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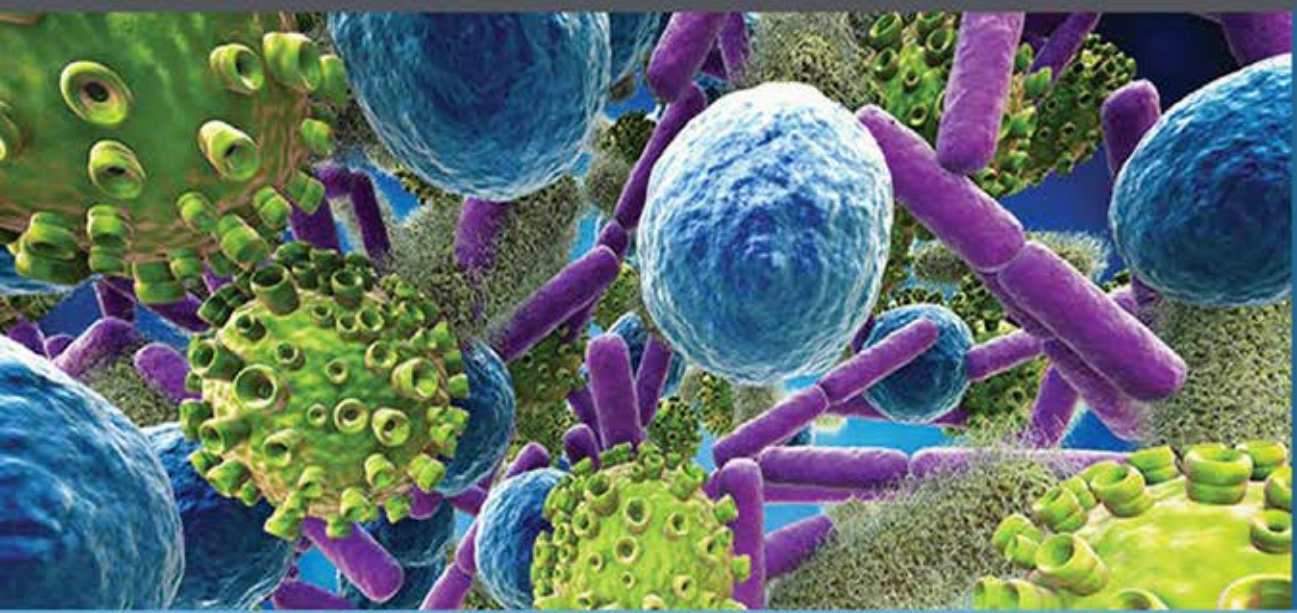

The Short Textbook of Medical Microbiology for Nurses

THIRD EDITION

Satish Gupte

JAYPEE

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Chapter 5

Media for Bacterial Growth

Culture media gives artificial environment simulating natural conditions necessary for growth of bacteria. The basic requirement of culture media are:

1. Energy source.
2. Carbon source.
3. Nitrogen source.
4. Salts like sulphates, phosphates, chlorides and carbonates of sodium, potassium, magnesium, ferric, calcium and trace elements like copper, etc.
5. Satisfactory pH 7.2 to 7.6.
6. Adequate oxidation-reduction potential.
7. Growth factor like tryptophan for *Salmonella typhi,* glutathione for gonococci, X and V factors for *hemophilus*.

The characteristics of an ideal culture medium are:

1. Must give a satisfactory growth from single inoculum.
2. Should give rapid growth.
3. Should be easy to grow.
4. Should be reasonably cheap.
5. Should be easily reproducible.
6. Should enable to demonstrate all characteristics in which we are interested.

Media used for obtaining the growth of bacteria are:

FLUID MEDIA

Bacteria grow very well in fluid media in 3 to 4 hours. Hence, they are used as enriched media before plating on solid media. They are not suitable for the isolation of organism in pure culture. We cannot study colony characters as well. Examples of fluid media are nutrient broth, peptone water, etc.

