OVERVIEW OF CULTURE MEDIA IN MICROBIOLOGY

A medium (plural: media) in microbiology terms is a solid or liquid preparation that supports the growth of a microorganism (bacteria, fungi and viruses). Media are to microorganisms what food is to man. For the effective propagation of microorganisms in the microbiology laboratory, microorganisms must be supplied with the requisite amount of nutrients that supports their growth, and these materials or nutrients are all contained in a bacteriological media (for bacteria). Some of the nutrients found in a growth media are proteins, carbohydrates, salt, peptone and agar. Agar is a solidifying agent found in virtually all the bacteriological media (except for broth medium). The media for the propagation of fungi and viruses are quite different from those used to culture bacteria. Only the protocol involved in the preparation of bacteriological media shall be expanded here. Some of the media used for the culture of bacteria in the microbiology laboratory include Nutrient agar (NA), MacConkey agar (MAC), Mueller-Hinton (MH) agar, Mannitol salt agar (MSA), Salmonella-Shigella agar (SSA) and cystein lactose electrolyte deficient medium (CLED) amongst others. While some of these media are differential or selective in nature (e.g. MAC, MSA, CLED & SSA), others such as NA and MH agar are known as general purpose media because they support the growth of virtually all types of bacteria. The differential or selective media as exemplified here only support the growth of certain types of bacteria. For example, MSA only support the growth of pathogenic S. aureus while SSA is used for the selective isolation of Salmonella & Shigella from stool samples. It is noteworthy to know that all media must be prepared according to the manufacturer’s instruction. Though the manufacturer’s instruction may vary from one type of media to another, the procedure still remains the same; and students are advised to stick to the manufacturer’s instruction of the particular media they seek to prepare.

Culture media are of different types; and they can be classified based on their consistency, based on their function and based on their composition.

Based on their consistency, culture media can be classified as:

1. **Solid culture media.** They contain agar as the solidifying agent. Agar is most commonly used to prepare solid media. Agar is polysaccharide extract obtained from seaweed; and it is an ideal solidifying agent because agar is bacteriologically inert (i.e. it has no negative influence on bacterial growth; and agar remains solid at 37°C. Agar is transparent; and at 100°C it transforms into a molten state.

2. **Liquid culture media.** Liquid culture media can also be called broth. They do not contain agar; and thus can remain in their liquid state even at a high and low temperature.

3. **Semi-solid culture media.** Semi-solid culture media contain agar but at a very little concentration (i.e. infinitesimally small) that does not allow the molten culture media to become solidified. Semi-solid culture media are neither solid or in liquid form. However, they are partially solidified. Typical examples of semi-solid culture media include Amies transport medium used for the transport of samples or microbial cultures.

Based on their function, culture media can be classified as:

1. **Basal media.** Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples of basal culture media include Nutrient
broth, nutrient agar and peptone water. *Staphylococcus* and *Enterobacteriaceae* grow in these types of culture media. Basal culture media can also be called general purpose media or simple media; and they support the growth of virtually all bacteria.

2. **Enriched Media.** Enriched media are culture media formed by adding blood, serum or egg (as growth factors). They are culture media that support the growth of fastidious bacteria (i.e. bacteria that require additional nutrient for growth). Examples of enriched culture media are blood agar and Lowenstein-Jensen media. *Streptococci* and *Haemophilus* are typical examples of bacteria that require additional growth nutrient for growth.

3. **Enrichment media.** They are liquid culture media that contain additional growth nutrient for the growth of bacteria. Unlike the enriched media, enrichment media are basically broth and they do not contain agar like the enriched media. Selenite F broth, an enrichment media for the culture of faecal samples prior to their subculture onto solid culture media is an example of enrichment culture media.

4. **Selective Media.** Selective culture media favour the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples of selective culture media include MacConkey agar, Lowenstein-Jensen media, and tellurite media (Tellurite inhibits the growth of most of the throat organisms except diphtheria bacilli in sputum samples). Antibiotic may be added to a medium for the inhibition of unwanted organisms. *Sabouraud dextrose agar (SDA)* is a selective culture media used for the cultivation of fungi in the laboratory. SDA contain chloramphenicol and cycloheximide which inhibit the growth of bacteria and saprophytic fungi respectively while allowing pathogenic fungi to grow.

5. **Differential Media.** Differential culture media are culture media that differentiates between two different classes of bacteria. For example, MacConkey agar is a differential media that supports the growth of lactose-fermenting bacteria (e.g. *Escherichia coli* and *Klebsiella pneumoniae*) from non-lactose fermenting bacteria (e.g. *Salmonella* and *Shigella*). An indicator is included in the culture medium; and it serves to differentiate the different colonies of bacteria growing on it. A particular organism causes change in the indicator, e.g. blood, neutral red, tellurite.

6. **Transport Media.** Transport media are used when specimen cannot be cultured soon after collection. They are used to transport samples or culture from their point of collection to the laboratory for further processing. Examples of transport culture media include Cary-Blair medium, Amies medium, and Stuart medium.

7. **Storage Media.** Storage media are used for storing the bacteria for a long period of time until a later time when they will be used again. Examples of storage culture media include egg saline medium, chalk cooked meat broth.

