Practical No. 2

Culture Media and Laboratory Equipments

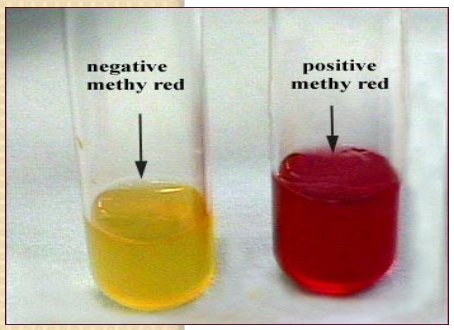
Department of Microbiology
College of Medicine

Culture Media

- Culture media
- Types of culture media
- Culture media components
- Preparation of culture media

Advantages of culture media

- To culture microorganisms.
- For pure culture isolation.



- For storage of stock cultures.
- To observe specific biochemical reactions.

- As transport media to preserve bacteria during transportation to the laboratory.
- For preparation of antigens (vaccines and diagnostic kits).

- Knowing the microscopic morphology and staining characteristics of a microorganism present in a clinical specimen, the microbiologist can make appropriate decisions as to how it should be cultivated and what biologic properties must be demonstrated to identify it fully.

- A suitable culture medium must be provided, and it must contain the nutrients essential for the growth of the microorganism to be studied.
- Most media designed for the initial growth and isolation of microorganisms are rich in protein components derived from animal meats.
- Many bacteria are unable to break down proteins to a utilizable form and must be provided with extracted or partially degraded protein material (peptides, proteoses, peptones, amino acids).
- Meat extracts or partially cooked meats are the basic nutrients of many culture media. Some carbohydrate and mineral salts are usually added as well. Such basal media may then be supplemented, or enriched, with blood, serum, vitamins, other carbohydrates and mineral salts, or particular amino acids as needed or indicated.

- **Types of Media used in Bacteriology laboratories:**

There are several types of media used in bacteriology labs. Several items or constituents could be added or removed from the routinely used media according to the purpose for use.

- **Media could be classified according to their composition;**

1. Natural medium: which contains natural constituents, such as; yeast extract, meat extract, peptones….etc. where the actual contents and their concentrations are not known.

2. Synthetic medium: This type of medium is prepared manually and the actual contents and their concentrations are exactly known.

3. Semi-synthetic medium: The contents of this medium are mainly synthetic with known concentrations, but is supplemented with some natural contents in order to enhance growth or for certain physiological studies. This is used for isolation as well as for physiological studies.

- **Types of media according to their solidity;**

1. Solid media: These types of media contains the actual concentration of agar (which is 2%) which makes the media solid, therefore encourages the formation of colonies on its surface and could be used for isolation and purification of mixed samples or cultures.

2. Liquid media: These types of media do not contain agar at all, and therefore the media remain in liquid form where the bacteria grow better and faster.

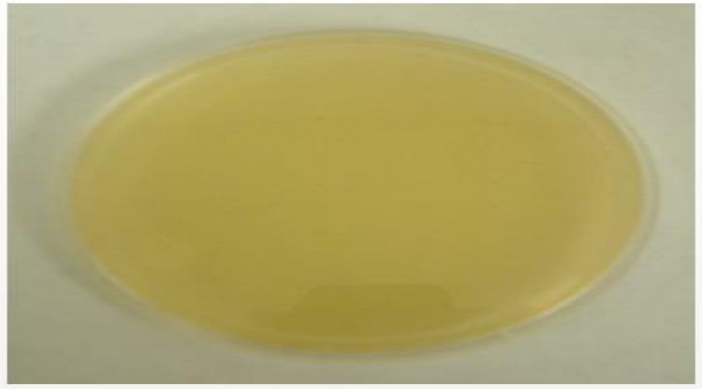
3. Semisolid medium: This type of medium contains half the actual concentration of agar (1% only), and is used mainly for studying motility of bacteria, since the agar molecules are not so condensed, therefore it will encourage the motile bacteria to pass through these molecules and go far from the inoculated area forming what is called brush –like growth around the stabbed area.