Types of Fluid Media (Table 5.1)

Broth

It is a clear transparent straw colored fluid prepared from meat extract or peptone. Following types of broth are in common use:

a. **Infusion broth:** Fat free minced beef meat is added to water and kept in refrigerator overnight. Fluid obtained after removal of meat is boiled for 18 minutes. To it peptone and 0.5% sodium chloride is added.
b. **Meat extract broth:** This is commercially available as Lab Lemco.
c. **Digest broth:** It is prepared from meat by enzymatic action. Nutritionally, it is more rich than infusion and extract broth. Addition of peptone is not required in digest broth. Hence, it is more economical. Enzymes used are trypsin, pepsin, etc.

Peptone

It is a protein partially digested with hydrolytic enzymes like pepsin, trypsin, papain, etc. Peptones supply nitrogenous material and also act as a buffer. Several bacteria can grow in 1% peptone water. Constituents of peptone are proteoses, polypeptides and amino acids.

Yeast extract: It is prepared by extracting autolyzed yeast with water. It has high contents of vitamin B.

The other examples of fluid media are sugar media (1% sugar in peptone water), glucose

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Table 5.1: Fluid media.

Medium	*Uses*
• Peptone water	Routine culture
	Demonstration of motility
	Sugar fermentation
	Indole test
• Nutrient broth	Routine culture
	Methyl red test
	Voges Pauskaur test
• Glucose broth	Blood culture
	Culture of bacteria like streptococci, etc.
• Enrichment media	
– Glycerole alkaline peptone water	Transport media for stool samples
– Selenite F broth	Culture of stool for *Salmonella* and *Shigella*
– Tetrathionate broth	Culture of stool for *Salmonella*
– Robertson cooked meat broth	Culture of anaerobic bacteria

broth (1% glucose in nutrient broth), bile broth (0.5% bile salts in nutrient broth), Hiss serum (1 part serum and 3 parts glucose broth), liquid MacConkey, glycerol saline and enrichment media (tetrathionate and selenite).

SOLID MEDIA (TABLE 5.2)

They are used to study colonies of individual bacteria. They are essential for isolation of organism in pure form.

a. **Agar:** It is important constituent of solid media. It is complex polysaccharide obtained from seaweeds (Algae geledium species). It melts at 80 to 100°C and solidifies at 35 to 42°C. It does not provide any nutrition to the bacteria. It acts only as solidifying agent. It is not metabolized by any pathogenic bacteria.

b. **Gelatin:** It is protein prepared by hydrolysis of collagen with boiling water. It is in liquid form at 37°C. It forms transparent gel below 25°C. The main use of gelatin is to test the ability of bacteria to liquefy it. This feature is important in the identification and classifications of bacteria. Blackening of media indicates hydrogen sulfide production.

Table 5.2: Solid media.

Medium	*Uses*
• Nutrient agar	• Routine culture
	• Antibiotic sensitivity test
• Blood agar	• Routine culture
• Chocolate agar	• Culture of fastidious bacteria like *Neisseria, H. influenzae, etc.*
• Loeffler serum slope	• Culture of *C. diphtheriae*
• Deoxycholate citrate agar	• Culture of *Salmonella* and *Shigella*
• MacConkey's medium	• Culture of intestinal bacteria
• Bile salt agar	• Culture of *Vibrio cholerae*
• TCBS	• Culture of *Vibrio cholerae*
• Lowenstein-Jensen medium	• Culture of *Mycobacterium tuberculosis* and atypical *Mycobacterium*

Classification of Media

Media have been classified in many ways:

A. i. Solid media
   ii. Liquid media
   iii. Semisolid media.

B i. Simple media
   ii. Synthetic media or defined media
   iii. Complex media
   iv. Semidefined media
   v. Special media.

Special medias are further divided as under:

i. Enriched media
ii. Enrichment media
iii. Selective media
iv. Indicator and differential media
v. Sugar media
vi. Transport media.

