علوم الحياة / كورس الأول

2018 / 10/ 6

المادة: احياء مجهرية عملي / 1

المرحلة: الثانية

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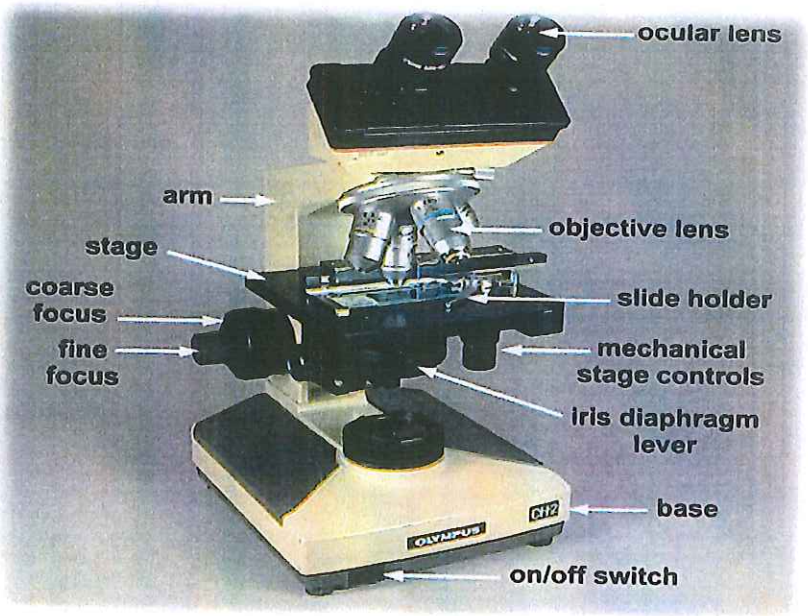
السعر (3500)

Lab-1- The Microscope

There are many types of microscopes. The most common (and the first to be invented) is the optical microscope, which uses light to image the sample. Other major types of microscopes are the electron microscope, the ultra microscope, and the various types of scanning probe microscope.

Compound light microscope:

It's widely used in microbiology laboratories .the limit of magnification is about 1000X, it consist of two systems:



1-The lens system: which consist of:

Eyepiece or Ocular lenses: *The lens the viewer looks through to see the specimen. The eyepiece usually contains a 10X or 15X power lens.*

Objective lenses: *One of the most important parts of a compound microscope, as they are the lenses closest to the specimen.*

A standard microscope has three, four, or five objective lenses

4X , 10X (Low) , 40X(high dry) and 100X(Oil)

2-Illumination system:

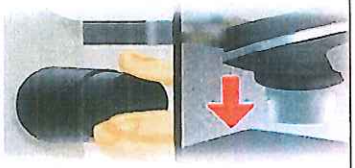
***Illumination**: The light source for a microscope. Older microscopes used mirrors to reflect light from an external source up through the bottom of the stage; however, most microscopes now use a low-voltage bulb.*

***Iris diaphragm**: Adjusts the amount of light that reaches the specimen.*

***Condenser**: Gathers and focuses light from the illuminator onto the specimen being viewed.*

Adjustment parts

Stage height adjustment (Stage Control): These knobs move the stage left and right or up and down.



***Coarse adjustment**: Brings the specimen into general focus.*

***Fine adjustment**: Fine tunes the focus and increases the detail of the specimen.*

Other parts:

***Nosepiece**: The viewer spins the nosepiece to select different objective lenses.*

***Specimen or slide**: The specimen is the object being examined.*

Most specimens are mounted on slides, flat rectangles of thin glass.

***Stage**: The flat platform where the slide is placed.*

***Stage clips**: Metal clips that hold the slide in place.*

***On/off switch**: This switch on the base of the microscope turns the illuminator off and on.*

***Base**: The base supports the microscope and it's where illuminator is located.*

***Arm**: The arm connects the body tube to the base of the microscope*

***Tube**: Connects the eyepiece to the objective lenses*

How to Use A Microscope

Step 1: Turn microscope ON.

Step 2: Rotate **low power objective** into place.

Step 3: Place slide onto ***stage*** – center the specimen over the hole in the stage. Secure the slide with ***stage clips***.

Step 4: Look through the ***eyepiece*** and turn the ***coarse adjustment knob*** until the specimen comes clearly into view. Adjust slide if necessary.

Step 5: Diagram what you see in the field of view.

