# CULTURE MEDIA FOR MICROBES

In everyday language, growth refers to an increase in size. We are accustomed to seeing children, other animals, and plants grow. Unicellular organisms also grow, but as soon as a cell called mother/parent cell, has approximately doubled in size and duplicated its contents, it divides into two daughter cells. Then the daughter cells grow, and subsequently they also divide. Because individual cells grow larger only to divide into two new individuals, microbial growth is defined not in terms of cell size but as the increase in number.

**Introduction to culture media**

Medium/media is the substance which provides nutrients for the growth.

Then what is culture media?

Culture media is also known as growth media. Nutrients on which microorganisms can be cultivated, transported and stored is called as culture media. It is a solid or liquid preparation that is required for the microbial growth. It becomes highly necessary if the microbe in question is needed to be cultured invitro. They are specific mixture or mixtures of nutrients and other substances that support the growth of microorganisms such as bacteria and fungi. They are a source of energy and certain environmental conditions in order to grow and reproduce. Culture media can vary in their form and composition determined by the species to be cultivated.

**Why should we culture microbes?**

The important reasons for us to culture microbe invitro are

a) it's utility in diagnosing infectious diseases,

b) to study morphology and its identification,

c) to obtain antigens from developing serological assays or vaccines,

d) viable count of microbes, and

e) solid media helps in separating bacterial mixtures.

Properties of culture media

1. Different nutrients for different microbes for good growth (there is no one media in which one can grow all the microbes).
2. It must contain sufficient moisture, suitable level of $O_2$ and pH adjusted as per requirement of the growing microbe.
3. Media must be sterile before use.

**Nutritional factors**

Much of the study of microbiology depends on the ability to grow and maintain microorganisms in the laboratory. Growth phases are displayed in different ways in colonies growing on a solid medium. Typically, a cell divides exponentially, forming a small colony. Growth of microorganisms is affected by nutritional factors, as well as by physical factors.

Nutrients needed by microorganisms include carbon, nitrogen, sulfur, phosphorus, certain trace elements and vitamins. There are two kinds of microbes based on their nutritional needs, they are: non-fastidious – those which can grow in minimal nutrients containing media; fastidious – those that requires special or specific needs.

**Composition of media**

1. Complex mixture of inorganic salts.
2. Organic supplements like vitamins and amino acids (Nitrogen source)
3. Carbon source
4. Growth regulators
5. Solidifying agents and
6. Water

**History**

It was Robert Koch who first cultured bacteria on the sterile surfaces of cut and boiled potato. But the problem here was that the microbes were growing but not very well. He then developed culture media using meat extracts and protein digests which were similar to body fluid (imitating the body fluids), and added gelatin to solidify the preparation. The individual bacteria produced separate colonies. Gelatin was not an ideal solidifying agent since it could be digested by many microbes and it liquifies above 28˚C. Fanny Hesse, wife of Walter Hesse – Koch's assistant, who was using it for making jellies, suggested agar as an alternative. It was ideal for two reasons a) it did not solidify until reaching 45-50˚C, and b) it did not melt until reaching 95-100˚C.

**Classification of bacterial culture on the basis of consistency**

1. Solid medium
2. Semisolid medium
3. Liquid (broth) medium

**Solid medium** – solid medium contains agar at concentration of 1.5-2.0% or some other, mostly inert solidifying agent. Solid medium has a physical structure and allows bacteria to grow in physically informative or useful ways (colonies). It is useful in isolating or for determining colony characters of the isolate. Eg: Nutrient agar, Blood agar, MacConkey agar etc.,

**Semisolid medium** – Semisolid medium is prepared at half the concentration as in that of solid medium or 0.5% or less. It has a soft custard like consistency. They are useful in cultivating microaerophilic bacteria (which requires oxygen to survive) or for the determination of bacterial motility (ability of bacteria to move independently using metabolic energy). Eg: Stuart's media, Amies media.

**Liquid (broth) medium** – These media contain specific amount of nutrients but don't have a trace of gelling agents such as gelatin or agar. Broth medium serves various purposes such as propagation of a large number of organisms, fermentation studies and various other tests. Eg: Nutrient broth, Peptone water.