C. Aerobic media and anaerobic media.

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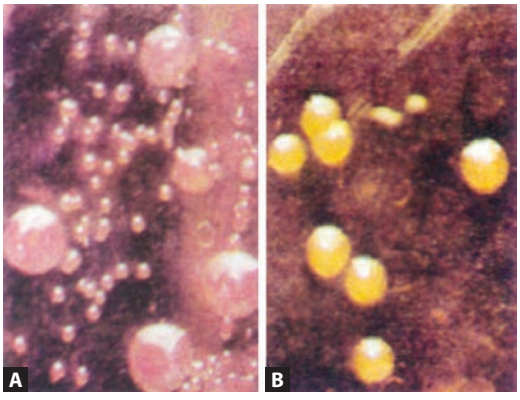
1. **Simple media:** It is also called basal media. It consists of meat extract, peptone, sodium chloride and water.
   a. *Peptone water:* It is prepared by adding 1 Gram peptone, 0.5 Gram sodium chloride to 100 mL of distilled water.
   b. *Nutrient agar:* Addition of 2% agar in nutrient broth constitute nutrient agar.
2. **Complex media:** These are ingredients for special purposes or for bringing out certain characteristics or providing special nutrient required for the growth of certain organisms.
3. **Synthetic or defined media:** These media are prepared solely from pure chemical substances and the exact composition of medium is known. They are used for research purposes.
4. **Special media**
   a. *Enriched media:* In these media substance like blood, serum or egg is added to basal medium, e.g., blood agar, chocolate agar, egg media and Loeffler serum slope.
   b. *Enrichment media:* Some substances are added to liquid media with the result that wanted organism grow more in number than unwanted organism. Such media are called, enrichment media, e.g., selenite F broth, tetrathionate broth.
   c. *Selective media:* This is like enrichment media with the difference that inhibiting substance is added to solid medium, e.g., deoxycholate citrate medium which contains nutrient agar, sodium deoxycholate, sodium citrates lactose and neutral red.
   d. *Indicator media:* The media contain an indicator which changes color when bacterium grow in them, e.g., *Salmonella typhi* reduces sulphite to sulphide in Wilson and Blair medium (colonies of *Salmonella typhi* have black and metallic sheen).
   e. *Differential media:* A medium which has substance enabling it to bring out differing characteristic of bacteria thus helping to distinguish between them, e.g., MacConkey's medium (peptone, lactose, agar, neutral red and taurocholate). It shows lactose fermenter as red colonies while nonlactose fermenter as pale colonies **(Figs. 5.1A and B)**.

      Blood agar is an enriched medium but also differentiates between hemolytic organisms and nonhemolytic organisms. So it also acts as a differential medium.
   f. *Sugar media:* The usual sugar media consist of 1% sugar concerned, in peptone water along with appropriate indicator. A small tube (Durham's tube) is kept inverted in sugar tube to detect gas production **(Fig. 5.2)**.
   g. Hiss's serum (25% serum) is used for organisms which are exacting in their growth requirement, e.g., pneumococci.
   h. *Transport media:* Delicate organisms like gonococci which may not survive the time taken for transporting the specimen to the laboratory or may be overgrown by nonpathogen (dysentery or cholera organism) special medium is required called transport medium, e.g., Stuart medium for gonococci and glycerol saline for stool.



Figs. 5.1A and B: (A) Lactose fermenter colonies on MacConkey media; (B) Nonlactose fermenter colonies on MacConkey media.

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Fig. 5.2: Sugar media with inverted Durham's tube.

Anaerobic media: These media are used to grow anaerobic organisms, e.g., Robertson's cooked meat medium.

Anaerobic indicators used is reduced methylene blue and it contains NaOH, methylenes blue, glucose.

Storage media: Lyophilization (freeze drying in vacuum) is the best method of preservation and storage of bacteria Dorset egg and semisolid agar may be used to preserve and store bacteria for a few months. For this loop charged with bacteria is inoculated on these media. After giving adequate incubation bacterial growth appears on the media which can be stored in the refrigerator. Robertson cooked meat media can also be used for preservation.

