

CULTURE MEDIA

Culture Media used in Microbiology



Microbiological Culture

Method of cultivating microbial organisms by letting them reproduce in predetermined culture media under controlled laboratory conditions

Culture Media

- Study of microorganisms depends on its ability to grow in the laboratory
- Growth of microorganisms is possible only if suitable culture media is available
- A culture medium is defined as a solid or liquid preparation used for the growth, transport and storage of microorganisms
- The effective culture medium must contain all the nutrients required for the growth of the microorganism.

How many types of growth media?

- There are two major types of growth media:
- **Cell culture:** which use specific cell types derived from plants or animals
- **Microbiological culture:** which are used for growing microorganisms, such as bacteria or yeast.

Nutrient Requirements

- Nutritional requirements vary greatly among the microorganisms
- In common all microorganisms need sources of energy, nitrogen, carbon, water, phosphorus, sulfur, and various minerals
- The exact composition of a satisfactory medium will rely on the species to be identified and cultivated

Reasons for Culturing

- Bacteria have to be grown (cultured) for them to be identified and subsequent clinical diagnosis
- Culturing on solid media is another convenient way of separating bacteria in mixture
- Bacteria have to be cultured in order to obtain antigens from developing serological assay for vaccines
- Certain genetic studies and manipulations of the cells also need that bacteria be cultured invitro

History of culture media

- Louis Pasteur used simple broths made up of urine or meat extracts
- Robert Koch realized the importance of solid media and used potato pieces to grow bacteria
- It was on the suggestion of Fannie Eilshemius, wife of Walther Hesse (who was an assistant to Robert Koch) that agar was used to solidify culture media

History of culture media

- Before the use of agar, attempts were made to use gelatin as solidifying agent. Gelatin had some inherent problems....
- It existed as liquid at normal incubating temperatures (35-37°C)
- Digested by certain bacteria

Agar

- Used for preparing solid medium
- Polysaccharide extract obtained from seaweed
- 2% agar is employed in solid medium
- Agar is an ideal solidifying agent as it ia:
- No nutritive value
- Bacteriologically inert i.e, no affected on the growth of bacteria
- Remains solid at 37⁰C
- Melts at 98⁰C
- It is transparent

Composition of culture media

- Provide similar environmental and nutritional conditions that exist in its natural habitat
- An artificial culture medium must provide all the nutritional components that a bacterium gets in its natural habitat
- A culture medium contains water, a source of carbon & energy, source of nitrogen, trace elements and some growth factors
- The pH of the medium must be set accordingly

Classification of Media

- Bacterial culture media can be classified based on
 - 1.Consistency
 - 2.Nutritional component
 - 3.Functional use

Classification of Culture Media

I. Based on the consistency

- a) solid medium
- b) liquid medium
- c) semi solid medium

II. Based on the ingredients

- a) simple medium
- b) complex medium
- c) synthetic or defined medium
- d) Special media

III. Based on functional use or application

1) Basal media

2) Special media

- Enriched media
- Selective media
- Enrichment media
- Differential media
- Transport media
- Indicator media
- Anaerobic media

Classification based on consistency

A) Solid media:

- An agar plate is a Petri dish that contains a growth medium (typically agar agar plus nutrients) used to culture microorganisms.
- Agar is the most commonly used solidifying agent
- Used for separating mixed bacteria
- Bacteria can be identified basing on colony character
- Used for the isolation of bacteria as pure culture
- Colony morphology, pigmentation, hemolysis can be appreciated.
- Eg: Nutrient agar, Blood agar

B) Liquid media:

- Contains no agar
- Used for profuse growth, inoculum preparation
- Eg: Nutrient broth
- Available for use in test-tubes, bottles or flasks
- Referred to as broths (e.g nutrient broth)
- Bacteria grow uniformly producing general turbidity
- Mixed organisms cannot be separated

C) Semi-solid agar:

- Contains 0.5% agar
- fairly soft and useful in demonstrating bacterial motility and separating motile from non-motile strains
- Eg: SIM

Different Types of Media

- **Basal/Simple media:** Basal media are basically simple media that supports most non-fastidious bacteria
- Ex: Peptone water, nutrient broth and nutrient agar
- NB consists of peptone, yeast extract, NaCl
NB + 2% agar = Nutrient agar

- **Complex media**

- Media other than basal media
- They have added ingredients
- Provide special nutrients
- specially prepared for research purposes
- composition of every component is well known.

- **Synthetic or defined media**

- Media prepared from pure chemical substances and its exact composition is known
- Eg: peptone water – 1% peptone + 0.5% NaCl in water

- **Enriched media**

- Enriched media are media that have been supplemented with highly nutritious materials such as blood, serum, egg yolk or yeast extract for the purpose of cultivating fastidious organisms.
- Addition of extra nutrients to basal medium makes them enriched media
- Used to grow bacteria that are exacting in their nutritional needs
- Ex: Chocolate agar, Blood agar

- **Enrichment media**

- Liquid media used to isolate pathogens from a mixed culture
- Media is incorporated with inhibitory substances to suppress the unwanted organism
- Eg: Selenite F Broth –for the isolation of Salmonella, Shigella
- Alkaline Peptone Water – for Vibrio cholerae