According to solidity or physical properties, media can be classified three types:

1. **solid medium (2 % of agar)**

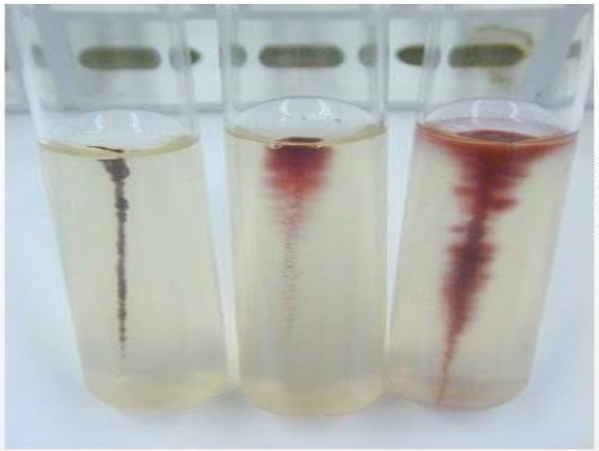
   agar plate /slant medium

2. **semi-solid medium (1 % of agar)**
3. **liquid medium(do not contain agar)**



SOLID

LIQUID



SEMI SOLID

Types of media according to their function

1. Isolation medium: This type of medium **all nutrients** that are required by most bacteria, and is used for growth of unknown bacteria **contains** from different samples.

Ex. nutrients agar

2. Selective medium: This type of medium **contains certain inhibitors** that will inhibit the growth of unwanted bacteria and do not affect the growth of the desired bacteria which will make the isolation and purification much easier and faster.

Ex. SS agar

3. Differential medium: In such type of medium, certain **chemicals or indicators** are added in order to differentiate between two groups of bacteria grown on this medium without needing to do further test for such purpose which will save time and efforts.

Ex. MacConkey agar

Isolation medium

All nutrients used for growth of unknown bacteria.

e.g. nutrients agar

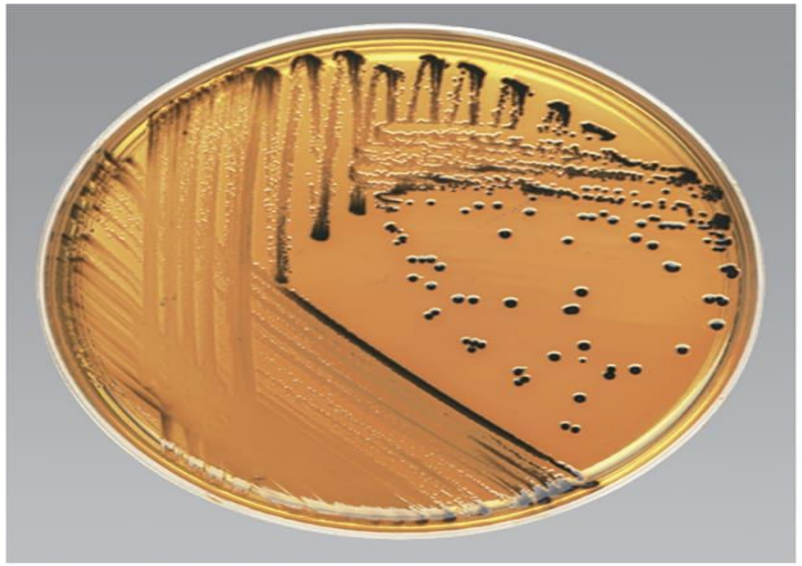
Figure 1: *S. bovis* grown on nutrient agar magnification 4.1X

nutrients agar

Selective Medium

the medium that can prevent the certain bacterial growth while permitting others.

e.g. SS agar (Salmonella Shigella agar)