**Based on their chemical composition, culture media can be classified as:**

1. **Simple culture media:** The nutritional composition of simple media is constant and known. They support the growth of bacteria that are able to grow with minimal requirements (i.e. non-fastidious bacteria).

2. Synthetic Media: Synthetic or defined media such are specially prepared media for research purposes; and the composition of every component of the media is well known.
3. **Complex culture media**: Complex media such as blood agar have ingredients whose exact components are difficult to estimate.

**GROWTH FACTORS**

This simplified scheme for use of carbon, either organic carbon or CO₂, ignores the possibility that an organism, whether it is an autotroph or a heterotroph, may require small amounts of certain organic compounds for growth because they are essential substances that the organism is unable to synthesize from available nutrients. Such compounds are called **growth factors**. They are required in small amounts by cells because they fulfill specific roles in biosynthesis. The need for a growth factor results from either a blocked or missing metabolic pathway in the cells. Growth factors are organized into three categories; and they are:

1. **Purines and pyrimidines**: required for synthesis of nucleic acids (DNA and RNA)
2. **Amino acids**: required for the synthesis of proteins
3. **Vitamins**: needed as coenzymes and functional groups of certain enzymes

**TYPES OF CULTURE MEDIA**

Media are of different types on consistency and chemical composition:

**A. On Consistency:**

1. **Solid Media.** Advantages of solid media: (a) Bacteria may be identified by studying the colony character, (b) Mixed bacteria can be separated. Solid media is used for the isolation of bacteria as pure culture. 'Agar' is most commonly used to prepare solid media. Agar is a polysaccharide extract obtained from seaweed. Agar is an ideal solidifying agent as it is: (a) Bacteriologically inert, i.e. no influence on bacterial growth, (b) It remains solid at 37°C, and (c) It is transparent.
2. **Liquid Media.** It is used for profuse growth, e.g. blood culture in liquid media. Mixed organisms cannot be separated.

**B. On Chemical Composition:**

1. **Routine Laboratory Media**
2. **Synthetic Media.** These are chemically defined media prepared from pure chemical substances. It is used in research work.

**ROUTINE LABORATORY MEDIA**

These are classified into six types: (1) Basal media, (2) Enriched media, (3) Selective media, (4) Indicator media, (5) Transport media, and (6) Storage media.

1. **Basal Media.** Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water. Staphylococcus and Enterobacteriaceae grow in these media.

2. **Enriched Media.** The media are enriched usually by adding blood, serum or egg. Examples: Enriched media are blood agar and Lowenstein-Jensen media. Streptococci grow in blood agar media.
3. **SELECTIVE MEDIA.** These media favour the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: MacConkey agar, Lowenstein-Jensen media, tellurite media (Tellurite inhibits the growth of most of the throat organisms except diphtheria bacilli). Antibiotic may be added to a medium for inhibition.

4. **INDICATOR (DIFFERENTIAL) MEDIA.** An indicator is included in the medium. A particular organism causes change in the indicator, e.g. blood, neutral red, tellurite. Examples: Blood agar and MacConkey agar are indicator media.

5. **TRANSPORT MEDIA.** These media are used when specie-men cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium.

6. **STORAGE MEDIA.** Media used for storing the bacteria for a long period of time. Examples: Egg saline medium, chalk cooked meat broth.

**COMMON MEDIA IN ROUTINE USE**

**Nutrient Broth.** 500 g meat, e.g. ox heart is minced and mixed with 1 litre water. 10 g peptone and 5 g sodium chloride are added, pH is adjusted to 7.3. Uses: (1) As a basal media for the preparation of other media, (2) To study soluble products of bacteria.

**Nutrient Agar.** It is solid at 37°C. 2.5% agar is added in nutrient broth. It is heated at 100°C to melt the agar and then cooled.

**Peptone Water.** Peptone 1% and sodium chloride 0.5%. It is used as base for sugar media and to test indole formation.

**Blood Agar.** Most commonly used medium. 5-10% defibrinated sheep or horse blood is added to melted agar at 45-50°C. Blood acts as an enrichment material and also as an indicator. Certain bacteria when grown in blood agar produce haemolysis around their colonies. Certain bacteria produce no haemolysis. Types of changes: (a) beta (p) haemolysis. The colony is surrounded by a clear zone of complete haemolysis, e.g. Streptococcus pyogenes is a beta haemolytic streptococci. (b) Alpha (a) haemolysis. The colony is surrounded by a zone of greenish discoloration due to formation of biliverdin, e.g. Viridans streptococci, (c) Gamma (y) haemolysis, or, No haemolysis. There is no change in the medium surrounding the colony.

**Chocolate Agar or Heated Blood agar.** Prepared by heating blood agar. It is used for culture of pneumococcus, gonococcus, meningococcus and Haemophilus. Heating the blood inactivates inhibitor of growths.

