substances that inhibit all but facilitate the isolation of a particular species.
- Used to isolate a particular bacteria from specimens where mixed flora is expected.
- They are solid media.
- **Potassium tellurite medium:** N.A. ,blood and pot.tellurite.
  - Pot.tellurite is less inhibitory to C.diphtheriae compared to other throat organisms.
  - Tellurite is converted to metallic tellurium which gives black colour to the colonies.

- **Lowenstein Jensen's medium**: Egg,malachite green,mineral salt solution containing glycerol.
  - Eggs enrich the medium,malachite green is least inhibitory for m.tuberculosis and glycerol favour the growth of human varities.
  - So used for the growth of M.tuberculosis human variety.

- **Mac conkey's agar**: P.W.,Agar,Sodium taurocholate, lactose,Neutral red.
  - Sod. Taurocholate is inhibitory for non intestinal organism.Lactose will be fermented by lactose fermenters producing acid and causes neutral red indicator to give pink colour.
  - Used for the growth of intestinal organism. And to differentiate lactose fermenting organism(E.coli,klebsiella) from Non lactose fermenters(salmonella,shigella,proteus).

- **Wilson and blair**:Glucose,sodium sulphite,Brilliant green,bismuth sulphite.
  - Brilliant green and bismuth sulphite inhibit intestinal commensals.
  - Salmonella typhi reduces sulphite to sulphide in the presence of glucose and produce black colonies.
- **TCBS(Thiosulphate citratebile sucrose agar)**:used for the growth of Vibrio cholerae that produce yellow colony.

- **Robertson's cooked meat medium**: Nutrient broth and pieces of meat.
  - Used for culture of anaerobic bacteria and for preservation of stock culture of aerobic bacteria.
  - If bacteria are saprophytic meat particles turns pink,and if bacteria are proteolytic meat particles turns black.
- **Saubauraud's medium**:glucose,peptone agar pH5.4
  - Used for the growth of fungi.



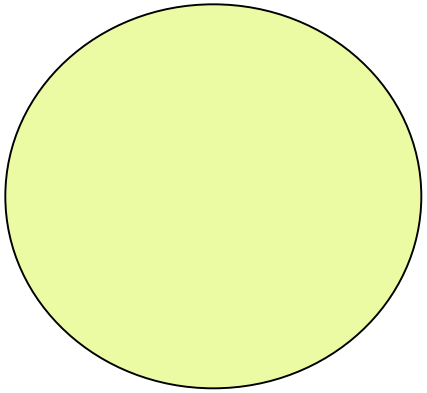
W B medium

Indicator media

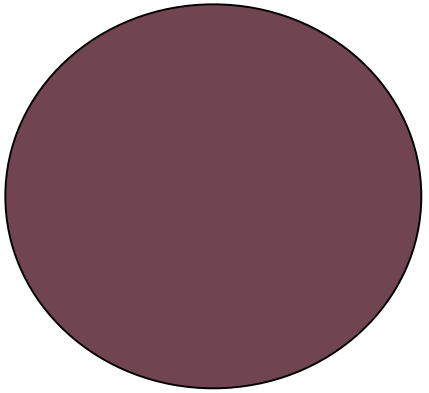
- **Sugar media**:1% sugar in Peptone water ,andrade's indicator.
  - Fermentation of sugar produces acid that is indicated by the development of pink colour.
  - Gas accumulates in the inner Durham's tube.
- **Wilson-blair medium**
- **Macconkey agar**
- **Potassium tellurite**
- **RCM**