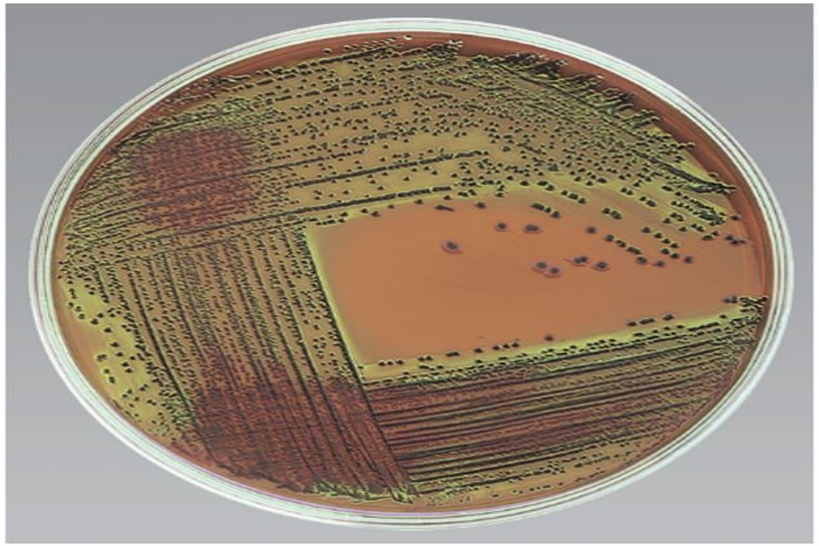


Salmonella Shigella agar

Differential Medium

Some special substrates and indicators are added into the media in order to produce a visual differentiation

e.g. EMB agar (Eosin-methylene blue agar).



Eosin-methylene blue agar

Maintenance medium

Cooked-meat medium is used for the preservation of anaerobic bacteria.

Cooked-meat medium

4. Maintenance medium: This type of medium contains certain nutrients that allow slow growth of bacteria, therefore it will stay longer time before they reach the stationary or the death phase. This type of medium is used for preservation of bacteria for longer time (1-2 months) before doing the subculture.

e.g. Cooked-meat medium is used for the preservation of anaerobic bacteria.

5. Enriched medium: In this medium certain nutrients or factors are added in order to allow specific bacteria to grow, because without such factors that bacteria will not grow. Such as the addition of blood, serum, hemolized blood….etc.

e.g. Blood agar

6. Biochemical test media: There are several types of such media where different biochemical tests are applied depending on the type of the medium and its contents. Such as the fermentation tests, enzyme activity, degradation of certain compounds….etc.

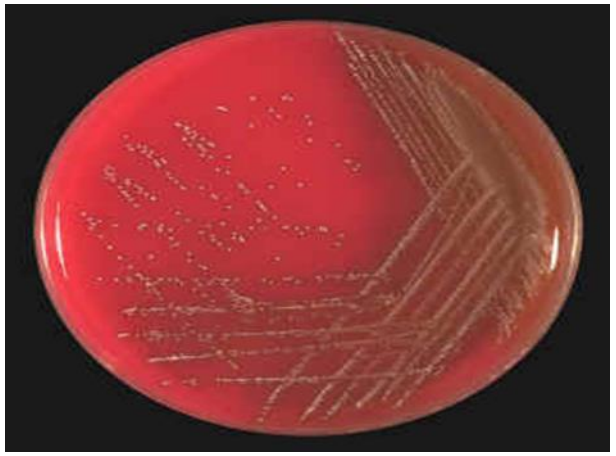
7. Antibiotic sensitivity test medium: This type of medium allows the growth of most bacteria and do not contain any growth inhibitors or materials that may interfere with the action of the antibiotics.

e.g. Müeller-Hinton agar

Enriched Medium

Additional or special nutrients (e.g., serum, growth factors, trace elements) are added to support some fastidious bacterial growth.

e.g. blood agar.