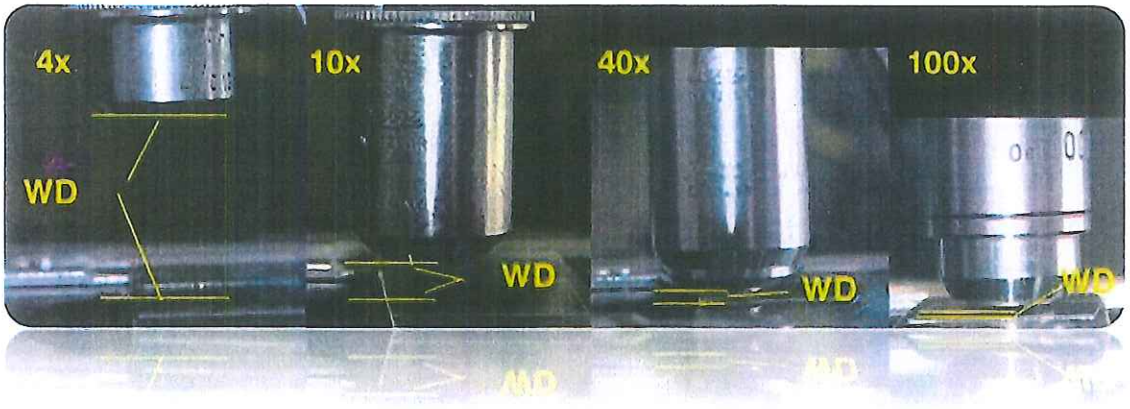
Step 6: Rotate **medium power objective** into place and repeat steps 4-5.

Step 7: Rotate **high power objective** into place and repeat steps 4-5 using the ***fine adjustment knob*** only.

Place one small drop of immersion oil on the spot of light.

Slowly rotate the 100X objective into alignment while checking to make sure it does not strike the slide.

If you were properly focused under the 40X objective, the 100X will rotate into place without striking the slide.



Notes

When convert to 100x power may be note the following

Don't *convert to 100x power unless put a drop of oil on slide in the point of light density*

Don't *move the slide from his place to put the drop of oil*

Don't *move the mechanical stage by using course adjustment to put the drop of oil*

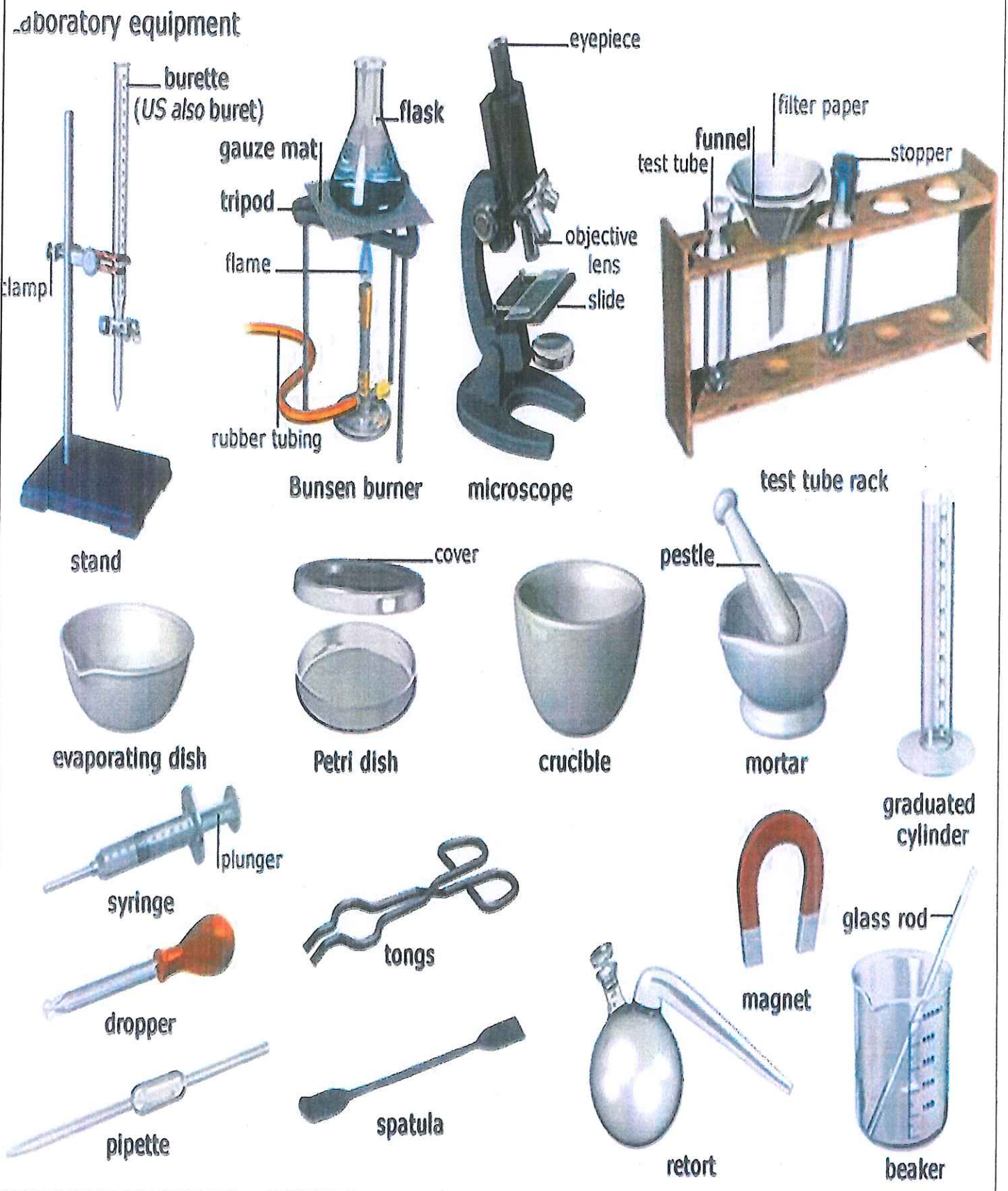
Only *use the fine adjustment to demonstrate the field(using of course adjustment led to break t slide)*

What are benefits (purpose) of using immersion oil with the oil lenses?