**MacConkey Agar.** Most commonly used for enterobacteriaceae. It contains agar, peptone, sodium chloride, bile salt, lactose and neutral red. It is a selective and indicator medium:

1. **Selective** as bile salt does not inhibit the growth of enterobacteriaceae but inhibits growth of many other bacteria.
(2) **Indicator** medium as the colonies of bacteria that ferment lactose take a pink colour due to production of acid. Acid turns the indicator neutral red to pink. These bacteria are called 'lactose fermenter', e.g. Escherichia coll. Colourless colony indicates that lactose is not fermented, i.e. the bacterium is non-lactose fermenter, e.g. Salmonella. Shigella, Vibrio.

**Mueller Hinton Agar.** Disc diffusion sensitivity tests for antimicrobial drugs should be carried out on this media as per WHO recommendation to promote reproducibility and comparability of results.

**Hiss's Serum Water Medium.** This medium is used to study the fermentation reactions of bacteria which can not grow in peptone water sugar media, e.g. pneumococcus, Neisseria, Corynebacterium.

**Lowenstein-Jensen Medium.** It is used to culture tubercle bacilli. It contains egg, malachite green and glycerol. (1) Egg is an enrichment material which stimulates the growth of tubercle bacilli, (2) Malachite green inhibits growth of organisms other than mycobacteria, (3) Glycerol promotes the growth of Mycobacterium tuberculosis but not Mycobacterium bovis.

**Dubos Medium.** This liquid medium is used for tubercle bacilli. In this medium drug sensitivity of tubercle bacilli can be carried out. It contains 'tween 80', bovine serum albumin, casein hydrolysate, asparagin and salts. 'tween 80 causes dispersed growth and bovine albumin causes rapid growth.

**Loeffler Serum.** Serum is used for enrichment. Diphtheria bacilli grow in this medium in 6 hours when the secondary bacteria do not grow. It is used for rapid diagnosis of diphtheria and to demonstrate volutin granules. It contains sheep, ox or horse serum.

**Tellurite Blood Agar.** It is used as a selective medium for isolation of Corynebacterium diphtheriae. Tellurite inhibits the growth of most secondary bacteria without an inhibitory effect on diphtheria bacilli. It is also an indicator medium as the diphtheria bacilli produce black colonies. Tellurite metabolized to tellbrism, which has black colour.

**EMB (Eosin-methylene blue) Agar.** A selective and differential medium for enteric Gram-negative rods. Lactose-fermenting colonies are coloured and nonlactose-fermenting colonies are nonpigmented. Selects against gram positive bacteria.

**XLD (Xylose Lysine Deoxycholate).** It is used to isolate Salmonella and Shigella species from stool specimens. This is a selective media.

**SS (Salmonella-Shigella) Agar.** It is a selective medium used to isolate Salmonella and Shigella species. SS Agar with additional bile salt is used if Yersinia enterocolitica is suspected.

**DCA (Desoxycholate Citrate Agar).** It is used for isolation of Salmonella and Shigella. The other enteric bacteria are mostly inhibited (a selective medium). It is also a differential (indicator) medium due to presence of lactose and neutral red.
**Tetrathionate Broth.** This medium is used for isolating Salmonella from stool. It acts as a selective medium. It inhibits normal intestinal bacteria and permits multiplication of Salmonella.

**Selenite F Broth.** Uses and functions are same as that of tetrathionate broth.

**Thiosulphate-Citrate-Bile-Sucrose (TCBS) Agar.** TCBS agar is a selective medium used to isolate Vibrio cholerae and other Vibrio species from stool.

**Charcoal-yeast agar.** Used for Legionella pneumophila. Increased concentration of iron and cysteine allows growth.

**Tellurite-Gelatin Agar Medium (TGAM).** It may be used as transport, selective and indicator medium.

**Alkaline peptone water.** See under Vibrio. (Chapter 51).

**Campylobacter Medium.** This selective medium is used to isolate Campylobacter jejuni and Campylobacter coli from stool.

**Cary-Blair Medium.** It is used as a transport medium for faeces that may contain Salmonella, Shigella, Vibrio or Campylobacter species.

**Amies medium** is used for gonococci and other pathogens.

**Peptone Water Sugar Media.** These indicator media are used to study 'Sugar fermentation'. 1% solution of a sugar (lactose, glucose, mannitol etc) is added to peptone water containing Andrade's indicator in a test tube. A small inverted Durham tube is placed in the medium. The media are colourless. After culture, change of a medium to red colour indicates acid production. Gas, if produced collects in Durham tube.

**Motility Indole Urea (MIU) Medium.** This is used to differentiate enterobacteria species by their motility, urease, and indole reactions.

**TSI (Triple sugar iron) Agar.** See chapter 53.

**KIA (Kligler Iron Agar).** This is a differential slope medium used in the identification of enteric bacteria. The reactions are based on the fermentation of lactose and glucose and the production of hydrogen sulphide (chapter 53).

**Christensen's Urea Medium.** This is used to identify urea splitting organisms, e.g. Proteus. A purple pink colour indicates urea splitting (See chapter 53).

**Bordet-Gengou Medium.** This medium is used for culture of Bordetella pertussis. Increased concentration of blood allows growth. It contains agar, potato, sodium chloride, glycerol, peptone and 50% horse blood. Penicillin may be added to it.