Lec(1) Microbiology 4/10/2010

Microscope

Microscope: Micro =small , scope =view

Bright Field Microscope:-

1- Commonly used in bacteriology laboratory.

2- It depends on light.

3- It consider as compound microscope, why ?

Bright Field Microscope components

1- Base:- The bottom support of the microscope.

2- Arm :-It helps in holding the microscope.

3- Light source:- A light source mounted under the stage.

4- Body tube :- It hold the projector lenses that direct the light toward the ocular lenses.

5- Nosepiece :- Hold the objectives lenses. (movable disk).

6- Coarse adjustment :-Used to obtain primary explaining specimen.

7- Fine adjustment :- Used to obtain final and fine explaining specimen.

8- Stage:- The flat plate where the slides are placed for observation.

9- Stage Clips:- Clips on the stage used to hold the slide in place.

10- Condenser :- Focuses the light through the specimen.

11- Iris diaphragm :-Vary the amount of light passing through the stage opening.

12-Condenser adjustment knob :- Used to move the condenser up and down.

13- Objective lenses :- primary magnification. (4 x, 10 x, 40 x, 100 x) .

14 - Ocular lenses : final magnification (Eye Pieces)x 10.

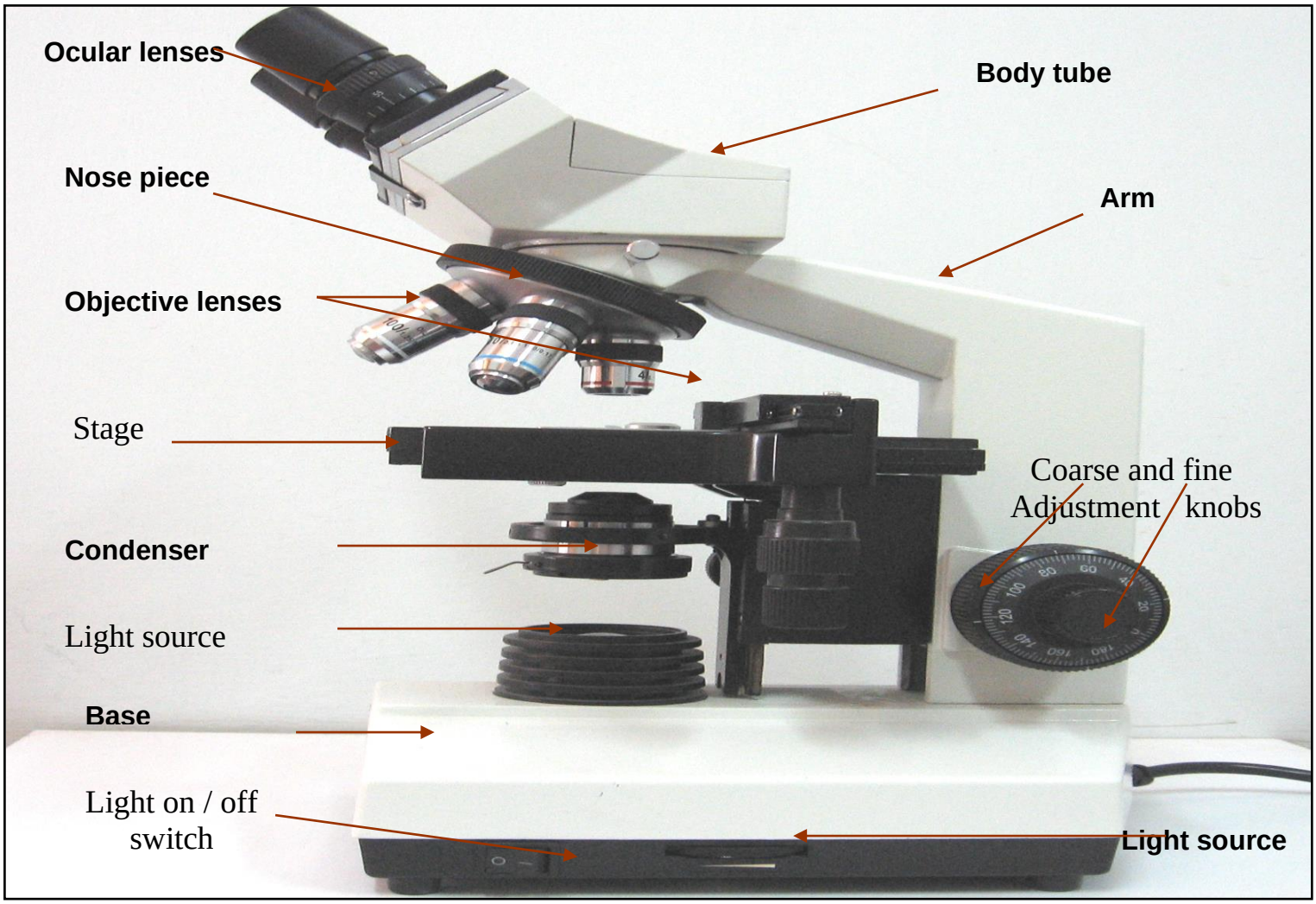

Figure 1.1 **Bright Field Microscope**