COLOUR OF MEDIA

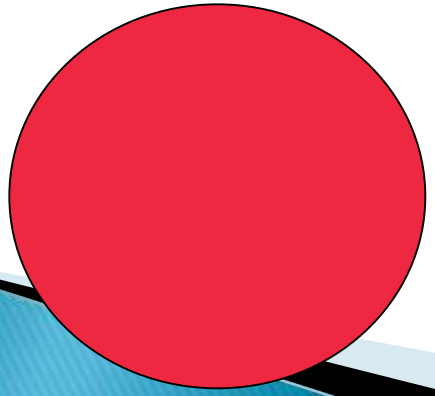
- NUTRIENT AGAR



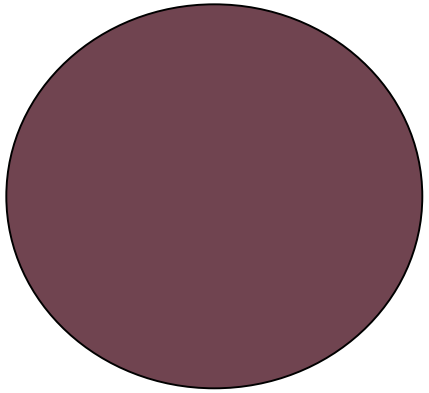
- CHOCOLATE AGAR



- BLOOD AGAR

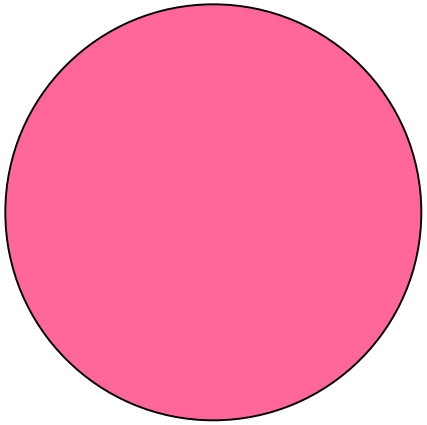


- PT CHOCOLATE AGAR

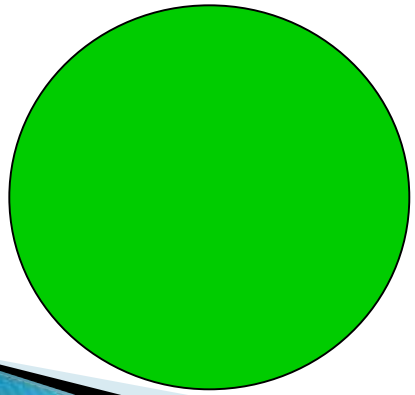


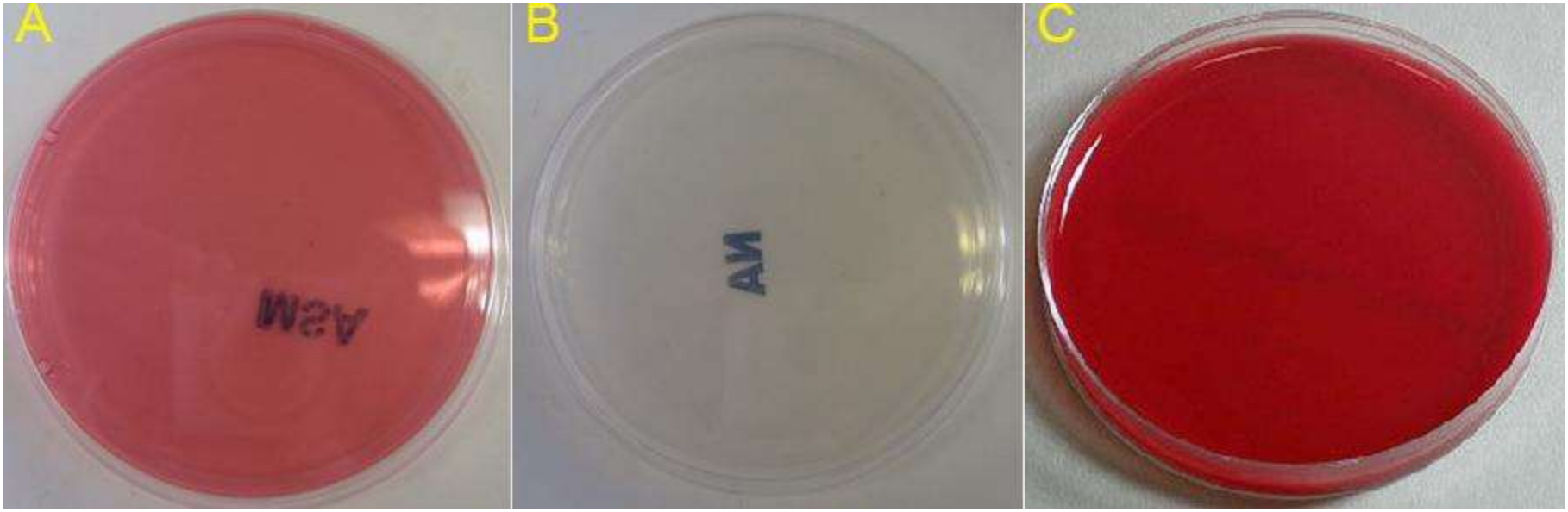
COLOUR OF MEDIA

- MACCONKEY AGAR



- WILSON-BLAIR

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THANK YOU