**Classification of bacterial culture on the basis of chemical composition**

1. Natural medium
2. Semisynthetic medium
3. Synthetic medium

**Natural medium** – Natural medium is composed of nutrients obtained from natural sources, sufficient for growth and proliferation of microorganisms. It is a type of media where neither the composition of which is unknown nor the quantity. In early years of invitro cultivation natural media were obtained from biological sources. Eg: Milk, urine, blood, vegetable juice, meat extracts, etc.

In natural media, the nutrients can be procured in three types of biological tissue, they are;

a. *Coagulants or plasma clots* – They are now available as liquid plasma kept in silicon ampoules or lyophilized plasma (a process in which plasma is frozen and dehydrated by sublimation under vacuum for several days). Plasma may also be prepared in lab by taking out blood from male fowl (rooster/cock) and adding heparin to prevent blood coagulation.
b. *Biological fluid* – Biological fluid is obtained in the form of serum from human adult blood, placental blood, cord blood (umbilical cord blood), horse blood or in the form of coconut water, amniotic fluid, pleural fluid, insect hemolymph serum (major extracellular fluid in insects), aqueous humor (from eyes-produced from ciliary body, low protein concentrations) etc.
c. *Tissue extract* – These are obtained from tissues like embryo, liver, spleen, tumor, bone marrow, embryo extract (chick embryo/bovine embryo is most commonly used), vegetable juices, meat extracts, etc. tissue extract should be used before a week or stored at 27˚C.

**Advantages**:

i. They are very easy to prepare.
ii. Since natural media consists of naturally occurring biological fluids, which are useful for wide range of microorganisms.

**Disadvantages**:

i. It has poor reproducibility due to lack of the exact composition.
ii. Since a few components are of unknown composition, its effect cannot be understood properly as there are high possibility of the unknown complex substance containing inhibitors.

**Synthetic medium** – it is also called as chemically defined medium. Here, the media is prepared from purified ingredients which is why all the chemicals or components used are known. There is no yeast, animal or plant tissue is present. These are prepared from pure chemicals and the exact composition of the medium is very well known. It is used for special studies like metabolic requirements. Eg: Peptone water, Richard solution, Raulin's media, etc.

Synthetic medium is further classified into five classes based on its composition,

1. Serum-containing medium
2. Serum-free medium
3. Protein-free medium

4. Chemically defined medium
5. Xeno-free media

***Serum-containing medium*** – In this medium serum is taken as a supplement to provide optimal culture medium. Serum provides carriers or chelators for labile or water insoluble nutrients, hormones and growth factors, protease inhibitors, binds and neutralizes toxic moieties. There is a debate that this should not be considered as one among the synthetic media as it contains serum, a complex of unknown composition. Eg: Fetal Bovine Serum, Potato Dextrose Agar, etc.

**Advantages**:

1. Contains various growth factors.
2. Provides attachment of cells.
3. Acts as buffering agent.
4. Helps in binding proteins.
5. Minimizes mechanical damages or damages caused by viscosity.

**Disadvantages**:

1. Lack of uniformity in composition.
2. Testing needs to be done to maintain the quality of each batch before using.
3. May contain some growth inhibiting factors.
4. Increased risk of contamination.
5. Interfere with purification and isolation of culture products.

Note:

Fetal Bovine serum – Bilirubin, cholesterol, creatinine, urea, NaCl, potassium salts, calcium salts, potassium salts, iron salts, glucose, protein, albumin, α-globulin and water.
Potato dextrose agar – Dextrose, potato extract, agar, chlortetracycline.

***Serum-free media*** – This is a type of media where there is no serum whatsoever. All the components are organic and inorganic components. Serum is replaced with iron salts which act as chelators. These media are specifically formulated to support the culture of a single cell type and incorporated defined quantities of purified components. Eg: Richard solution, Raulin's media, Czapek's agar, etc.

**Advantages**:

1. Growth can be controlled of the cultured cells as required by changing the composition of the media.
2. Easier downstream processing of products from cultured cells.
3. Toxic effects of serum are avoided.
4. There is no danger of degradation of sensitive proteins by serum proteases.