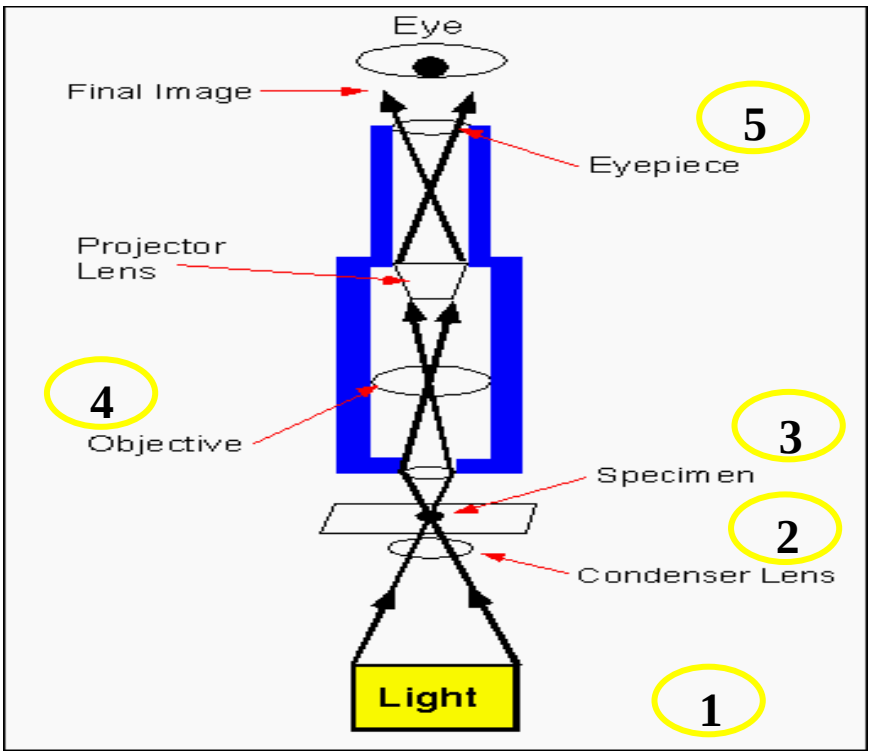

Figure 1. 2**Mechanism of Light pathway**

Other types of the microscopes

1- Dark field microscope.
2- Phase- contrast microscope.
3- Fluorescent microscope
4- Electron microscope
5- Scanning electron microscope

Type of Microscope	Features	Best Used for
Bright field	Uses visible light	Observing dead stained specimens and living organisms with natural color
Dark-field	Uses special condenser allowed the light rays to pass through terminal reflect off the specimen therefore the bacteria appearance bright on the ground dark.	Observing living organisms
Phase - contrast	Uses a condenser that increases contrast between the bacteria and the surrounded media .	Observing cell wall and larger structure in cytoplasm.
Fluorescent	Uses mercury lamb sources to ultraviolet light and special fluorescent dyes conjugated with antibody. The bacteria appearance bright fluorescent (yellow green).	Observing specimens or antibodies in clinical studies
Electron microscope	Uses electron beams and electromagnetic lenses to view thin slices of cells.	Observing virus and smallest parts of bacteria such as flagella and cell wall.
Scanning electron microscope	Uses electron beams and electromagnetic lenses	Giving a three-dimensional view of exterior surfaces of cells