Culture Techniques

In clinical laboratory indications for culture are:

a. Isolation of bacteria in pure culture.
b. To demonstrate their properties.
c. To obtain sufficient pure growth for preparation of antigen and for other tests.
d. For typing of bacterial isolate by method like bacteriophage and bacteriocin susceptibility.
e. To determine sensitivity to antibiotics.
f. To estimate viable count.
g. To maintain stock culture.

METHODS OF ISOLATING PURE CULTURE

1. Surface plating.
2. Use of enriched and selective media.
3. Pretreatment of specimens with appropriate bacteriocidal agents.
4. By heating liquid medium.

Methods of Culture

1. **Streak culture** (surface plating) is the method routinely employed for the isolation of bacteria in pure culture. A platinum loop with 2½" long wire and loop with diameter 2 mm **(Fig. 5.3)** is charged with specimen to be cultured and is placed on the surface of dried plate of solid media towards peripheral area **(Fig. 5.4)**. The inoculum is spreaded thinly over the plate in series of parallel lines in different segment of the plate. On incubation we may find confluent growth at the site of primary inoculum. Well-separated colonies are obtained over the final series of streaks.
2. **Lawn or carpet culture:** Lawn cultures are prepared by flooding the surface or plate with suspension of bacteria. It provides uniform surface growth of bacteria. It is useful for bacteriophage typing and antibiotic sensitivity test.
3. **Stroke culture:** It is made in tubes containing agar slopes. It is used for providing a pure growth of bacterium for slide agglutination.
4. **Stab culture:** It is prepared by puncturing with charged long, straight wire (4" long). Stab culture are employed mainly for demonstration of gelatin liquefactions and for maintaining stock culture.
5. **Pure plate culture:** 15 mL of agar medium is melted and left to cool in water bath at 45

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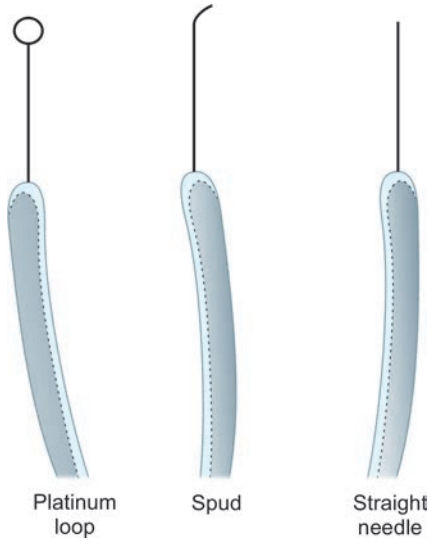

Fig. 5.3: Types of inoculation loops.

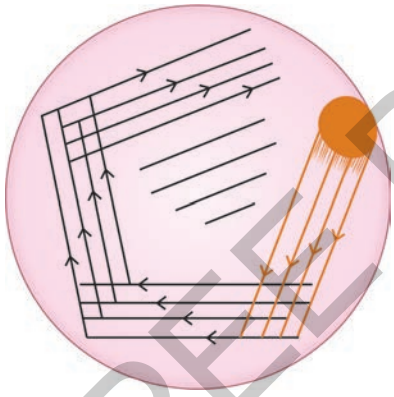

Fig. 5.4: Method of streaking culture plate.

to 50°C. Appropriate dilution of inoculum is added in 1 mL volume to molten agar and mixed well. Content of tube is poured in Petri dish. It is allowed to set and after incubation colonies will be seen distributed throughout the depth of medium. This method gives viable bacterial count in a suspension. It is the recommended method for quantitative urine culture.

6. **Liquid culture** in a tube, bottle or flask may be inoculated by touching with a charged loop. Liquid cultures are preferred when large and quick yield is required. The major disadvantage of liquid culture is that it does not provide pure culture from mixed inocula.

