Al- Mustaqbal university collage
Department of radiology
technologies
1.St stage
Lecture 7

Culture Media & Smearing

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Bacterial Cell Wall

Any bacterial cell whether it is a coccus or a bacillus will have some structures common.

These structure are cell wall, cell membrane, cytoplasm, ribosomes and the chromosome.

Other intra-cellular structures such as plasmid, inclusion bodies and extra-cellular structures such as capsule, fimbriae and flagella are possessed only by some bacteria.

A gelatinous polysaccharide or polypeptide outer covering of certain bacteria is called glycocalyx, that surround the outside of the cell envelope.

Bacterial Cell Wall

The chemical nature of bacterial capsules is diverse but majority of them are polysaccharides, these polymers are composed of repeating oligosaccharide units.

However, the capsule of *Bacillus anthracis* is composed of polypeptide (polyglutamic acid). While *Yersinia pestis* produces a capsule of mixed amino acids.

Capsules may be weakly antigenic to strongly antigenic, depending on their chemical complexity.

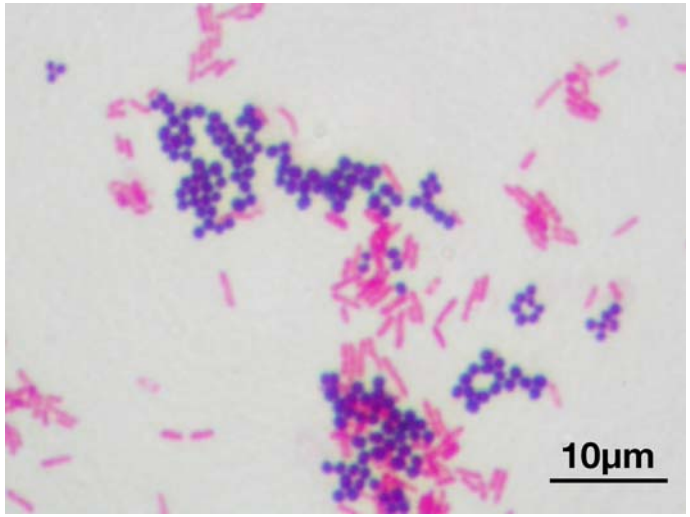
Bacterial Cell Wall

Based on the how the bacteria reacts to the Gram stain, there are two main categories of bacterial infections:

1. **Gram-positive**
2. **Gram-negative**

A Gram stain is colored purple so when the stain combines with bacteria in a sample, the bacteria will:

- ✓ **either stay purple (Gram positive)**
- ✓ **or turn pink or red (Gram negative)**



Gram-positive Bacterial Cell Wall

Most gram-positive cell walls contain additional substances such as teichoic acid and teichuronic acid, These are water soluble polymers of ribitol or glycerol.

There are two types of teichoic acid, wall teichoic acid (linked to peptidoglycan) and lipoteichoic acid (linked to membrane).

Some gram-positive bacteria may lack wall teichoic acid, but all contain lipoteichoic acid.

The teichoic acid constitutes major antigens of cells that possess them. Teichoic acid binds to Magnesium ions and plays a role in supply of this ion to the cell.

Teichuronic acids are produced in place of teichoic acid when phosphate is limiting.

Gram-negative Bacterial Cell Wall

Gram negative cells consist of a relatively thin layer of peptidoglycan (approximately 10 nm) and do not retain the primary dye in Gram stain and hence appear pink.

Gram negative bacteria have an additional outer membrane, that is the major permeability barrier in Gram negative bacteria.

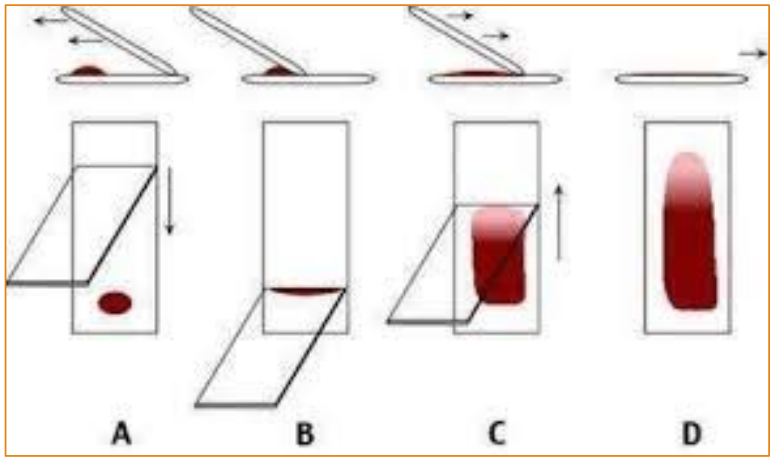
The space between the inner and outer membranes is known as the periplasmic space, which contains digestive enzymes and other transport proteins

Smear Preparation

The process of making a smear preparation is an important skill in the microbiology laboratory and is usually the first step in most staining procedures.