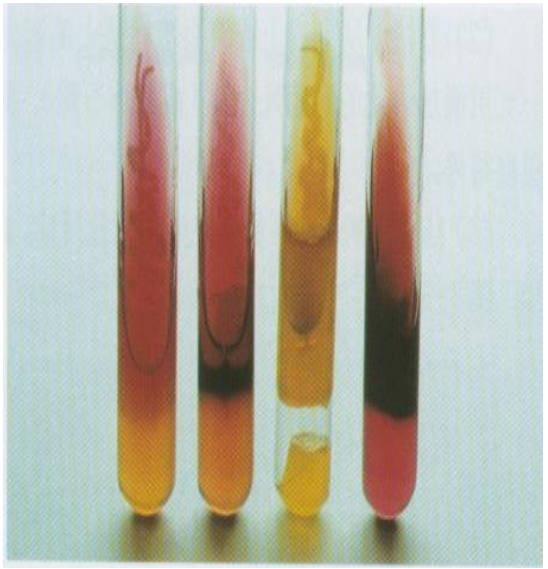


Biochemical test:

different compound.

e.g. Simmon citrate agar , Triple sugar iron slant

Simmon citrate agar



Triple sugar iron slant

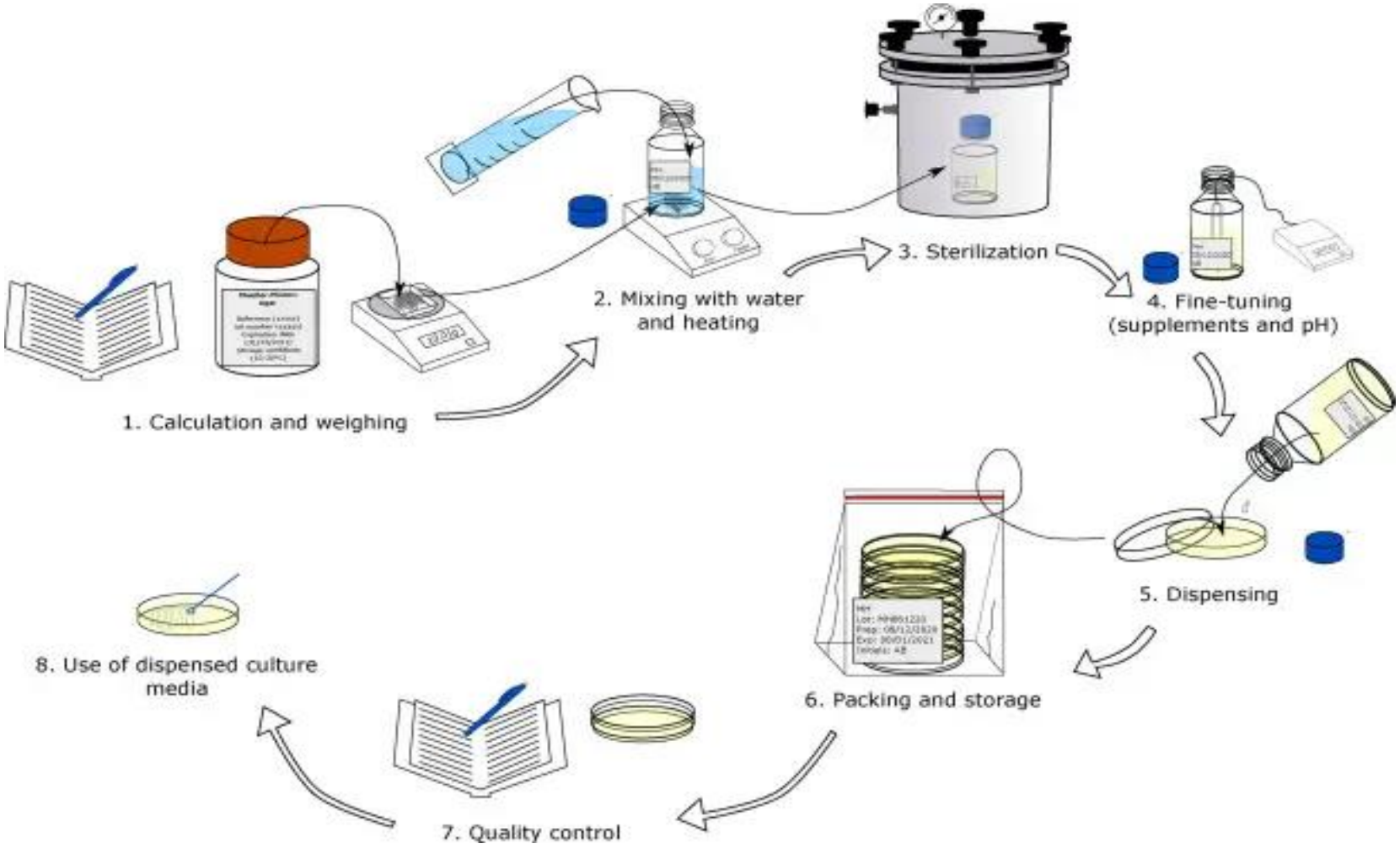
Antibiotic sensitivity test medium:

Allows the growth of most bacteria .

e.g: Müeller-Hinton agar is most frequently used in this antibiotic susceptibility test.

Müeller-Hinton agar

Preparation of Culture Media



Instruments and equipment's

Instruments and equipment's usually used in bacteriology laboratories;

1. **Microscope (with at least 1000X)**
2. **Incubator**
3. **Hot air oven**
4. **Autoclave**
5. **Anaerobic jar**
6. **Microflow (laminar flow)**
7. **Fume hood**
8. **Hot plate with magnetic stirrer**
9. **Electronic Balance**
10. **Top plate balance**
11. **Shaking Incubator**
12. **Shaking water bath**
13. **Bunsen burner**
14. **Wire loop & wire needle**

Equipments

1- Autoclave:- used for sterilization

equipment with high temperature, pressure and steam to sterilize the culture media and some of metal tools and glass wares.

- **Temperature: 121 C**
- **Pressure: 1 atm. (15 pound/1 itch)**
- **Time: 10 - 15 min.**
- **30 min. (for Petri-Dish cultured media and contaminated tools & glass wares)**
- **Sterilization by Autoclave called (Wet heat sterilization).**

Bacterial cell is killed by protein denaturation.

2-Hot air oven

Oven: used to sterilize the glass wares and some metal tools by (Dry heat sterilization).

Temp.: 180

Time: One hour & half.

3-Water bath :- It's a laboratory equipment made from a container filled with heated water .

- It is used to incubate samples in water at a constant temperature over a long period of time.

4-Incubator

used to provide suitable conditions for microorganism growth temperature , Co2, humidity and oxygen content of the atmosphere inside.

5- Refrigerator

Used to maintain the sterilized media and broth when not used to avoid the contamination , and also to preserve the bacterial culture for long time by preventing the growth at 4C°.

6-Biosafety cabinet (BSC)

Also called a biological safety cabinet or is an enclosed, ventilated laboratory workspace for safely working with materials contaminated with (or potentially contaminated with pathogens).

7- Centrifuge

A machine with a rapidly rotating container that applies centrifugal force to its contents.