Description of colonies of bacteria: Colonies of bacteria are described as follows:

Shape: Circular, irregular, radiate or rhizoid.

Surface: Smooth, rough, fine or coarsely granular, papillate, glistening, etc.

Size: Surface of colony is measured in millimeter. It measures 2 to 3 mm and if very small then 0.5 to 1 mm.

Elevation: Raised, low convex dome, umbilicate **(Fig. 5.5)**.

Some bacteria produce spreading growth, e.g., proteus, clostridia, etc.

Edges: Mostly edges are entire, e.g., *Klebsiella*, *Escherichia coli, Staphylococcus*. Sometimes edges may be crenated, fimbriated (*B. subtilis*) or effuse **(Fig. 5.6)**.

Color: Some organisms may produce pigmented colonies, e.g., staphylococci, *Pseudomonas aeruginosa.*

Opacity: Colonies on nutrient agar may be transparent, translucent or opaque.

Consistency: Colonies may be hard or firm, e.g., *Mycobacterium tuberculosis*, friable and membranous, e.g., *B. subtilis.* Mostly they are soft and butyrous, e.g., *Escherichia coli.*

Changes in the medium: Some organisms produce beta type of hemolysis around the colony, e.g., *Staphylococcus aureus* and *Streptococcus pyogenes.* Few bacteria produce soluble pigment that diffuses into the medium, e.g., *Pseudomonas aeruginosa*.

Emulsifiability: Growth of bacteria like *Escherichia coli, Salmonella* is easily emulsifiable whereas growth of *Neisseria catarrhalis* is not emulsifiable.

Growth in liquid media is described as:

1. *Turbid*
2. *Deposit:* Growth of *Streptococcus pyogenes* is characterized by deposits at the bottom of tube.

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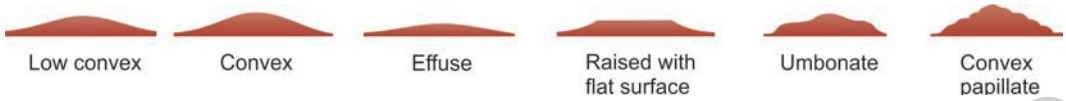

Fig. 5.5: Elevation of colonies.

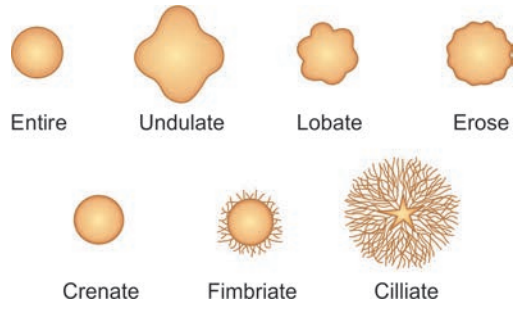

Fig. 5.6: Edges of colonies.

3. *Surface growth:* Surface growth is related to aerobic, nature of organism.
4. *Color changes:* Some organisms produce water soluble pigment which after diffusion change the color of medium, e.g., *Pseudomonas aeruginosa.*

METHODS OF ANAEROBIC CULTURE

Obligate anaerobes grow only in absence of free oxygen. These bacteria lack mechanism of oxidation through respiratory enzymes like cytochrome oxidase, catalase and peroxidase resulting in H_2O_2 accumulation. This H_2O_2 is toxic for the growth of anaerobic bacteria.

Clostridium tetani are strictly anaerobic. A number of methods are described for achieving anaerobiosis on the basis of following principles:

a. Exclusion of oxygen.
b. Production of vacuum.
c. Displacement of oxygen with other gases.
d. Absorption of oxygen by chemical or biological means.
e. Reduction of oxygen.

1. Cultivation in vacuum was tried by incubating culture in vacuum desiccator. It has disadvantages:
   a. Some oxygen is always left behind.
   b. Fluid culture may boil over and media get detached.
2. Displacement of oxygen with gases like hydrogen, nitrogen, helium or carbon dioxide was also tried. Anaerobiosis is never complete.