The quality of the smear will directly affect the quality of the subsequent staining procedure.

The smear preparation differs slightly depending on the specimen or culture.



Smear Preparation Requirements

1. **Personal protective equipment**
2. **Sharps container**
3. **Biological waste container**
4. **Microscope slides with frosted-edge**
5. **Pencil or wax pencil**
6. **Sterile saline or water**
7. **Sterile pipettes**
8. **Loops or applicator sticks**
9. **Slide warmer, Bunsen burner, or methanol**

Culture Media

Culture media are mediums that provide essential nutrients and minerals to support the growth of microorganisms in the laboratory.

Microorganisms have varying nature, characteristics, habitat, and even nutritional requirements; thus, it is impossible to culture them with one type of culture media.

However, there are also microorganisms that can't grow on a culture media at all in any condition – these are called obligate parasites.

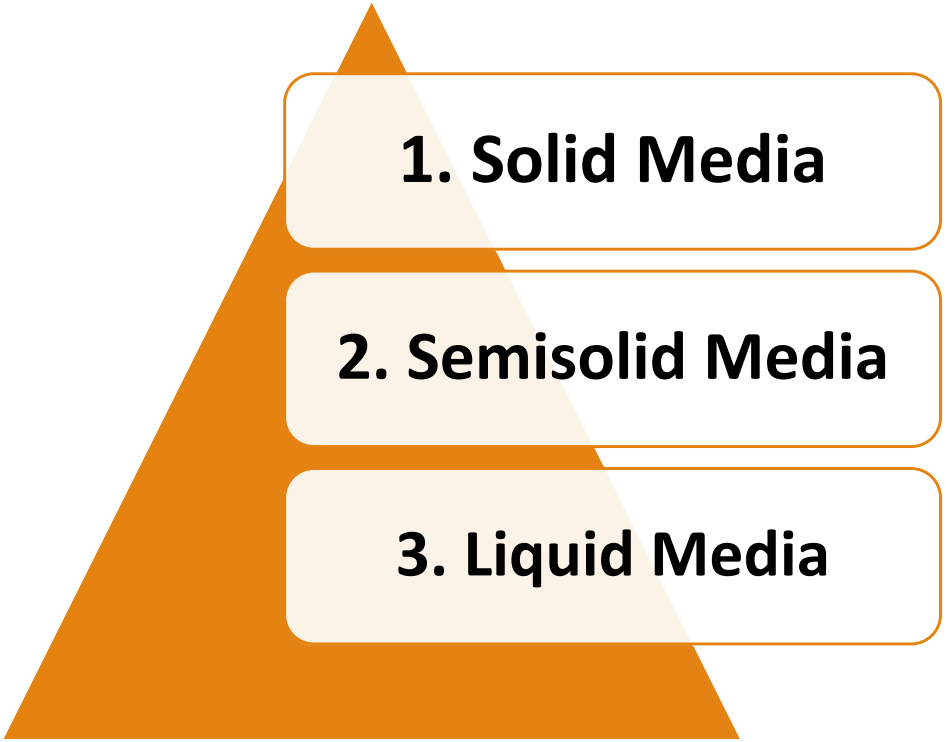
Culture Media

Culture Media

Culturing microorganisms is essential for diagnosing infectious diseases, obtaining antigens, developing serological assays for vaccines, genetic studies, and identification of microbial species.

Furthermore, it's also essential for isolating pure cultures, storing culture stock, studying biochemical reactions, testing microbial contamination, checking antimicrobial agents, and testing antibiotic sensitivity.

General Types of Culture Media



Solid Media

In these media, the agar which is an unbranched long chain of polysaccharides is added in the concentration of 1.5-2.0%.

The agar-containing media solidifies at 37 ºC. Sometimes, in the place of agar, some other inert solidifying agents are used, such as gellan gum.

Solid media are used to:

1. **Grow microorganisms in their full physical form**
2. **Prepare bacterial pure cultures,**
3. **Isolate bacteria to study colony characteristics**

Solid Media

The bacterial growth on solid media varies in appearance as mucoid, round, smooth, rough, filamentous, irregular, and punctiform.

The media is not hydrolyzed by microorganisms and is free from growth-inhibiting substances.

Examples of solid media are blood agar, nutrient agar, McConkey agar, and chocolate agar.