**Disadvantages**:

1. Cell proliferation is low.
2. Cultured cells may need more than one type of media to obtain desired cell culture products.
3. A greater control over the pH, temperature, etc. are necessary than to that of serum containing media.
4. Growth rate and maximum cell density attained are lower than those with serum. containing media.

Note:

**Richard media** – Potassium nitrate, monopotassium dihydrogen phosphate, $MgSO_4$, $FeCl_2$, sucrose, agar, water.

Raulin's media. – Ammonium tartarate, tartaric acid, glucose, water, agar, $(NH\ ^4)_2HPO_4$, $(NH_4)_2SO_4$, $K_2CO_3$, $MgCO_3$, $FeSO_4$, $ZnSO_4$

Czapek's media – $NaNO_3$, $K_2PO_4$, $MgSO_4$, KCl, $FeSO_4$, agar, water.

***Protein-free media*** – In this media, there is no protein content or its derivatives but can give good cell growth. It is greatly used in industries to produce and isolate proteins and its derivatives. Minimum essential media (MEM), Roswell Park Memorial Institute Medium (RPMI), etc.

Note:

Minimum essential media – NaCl, KCl, $CaCl_2$.H2O, $NaHCO_3$, $NaH_2PO_4.H_2O$, $MgSO_4.7H_2O$, Phenol red, Chlorine chloride, L-glutamine, L-cystein, aminoacids,vitamins.

Roswell Park Memorial Institute Medium – $Ca(NO_3)_2.4H_2O$, $MgSO_4$, KCl, $NaHCO_3$, NaCl, $Na_2HPO_4$, vitamins, amino acids.

***Chemically defined media*** – Media whose compositions are precisely chemically defined. It contains pure organic and inorganic compounds that vary little from one source to another.

Molecular content of the media is specified by an exact formula. Eg: Peptone, Minimal glucose media, etc.

***Xeno-free media*** – The media that contains the derivatives of the cell that is cultured. This is a common media that is used in mammalian cell culture. For example, mammalian cell can be cultured in the media containing human serum or any such derivatives. There is no known xeno-free media for microbial culture.

**Semisynthetic medium** – In this media used the composition is partially known. It can contain chemically defined or known substances but is in association with a few other components that are complex like yeast, casein hydrolysate, etc. in unknown proportion. It is also called as complex media. Most of the culture media used for routine diagnostic work are semisynthetic media. Eg: Oatmeal agar, potato dextrose agar, blood agar, etc.

**Advantages**:

i. This media is used for isolation and physiological studies.

**Disadvantages**:

i. Since a few components are of unknown composition, its effect cannot be understood properly as there are high possibility of the unknown complex substance containing inhibitors.

Note:

Oatmeal agar – Oatmeal, agar, water.

Potato dextrose agar – Dextrose, potato extract, agar, chlortetracycline.

Blood agar – Pancreatic digest of casein, papaic digest of soy meal, NaCl, agar, water.

**Classification of bacterial culture on the basis of application (functional media types):**

1. Basal medium
2. Enriched medium
3. Enrichment medium
4. Differential medium
5. Indicator medium
6. Transport medium
7. Storage medium

**Basal medium** – Basal medium is a simple medium that supports most of the non-fastidious microbes. It is used to grow or culture the microbe that do not need enrichment. Eg: *Staphylococcus*, Enterobacteriaceae, etc.

It is used for the primary isolation of microorganisms. This isolation of separate colonies is for studying a) colony morphology, b) pigmentation present in them, c) biochemical identification tests. It can also be used as a means for producing bacterial lawns needed for antibiotic tests. Enumeration of the organisms in water, sewage, dairy products, feces and other materials.

Components that are to be present in basal media are; nitrogen, carbon and minerals that provide minimum adequate nutrition.

Eg: Peptone water, Nutrient broth, Nutrient agar, etc.

Note:

Peptone water – Peptone, NaCl, $H_2O$ (used for sugar fermentation test, indole test).

Nutrient broth – Peptone water, meat extract (routine culture).

. Nutrient agar – Nutrient broth, agar (isolation and purification of culture).