Figure2.1 **Dark field Microscopy**

Figure2.2 **Phase-Contrast Microscopy**

Figure2.3 **Fluorescence Microscopy**

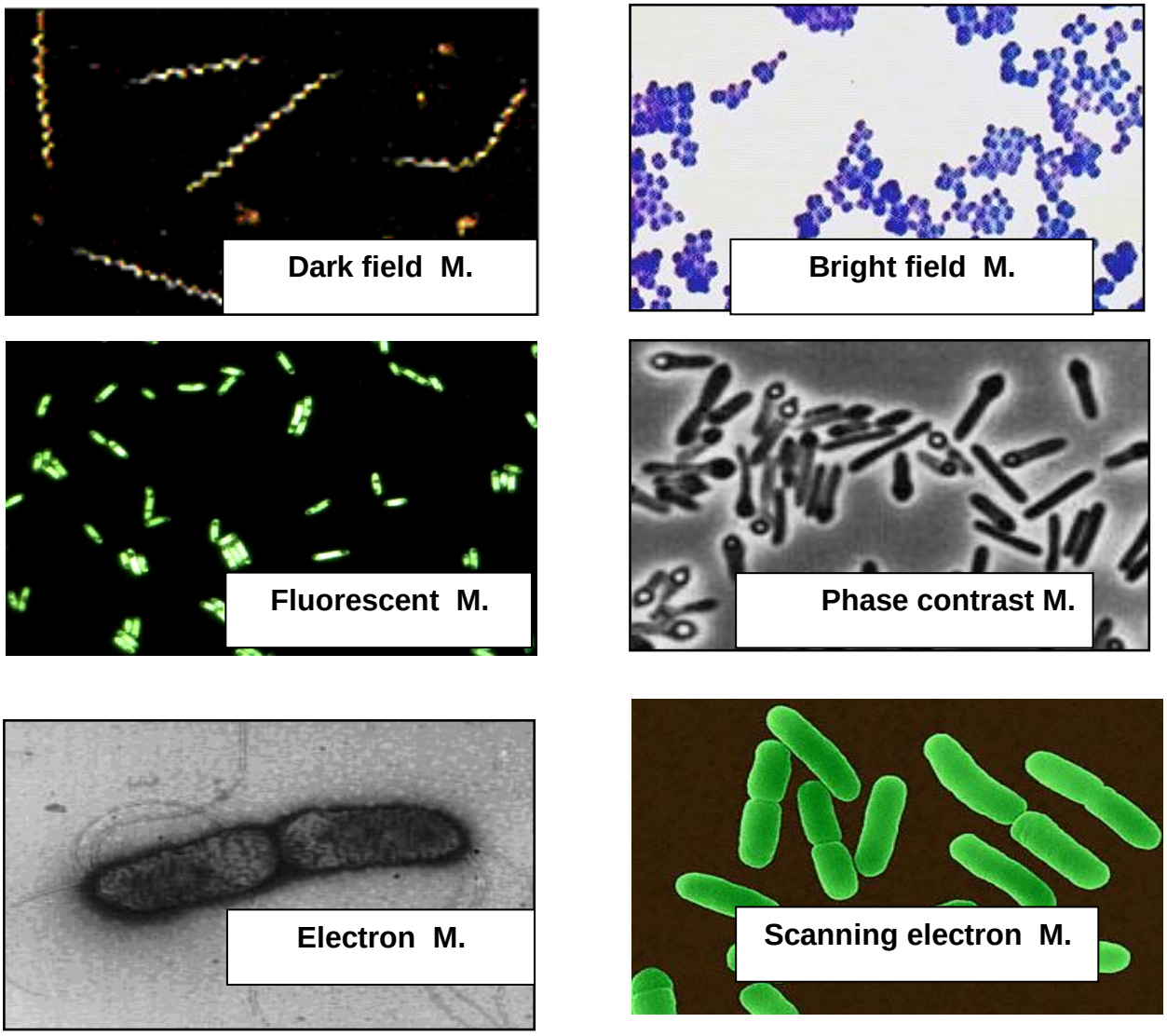

Figure1.6 **Microscopic picture by used types of microscopes**

Lec(2) Microbiology /10/2010

Culture media

A culture medium (*media,* plural):- is nutrient material prepared in the laboratory for the growth of bacteria, molds, and other microorganisms.

Agar:- is a complex polysaccharide derived from seaweed (red algae). The melting point is 97-100 °C and solidify at 42 °C. Usually agar is used in 1.5-2% (final concentration) to solidify the liquid medium. 0.5-1% to make the medium semisolid. 5% to decrease bacterial motility(prevents swarming, *proteus*).Gelatin medium (12-15%) is solid at 4 °C and Liquid at 25 °C.