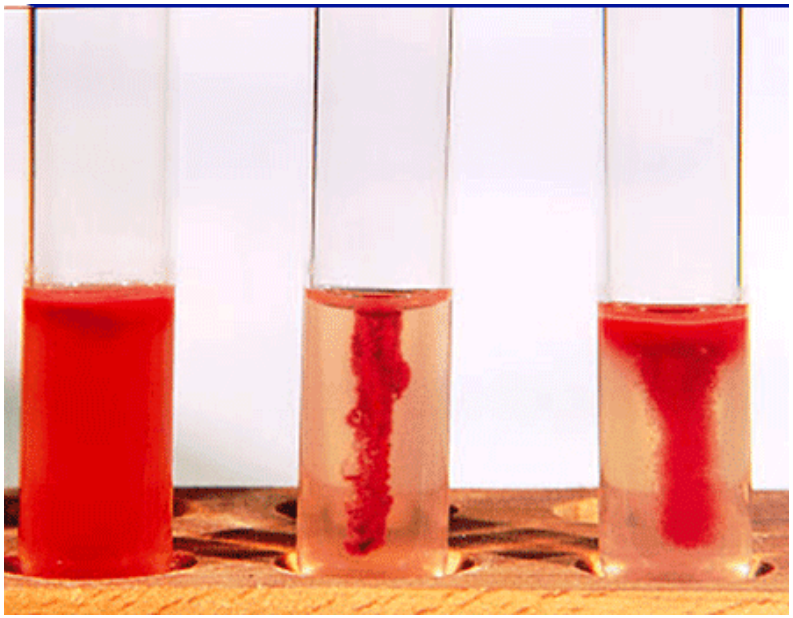
Blood Agar

Semisolid Media

This media has 0.2-0.5% agar concentration, and due to the reduced agar concentration, it appears as a soft, jelly-like substance.

It's mainly used to study the motility of microorganisms, distinguish between motile and non-motile bacterial strains (through U-tube and Cragie's tube), and cultivate microaerophilic bacteria – bacteria on this media appear as a thick line.

Examples of semi-solid media are Stuart's and Amies media, and Mannitol motility media.



Mannitol Motility Media

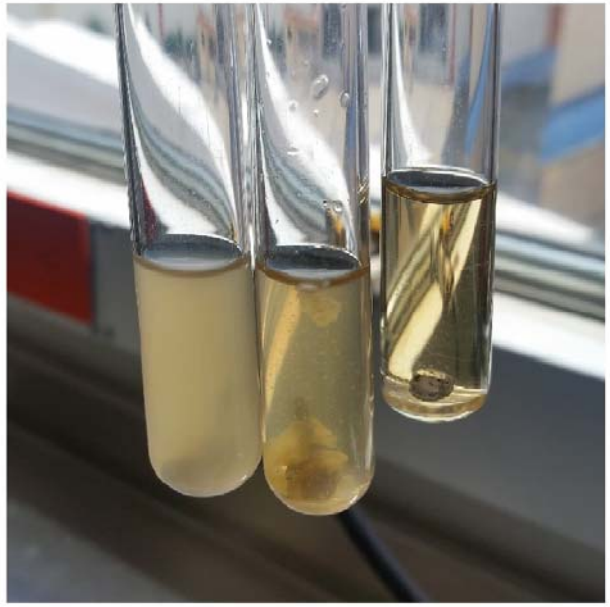
Liquid Media

These media do not contain any traces of solidifying agents, such as agar or gelatin, and large growth of bacterial colonies can be observed in the media.

Liquid media are also called broths, they allow for uniform and turbid growth of bacterial strains when incubated at 37ºC for 24hrs.

The media is used for the profuse growth of microorganisms and fermentation studies.

Examples include Tryptic soy broth, phenol red carbohydrate broth, MR-VP broth, and nutrient broth.



Tryptic Soy Broth

Classification of culture media based on application/chemical composition

1. Basal media:

These are routinely used simple media having carbon and nitrogen sources that boost the growth of many microorganisms. They are also known as general-purpose media and are considered non-selective media.

The basal media do not require enrichment sources for the growth of non-fastidious bacteria and are suitable for growing Staphylococcus and Enterobacteriaceae.

They are generally used to isolate microorganisms in labs or in sub-culturing processes. Examples are nutrient broth, nutrient agar, and peptone water.

Classification of culture media based on application/chemical composition

2. Enriched media:

This media is prepared by adding additional substances like blood, serum, or egg yolk in the basal medium. It's used to grow fastidious microorganisms as they require additional nutrients and growth-promoting substances.

Examples are chocolate agar, blood agar, and Loeffler's serum slope.

Chocolate media is used to grow *N. gonorrhea* while blood agar (which is prepared by adding 5-10% blood by volume to a blood agar base) is used to identify hemolytic bacteria.

Classification of culture media based on application/chemical composition

3. Selective media:

This media allows the growth of certain microbes while inhibiting the growth of others.

It's an agar-based medium that is used to isolate microorganisms in labs.

The selective growth of microbes is decided by adding substances like antibiotics, dyes, bile salts, or by pH adjustments.

THANK YOU FOR YOUR ATTENTION