- Enriched media contains the nutrients required to support the growth of a wide variety of organisms including some fastidious ones
- Used to grow as many different types of organisms as are present in the specimen
- Enrichment media promotes the growth of a particular organism by providing it with essential nutrients and rarely contains certain inhibitory substances to prevent the growth of normal competitors
- Ex: Selenite F Broth –favors the growth of Salmonella and also prevent the growth of normal competitors like E.coli.
- E.coli does not die in the medium but they donot flourish like salmonella does

Blood agar and Chocolate agar



Blood agar



Chocolate agar



- **Selective media enrichment media**
- Selective media allows the growth of certain type of organisms, while inhibiting the growth of other organisms.
- Designed to inhibit unwanted commensal or contaminating bacteria and help to recover pathogen from a mixture of bacteria
- This selectivity is achieved in several ways. For example, organisms that have the ability to utilize a given sugar are screened easily by making that particular sugar the only carbon source in the medium for the growth of the microorganism.
- Like wise Inhibitory substance is added to solid media
- Any agar media can be made selective by addition of certain inhibitory agents (antibiotics, dyes, chemicals, alteration of pH or a combination of these) that don't affect the pathogen.

- **Examples of Selective media**

- **Thayer Martin Medium** selective for *Neisseria gonorrhoeae*
- **EMB agar:** selective for gram-negative bacteria. The dye methylene blue in the medium inhibits the growth of gram-positive bacteria; small amounts of this dye effectively inhibit the growth of most gram-positive bacteria
- nutrient agar supplemented with the antibiotic penicillin can be used to select gram negative bacteria
- **Campylobacter Agar (CAMPY):** selective isolation of *Campylobacter jejuni* from fecal or rectal swabs
- LJ medium – *M.tuberculosis*
- Wilson and Blair medium – *S.typhi*
- Potassium tellurite medium– *Diphtheria bacilli*
- Mac Conkey's medium for gram -ve bacteria

Differential media

- Differential media are widely used for differentiating closely related organisms or groups of organisms
- Media are designed in such a way that different bacteria can be recognized on the basis of their colony colour
- Because of the presence of certain dyes, metabolic substrates, or chemicals in the media, those bacteria that utilize them appear as differently coloured colonies
- The organisms will produce certain characteristic changes or growth patterns that are used for identification or differentiation of microorganism.
- Ex: Mac Conkey agar, Eosin Methylene Blue (EMB) agar

- **Differential media**

- A media which has substances incorporated in it enabling it to distinguish between bacteria
- Eg: Mac Conkey's medium Distinguish between lactose fermenters & non lactose fermenters
- Lactose fermenters – Pink colonies
- Non lactose fermenters – colourless colonies

McConkey agar

- Selective medium for Gram negatives
- Differential medium for lactose fermenter bacteria
- Positive for lactose fermentation = Colonies bright pink
- Negative for lactose fermentation = colorless colonies







- **Anaerobic media**

- These media are used to grow anaerobic organisms
- Eg: Robertson's cooked meat medium, Thioglycolate medium

- **Transport media**

- Media used for transporting the samples
- Delicate organisms may not survive the time taken for transporting the specimen without a transport media
- Eg: Stuart's medium – non nutrient soft agar gel containing a reducing agent
- Buffered glycerol saline- enteric bacilli

- **CHOCOLATE AGAR:** Non-selective, enriched growth medium. containing red blood cells that have been lysed by slowly heating to 80 °C
- Used for growing fastidious bacteria, such as *Haemophilus influenzae*
- **BLOOD AGAR:** Contains mammalian blood (usually sheep or horse), typically at a concentration of 5– 10%
- Blood agar plates are enriched, differential media used to isolate fastidious organisms and detect hemolytic activity

- **Enrichment media** liquid media that also serves to inhibit commensal in the clinical specimen. Selenite F broth and alkaline peptone water are used to recover pathogens from fecal specimens

Mannitol Salt Agar (MSA)

- Mannitol salt agar is both a selective and differential media used for the isolation of pathogenic *Staphylococci* from mixed cultures.
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- **Components:**
- **7.5% NaCl** – selects for species of *Staphylococcus*. This concentration of salt is too high for most other bacteria to withstand and , therefore, inhibits their growth.
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- **Mannitol** – alcohol of the carbohydrate mannose. Mannitol fermentation produces acid end products which turn the medium yellow. Yellow indicates mannitol positive and no color change indicates mannitol negative.
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- **Phenol red pH indicator** – yellow in acid pH (The same indicator that is used in phenol red carbohydrate fermentation broths).

Blood Agar

- A nutrient culture medium that is enriched with whole blood and used for the growth of certain strains of bacteria.
- Contains mammalian blood (usually sheep or horse), typically at a concentration of 5–10%.
- **BAP are an enriched, differential media used to isolate fastidious organisms and detect hemolytic activity.**
- β -hemolytic activity will show complete lysis of red blood cells surrounding colony,
- while α -hemolysis will only partially lyse hemoglobin and will appear green.
- γ -hemolysis (or *non-hemolytic*) is the term referring to a lack of hemolytic activity.



Different types of hemolysis on Blood Agar

Enriched, selective, and differential media help establish the presence of pathogens

- Many selective media are also differential media