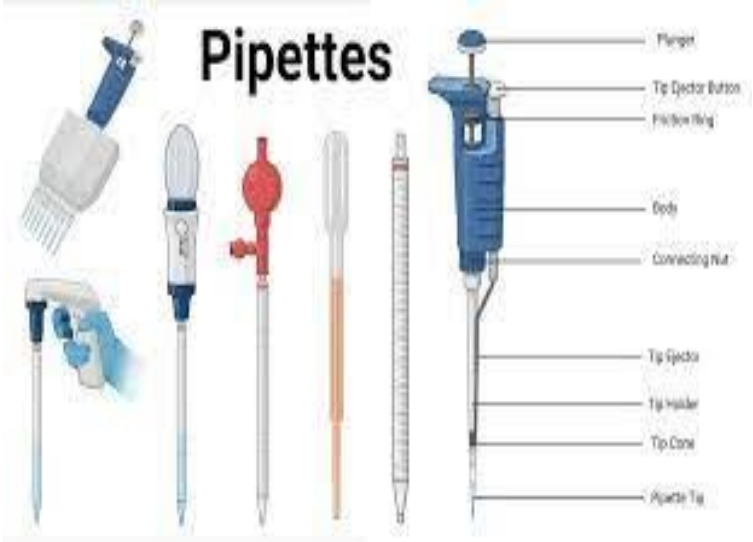
Used for separating substances of different densities.

e.g. to separate red blood cells and other blood components from whole blood.

Instrument

1. Loop: used for transfer of bacterial cells from medium to another, sterilized by the flame of burner.

2. Pipette: used for transfer of cultured and uncultured broth from tube or flask to other, and placed in the Can. Sterilized in Autoclave.



4 - Petri-Dish(Petri-plate):-

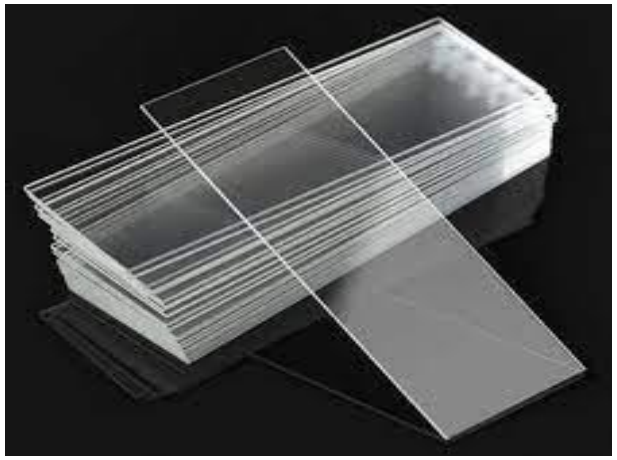
Used for place the solid medium in it.

glass petri-dish used for many times and sterilized by oven or autoclave while sterilized plastic plates used for one time.

5 - Spreader: used for spreading bacterial cells on the surface of solid medium in Petri dish, sterilized by the flame of burner.

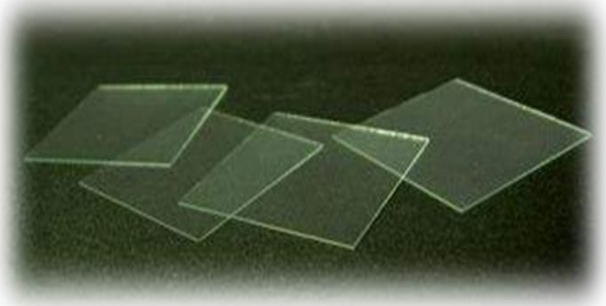
9- Slide

Used for examination of bacterial smear under microscope , it used for one time.



10- Cover-Slips

Placed on the slide , the bacterial smear may be between the cover and the slide, it used for one time.



11- Flask

Used for place cultured and uncultured broth in it , sterilized after plugs with cotton by autoclave.

13- Beaker

Used for graduated the volume of liquids. Sterilized by oven.

14- Cylinder(Graduated Cylinder)

Used for graduated the volume of liquids, sterilized by oven.

15- Washing bottle

Used to fill with liquid (specially distilled water)for washing and homogenizing the glass wares and washing the slide during staining , don’t need sterilization.

16- pH paper

Used to know the pH of the medium or any liquids

17- Rack

May be wooden , metallic or plastic used to stand and hold the tube.

18- Burner

May be gaseous or alcoholic , used for sterilized the loop , needle and other metallic tools by flame (dry heat sterilization).

Thank You