Advantage of culture media are:-

1-For pure culture isolation.
2-For storage of stock cultures.
3-To observe specific biochemical reactions.
4-As transport media to preserve bacteria during transportation to the laboratory.
5-For preparation of antigens (vaccines and diagnostic kits).

Types of culture media:-

A- According to physical consistencies:

1- Liquid media.
2- Solid media.
3- Semi solid media.

B- According to the purpose of application:-

1- **Simple media**:- Contains the essential nutrients as source of nitrogen and carbon such as: Nutrient broth , Peptone water, nutrient agar.

2- **Differential media:-** are media that contain substances that cause some bacteria to take on a different appearance from other species, allowing one to differentiate one species from another, e.g.

a- **MacConkey agar:-** Differentiate between lactose fermenting and non-lactose fermenting bacteria.

b- **Blood agar:-** Differentiate between hemolytic and non-hemolytic bacteria.

3- Selective media:- are media that contains inhibiting materials for growth of some bacteria and at the same time it is activating for some other types, such as

a- bismoth sulphate agar:-used to isolate *Salmonella* .it contains bismuth sulphate which works as indicator, and also contain Brilliant green material which is used as inhibiting factor to other bacteria .

b- manitol salt agar:- used to isolate *Staphylococcus*, it contains high concentration of NaCl as inhibitor and manitol sugar which works as differential agent between staph. fermenting (yellow) and non-fermenting staph. (reddish)

c. Salmonella Shigella agar:-used to grow in Salmonella and Shigella, it contains bile salt and brilliant green agar are working as inhibitor and also it contains neutral red and thiosulphate to produce H_2S gas .

4- Enriched media:- are media that used to grow most types of bacteria, it contains organic compounds, vitamins, salts and yeast, such as

a. blood agar
b.chocolate agar (heated blood agar)
c. brain heart infusion agar
d. serum agar
e. extract animal tissue.

5- Transport media:- Simple media used for transport samples from different regions to the lab. e.g. Stuart transport medium

The names some of the Labroatory Culture media

1- Nutrient agar (simple, solid)
2-Nutrient broth (simple, liquid)
3- Peptone water (simple, liquid)
4-Gelatin medium (semi solid)
5-MacConkey agar (selective and differential)
6-Mannitol salt agar (selective and differential) for isolation of *staphyl*
7-Eosin Methylene blue agar (selective and differential) for isolation of *E. coli.*
8- Blood agar (enriched and differential)
9- Brain heart infusion agar (enriched, solid)
10- Brain heart infusion broth (enriched, liquid)
11- Salmonella Shigella agar (selective) for isolation of *Salmonella and Shigella.*
12- Brilliant green agar (selective) for isolation of *Salmonella.*
13- Lowenstein – Jensen medium (selective) for isolation of *Mycobacterium tuberculosis.*

Method of Preparation

1- Measure the amount of dehydrated medium that you need.
2- Dehydrated medium is dissolved in a measured amount of distilled water and pH adjusted.
3- Sterilize the medium using autoclave.
4- Cool after autoclaving.
5- Flame flask opening.
6- Pour in Petri dishes.
7- Flame medium surface.
8- Fame Petri dish cover.
9- Leave for cooling.
10- Put in special bags
11- keep in refrigerator.

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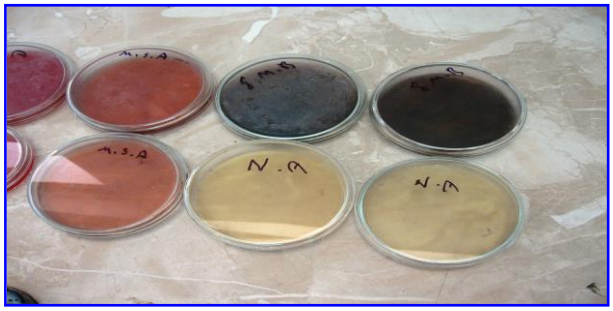

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A culture:- is growing of microorganisms on a culture medium.

Pure culture:- is growing of one type of microorganisms on a culture medium. To be able to study the cultural, morphological, and physiological characteristics of an individual species.

A colony :- is a large number of bacterial cells on solid medium, which is visible to the naked eye.