   Candle jar is again ineffective. Here inoculated plates are placed, inside airtight container and lighted candle is kept before lid is sealed. A burning candle should use up all the oxygen before it gets extinguished. Unfortunately some oxygen is always left behind. It also provides concentration of carbon dioxide which stimulates growth of bacteria other than anaerobes.
3. Absorption of oxygen by chemical or biological mean:
   a. Pyrogallic acid and sodium hydroxide.
   b. Chromium and sulfuric acid.
   c. GASPAK is now the method of choice consisting of an envelope and jar. Envelope is placed inside jar. GASPAK envelope contains 3 tablets, one each of citric acid, sodium carbonate and sodium borohydrate. It generates hydrogen and carbon dioxide on addition of water. Hydrogen combines with oxygen to produce water, and thus creation of anaerobiosis.
   d. McIntosh and Fildes anaerobic jar **(Fig. 5.7)**: It consists of glass or metal jar with metal lid which can be clamped airtight with screw. The lid has two tubes, one acts as a gas inlet and the other one as outlet. Additionally lid has two terminals which can be connected to electrical supply.

      Inoculated culture plates are placed inside jar. Outlet tube is connected to vacuum pump and air inside is evacuated. The outlet tap is closed and inlet tube is connected with hydrogen gas

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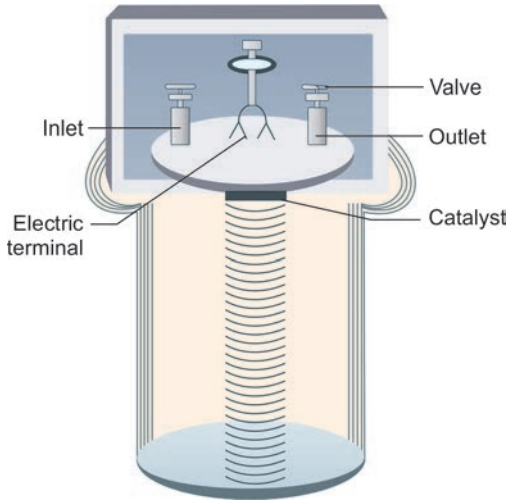

Fig. 5.7: McIntosh and Fildes jar.

cylinder. After filling of jar with hydrogen, electric terminals are connected so that palladinised asbestos is heated. This acts as catalyst for combination of hydrogen and residual oxygen. It ensures complete anaerobiosis. At the same time, it also carries risk of explosion.

An indicator should also be kept for verifying anaerobic condition in jar. Reduced methylene blue is used for this purpose. It is colorless anaerobically and regains blue color on exposure to oxygen.

4. *Anaerobic glove box:* This is a self-contained anaerobic system with provisions of circulation of hydrogen, nitrogen and carbon dioxide within it and catalytic conversion of residual oxygen to water. It is expensive. It is recommended for total anaerobic gut flora studies.
5. *Reduction of oxygen* in medium is achieved by using various reducing agents:
   a. 1% glucose.
   b. 0.1% thioglycolate.
   c. 0.1% ascorbic acid.
   d. 0.05% cystine.
   e. Broth containing iron pieces flamed red hot.
   f. Broth containing fresh animal tissue, e.g., rabbit kidney, spleen, etc.
   g. Robertson cooked meat medium produces anaerobiosis as under:
      i. Unsaturated fatty acids present in meat utilize oxygen for autoxidation.
      ii. Glutathione and cystein are reducing agents of meat and use oxygen.
      iii. Sulfahydryl compounds of cystein also precipitate for reduced oxidation reduction.

It consists of fat free minced meat in broth. It permits growth of even strict anaerobes. The meat itself contains reducing substances, particularly glutathione, which helps in the growth of anaerobes. Further fresh entry of oxygen into the medium is prevented by layering the top with sterile liquid paraffin. It indicates sacchrolytic (meat being red) and proteolytic (meat being black) activities.

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