Note:

Purpose of

a) Beef extract – aqueous extract of lean beef tissues, contains water soluble substances of animal tissue which include carbohydrates, organic nitrogen compounds, water soluble vitamins and salts.

b) Peptone – It is made by digesting proteinaceous materials. Eg: meat, gelatin, casein; using acids or enzymes. It is the principal source of organic nitrogen and may contain carbohydrates/vitamins. Depending upon the nature of protein and method of digestion, peptones differ in their constituents and their ability to support growth of microbes.

c) Agar – Obtained from certain marine algae used as a solidifying agent for media and does not have nutritive value.

d) NaCl – To maintain osmotic equilibrium of the medium and prevent the change in pH.

Microbes that grow in basal media are: *E. coli*, *Pseudomonas*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Yersinia*, etc.

**Enriched media** – In enriched media extra nutrients from blood, serum, egg yolk, etc. are added to basal medium. this makes the media enriched to grow the fastidious bacteria. They contain nutrients required to support the growth of wide variety of organisms commonly used to harvest as many different types of microbes that are present in the specimen. Eg: Chocolate agar, Blood agar, Columbia agar, Trypic soy agar, etc.

It is an excellent medium for cultivation of fastidious bacteria that require particular nutrients. About 5% of defibrinated mammalian blood (human, sheep or horse) is added to the autoclaved basal media to prepare Blood Agar Media. This inhibits a few bacteria like *Neisseria* and *Haemophilus*.

The blood added to the medium provides growth factors.

Note:

Blood agar media – Casein enzymatic digest, peptic digest of animal tissue, yeast extract, NaCl, agar, sheep blood.

Trypic agar – Peptone, tryptose, NaCl, agar, blood.

Trypic soy agar – Tryptose, soytone, NaCl, agar, blood.

Columbia agar – Pancreatic digest of casein, meat peptic digest, heart pancreatic digest, yeast extract, maize, starch, NaCl, agar.

Chocolate agar is very similar to blood agar except that, during the preparation, the red blood corpuscles are lysed with a molten agar base. This specific agar is used in isolation and cultivation of fastidious microbes *Haemophilus* and *Neisseria* species.

Disadvantage – Nonpathogenic organisms may over grow pathogenic organisms.

**Enrichment media** – In this media, the stimulating effect on the bacteria to be grown or inhibits its competitors. This result in an absolute increase in the number of wanted bacteria related to other bacteria. The media contains specific nutrients required for the growth of particular bacterial pathogen that may be present alone or with other bacterial species in specimen. It is used to enhance the growth of particular bacterial pathogen from mixture of organism by using nutrient specificity. Eg: Selenite F broth.

Note:

Selanite F Broth – Casein enzymatic hydrolysate, lactose, sodium phosphate, sodium hydrogen selenite.

Note:

Casein enzymatic hydrolysate – Nitrogen, vitamins.

Lactose – Carbohydrates.

Sodium phosphate – Lessens selenite toxicity.

Sodium hydrogen selenite – Reduced by bacteria and alkali is produced.

Bacteria that grow in this media are *Salmonella*, *Shigella*, *Vibrio cholerae*.

**Difference between enriched media and enrichment media are:**

| Enriched media | Enrichment media |
|---|---|
| The media, which contain the nutrients required to support the growth of a wide variety of organisms, including some fastidious ones. | The liquid media that inhibits the growth of the unwanted bacteria. |
| Allow the growth of a wide variety of microorganism. | Allow the growth of a particular type of microorganism in the medium |
| Solid media | Liquid media |
| Contain extra nutrients in the form of egg yolk, blood, serum, etc. | Contain added antibiotics, dyes, chemicals or altered pH. |
| Facilitate the growth of fastidious microorganisms. | Inhibit the growth of unwanted commensal or contaminating bacteria. |
| Examples: Blood agar, chocolate agar, Loeffler's serum, etc. | Examples: Selenite F broth, tetrathionate broth, alkaline peptone water, etc. |

**Selective media** – This is a media that is used for growing selected microorganism. For example, if a microorganism is resistant to a certain antibiotic, such as ampicillin or

tetracycline, then that antibiotic can be added to the medium in order to prevent other cells, which do not possess resistance.