Subculturing:- is transferring of Microorganisms from one culture medium to another by using specific procedures.

Bacterial growth can be observed in three main forms:-

1-Bacterial growth in liquid media:-

These media are used for the propagation of large numbers of microorganisms.

1-Turbidity:- Most bacteria produce turbidity as a result of growth in liquid media like *E. coli*

2- Sediment formation:- *Staphylococcus*

3- Slime:- *Klebsiella*

4- Pellicle formation:- *Bacillus* .

5- Gas: -*E. coli*

6- Exopigmentation:- *Pseudomonas*

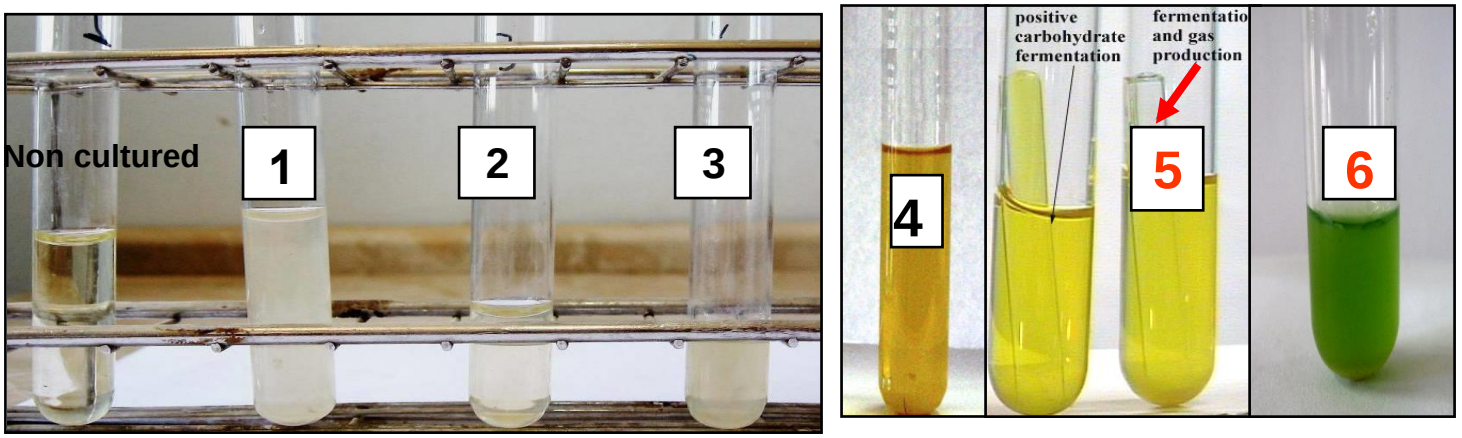


Figure3.1 **Bacterial growth in liquid media**

2. Bacterial growth on solid media:-

These media are used for developing surface colony growth of bacteria and molds when trying to isolate microorganisms from mixed cultures.

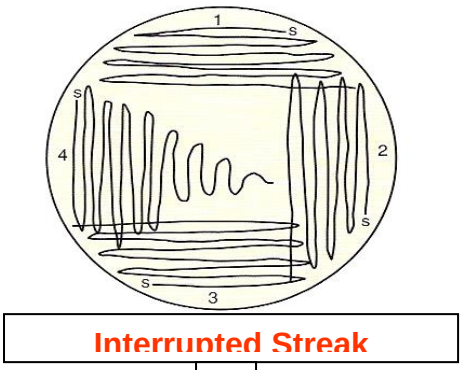
1- Streak – plate method.
2- Pour –plate method.
3- Spreading - plate method.

} Methods used for pure culture techniques

4-Agar – slop method:- A method used for preservation of bacterial stocks and performing biochemical tests.

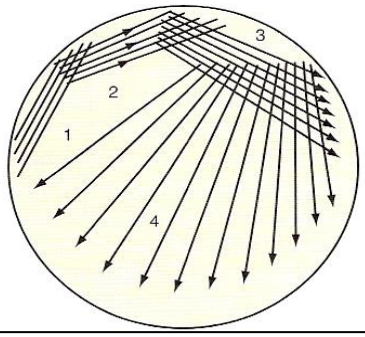
Streak Patterns:-

1- Interrupted Streak**.**
2-Cross Streak.
3-Radiant Streak.
4-Continuous Streak.