Eg: Media lacking an amino acid such as proline in conjugation with E. coli unable to synthesize it were commonly used before the emergence of genetics and genomics to map bacterial chromosomes.

Selective growth media are also used in cell culture to ensure the survival or proliferation of cells with certain properties, such as antibiotic resistance or the ability to synthesize a certain metabolite.

Normally the presence of specific gene or an allele of a gene confers upon the cell, the ability to grow in selective medium with the help of marker.

Selective growth media for eukaryotic cells commonly contain neomycin to select cells that have been successfully transfected with a plasmid carrying the neomycin resistance gene as marker.

Glancycovir is an exception to the rule as it is used specifically, kill cells that carry its respective marker, the Herpes Simplex Virus Thymidine Kinase (HSVTK).

Eg: Eosin methylene blue, MacConkey agar, Hektoen enteric agar, mannitol salt agar, terrific broth, xylose lysine desoxyscholate, etc.

Note:

Eosin methylene blue – Contains methylene blue which is toxic to gram-positive bacteria. Allows growth of gram-negative bacteria.

MacConkey agar – Allows growth of gram-negative bacteria.

Hektoen enteric agar – Allows growth of gram-negative bacteria.

Mannitol salt agar – Allows growth of gram-positive bacteria.

Terrific broth – Used with glycerol in cultivating recombinant strains of E. coli.

Xylose lysine desoxyscholate – It is selective for gram-negative bacteria.

Various techniques are employed to make a medium selective. They are;

i. Addition of antibiotics.
ii. Addition of chemicals.
ii. Addition of dyes.
iv. Altering the pH.
v. Combination of any of the above.

Note: Selective media – suppression of unwanted microbes; encouraging desired microbes. Enrichment media – similar to selective media but designed to increase numbers of desired microbes to detectable levels.

**Differential media** – It is also called as indicator media. It contains specific ingredients or chemicals that helps us to visually distinguish which species do or do not carryout a specific biochemical process or appearance of the colony or surrounding media. It is used to differentiate closely related organisms or groups of organisms.

Presence of certain dyes or chemicals in the media. The organisms will produce characteristic changes or growth patterns that are used for identification or differentiation.

Eg: Blood agar is a differential medium that distinguishes bacterial species by their ability to break down red blood corpuscles included in the media.

Often used to distinguish between the different species of pathogenic Streptococcus bacteria.

Different type of *Streptococcus* has predictable pattern of hemolysis.

α-Hemolytic *Streptococcus* (causes pneumonia) – they produce narrow band of slimy discoloration (greenish or bluish) around the colony during partial breakdown of the red blood cells.

Eg: *Streptococcus pneumoniae*, and many oral *Streptococci*.

β-Hemolytic *Streptococcus* (potentially deadly infection in new-borns) – They produce completely clear zone around the colonies. Here there is complete breakdown of red blood cells that produces characteristic clear zone.

Eg: *Streptococcus pyogenes, S. agalactiae* etc.

Apart from Streptococci, other bacteria that can undergo complete hemolysis are, *Stephylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*.

γ-Hemolytic *Streptococcus* (Group D Streptococci) – In this type, there is absolutely no hemolysis.

Eg: *Streptococcus bovis*.

Other than *Streptococcus bovis*, other bacteria that are found in gastro-intestinal tract will not breakdown of red blood cells.

Examples for differential media are; MacConkey agar, Eosin methylene blue, etc. Difference between selective and differential media are;

| Selective media | Differential media |
|---|---|
| Selective media refers to a type of growth media that allows the growth of selected microorganisms in the medium. | Differential media refers to a type of growth media that allows the differentiation of closely-related microorganisms. |
| Used to isolate a particular strain of microorganisms. | Used to identify and differentiate closelyrelated microorganisms. |
| Use specific growth characteristics of a particular microorganism to select it from the others. | Use unique growth patterns of microorganisms to differentiate them from others. |
| Only allow the growth of a single microorganism in the medium. | Allow several closely-related organisms to grow in the medium. |
| Do not use indicators. | Use indicators. |

Note:

MacConkey agar – Peptone (pancreatic digest of gelatin), proteose peptone (meat and casein), lactose monohydrate, bile salts, NaCl, neutral red, crystal violate, agar.

MacConkey agar is both differential and selective media. It is used in detection and isolation of all types of dysentery, typhoid, paratyphoid organisms. Generally, it is used in detection or differentiating strains of *Salmonella typhosa* from coliform group (rod shaped gram-negative bacteria that are non-spore formation and motile/non-motile bacteria that can ferment lactose with the production of acid and gas). This medium supports the growth of all Salmonella and Shigella strains.

When coliform bacilli are grown on this medium, there is a characteristic brick red coloration surrounded by a zone of precipitated bile (acid end products act on bile salts and neutral red is absorbed by the precipitated salts).

Dysentry, typhoid and paratyphoid bacilli do not ferment lactose but give an alkaline reaction which does not give any coloration.

Gram-positive organisms are inhibited because of crystal violet and bile salts.

Eosine methylene blue agar – This media helps in detection and isolation of intestinal pathogens. Allows differentiation between the organisms that ferment lactose and those that do not.
Saccharose is also added because, certain members of enterobacteria/coliform group

ferments saccharose more readily than lactose.

Methylene blue inhibits gram-positive bacteria.

*E. coli* colonies have dark center and greenish metallic sheen

*Enterobacteria aerogenes* gives pinkish-mucoid colonies.

*Salmonella*, however, do not ferment saccharose and produce noncolored colonies.

**Storage media** – This is also called as maintenance media. It is used for storin bacteria for a long period of time. It gives satisfactory maintenance of viability and physiological characteristics of microorganisms.

Eg: Egg saline medium, chalk cooked meat broth, etc.

**Transport media** – The clinical specimens need to be transported to the laboratory immediately after collection to prevent over growth of contaminating microbes and without allowing them to multiply.

Transport media prevents desiccation, maintain pathogen to commensal ratio inhibit unwanted bacteria. Some of these media are semisolid (Stuart's media and Amie's media).

Eg: Cary Blair medium, Venkataraman Ramakrishnan medium, Stuart's medium, Amie's medium.

Note:

Cary Blair medium – Sodium thioglycolate, disodium hydrogen phosphate, NaCl, Agar. This media can be used as storage media as well. *Shigella*, *Salmonella*, *Vibrio cholerae*, *Campylobacter* (from feces/rectal swab).

Venkataraman Ramakrishnan medium – Crude-sea salt, peptone. This media is majorly used to transport feces from suspected cholera patients.

Stuart's medium – Sodium glycerophosphate, Sodium thioglycolate, $CaCl_2$, methylene blue, agar. This medium is used to grow *Streptococcus pyogenes*, *Bacteroides fragilis*, *Pseudomonas aeruginosa*, etc. The limitation to this media is that, it undergoes slight degree of oxidation indicated by blue color at the upper periphery of the medium. If the medium exhibits a distinct blue color throughout. Sodium glycerophosphate is a buffer; however, some organisms metabolize glycerophosphate with resultant promotion of bacterial growth. All specimens should be transported to the laboratory without delay and maintained at room temperature until processed. Chilling may be detrimental for a few organisms.

Amies medium – NaCl, KCl, $CaCl_2$, $MgCl_2$, monopotassium phosphate, disodium phosphate, sodium thioglycolate, charcoal, agar. Amies transport medium provides a reduced environment due to the presence of sodium thioglycolate and the small amount of agar. Charcoal helps to neutralize materials that ae toxic to sensitive pathogens like *Neisseria gonorrhoeae*.

**Reference:**


- https://conductscience.com/culture-media/.
- https://bio.libretexts.org/Bookshelves/Microbiology/Book%3A_Microbiology_(Boundless)/6%3A_Culturing_Microorganisms/6.3%3A_Culturing_Bacteria/6.3A%3A_Culture_Media.
- https://www.slideshare.net/NAMDEOSHINDE15/culture-media-165080927.
- Sharma P. D., (2015), Microbiology and plant pathology, (3rd ed.), Rastogi Publication.