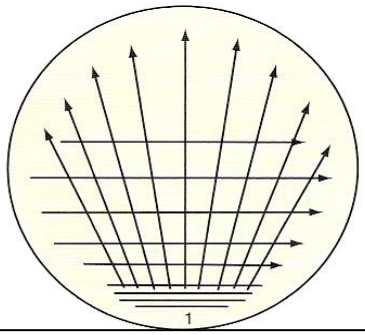
Interrupted Streak

1



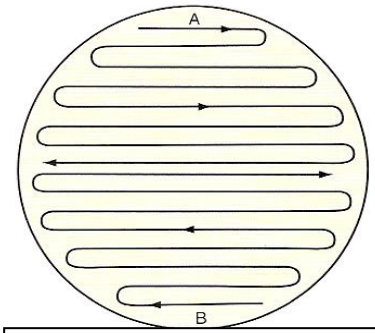
Cross Streak

2



Radiant Streak

3



Continuous Streak

4

Figure3.2 four different streak techniques

2. Pour - Plate Method:-

1-The original sample is diluted several times to decrease or dilute the population sufficiently.

2- 0.1 ml of each dilution is then dispensed into the bottom of a Petri plate.

3- Agar pours are then added to each plate.

4- The surface colonies are circular and large, subsurface colonies are lens shaped and much smaller.

3. Spreading - Plate Method:-

1- Pipette 0.1 ml of the diluted broth onto the surface of a plate of nitrate agar.

2- Spread the inoculum over the surface of the agar with a bent glass rod.

3- Incubate the plate, inverted, at 37° C for 24 hours.

4. Agar - Slop Method :-

1- The test tubes are held at a slant (angle less than 30°) and are allowed to solidify on an angle, called a **slant**. This method is used for increases the surface area for organism growth.

2- The test tubes are held at a slant (angle between 30°- 40°) and are allowed to solidify on an angle, called a **slant - butt.** This method is used for preservation of bacterial stocks and performing biochemical tests.

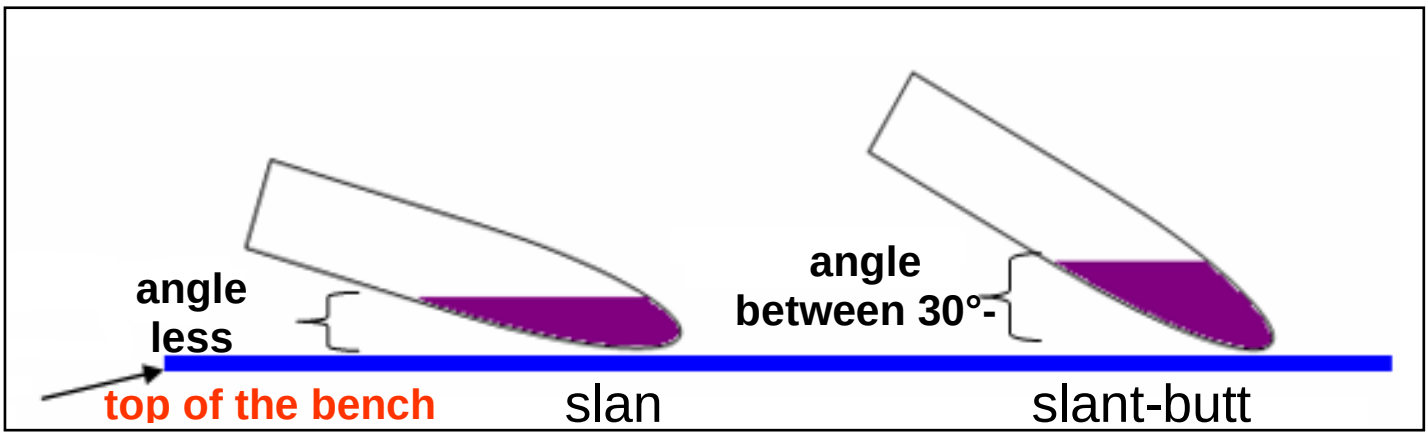


Figure3.3**Agar - Slop Method**

3. Growth in Semisolid Media:-

These media are used for:

1- Motility test, to Determine whether certain bacteria are motile.

2-Gelatin hydrolysis test, as certain bacteria have the ability to hydrolyze Gelatin.

A- Motile bacteria.

B- Non motile bacteria.

C- Uninoculated medium.