To avoid *the mechanical contact between the slide and lenses*

The diffraction factor *of oil approximately equal to diffraction factor of glass so the light hadn't refract and led to increase the resolving power of microscope*

Tools and Equipment



Tools

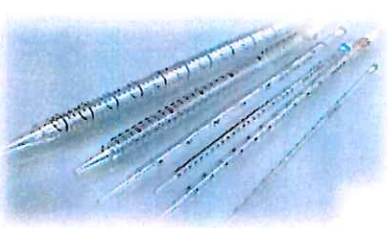
1-Loop

The loop is used in the cultivation of microbes on plates by transferring inoculums for streaking. Touching a broth or a culture plate will gather enough microbes (0.01ml) for inoculation. The inoculation loop is sterilized with flame or another heat source.

2-Can

Used for preserve the pipette from any contamination, sterilized with pipettes by autoclave

3-Pipette



Used for transfer of cultured and uncultured broth from tube or flask to other and placed in can sterilized by autoclave inside the can.

4-Spreader (L-shape)

Used for spreading bacterial cell on surface of solid medium in petriplate ,before using placed in alcohol and then sterilized by flame of burner.

5-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.

6-Swab

Used for swabbing bacterial cells on the surface of solid medium in Petri plate, must be placed in test tube and sterilized by autoclave, it used for one time.

7-Test tube

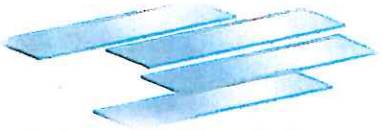
Used to place the liquid or solid or semisolid medium for stabbing or placed as slant for culture of bacteria ,it sterilized by autoclave.

8-Needle

Used for transfer of bacterial cells to a solid medium or semi-solid medium by stabbing , sterilized by the flame of burner before and after use.

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.

12-Cotton plugs

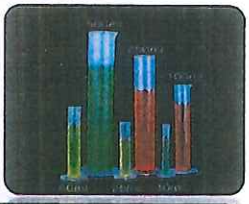
A piece of coiled cotton used to close the upper part of flasks and tubes.

13-Beaker

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

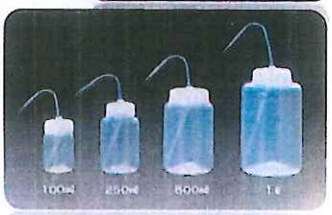
14-Cylinder(Graduated Cylinder)

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.

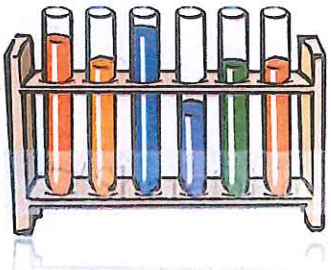


16-pH paper

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.



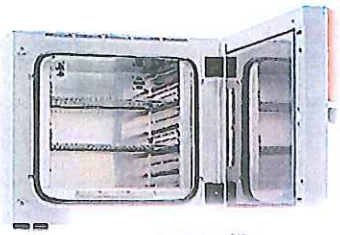
18-Burner

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


Equipments

1-Autoclave

Wet heat sterilization= death by protein denaturation
It's an equipment with:
High temperature(121C°)
High pressure 1 atm(15 pound/inch^2) used to sterilize media
(with sugarfor 10min) (uncultured mediafor 15min),
(cultured media and contaminated glass wares for 30 min)



2-Oven

Dry heat sterilization=death by oxidation
Equipment with high temperature only (180C°) for (90 min)
used to sterilized some of metallic tools and glass wares

3-Incubator

Is a device used to grow and maintain microbiological cultures or cell cultures. The incubator maintains optimal temperature ,humidity and other conditions such as the carbon dioxide (CO2) and oxygen content of the atmosphere inside

4-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°.

5-Biosafety cabinet (BSC)

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

6-Waterbath

Is 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

7-Centrifuge

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

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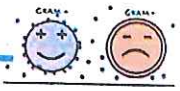

Lab Report

Date /

Student(s) Name(s) /

1- .....

2- .....

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Method Name

Materials

Detergent

☐

Sponge

☐

Burner

☐

Lighter

☐

Marker

☐

Loop

☐

Slides

☐

Agar Medium

☐

Broth Medium

☐

Stain Set

☐

Lab-2- Culture Media

Growth medium or culture medium *is combination of substances designed to support the growth of microorganisms or cells, Different types of media are used for growing different types of cells.*

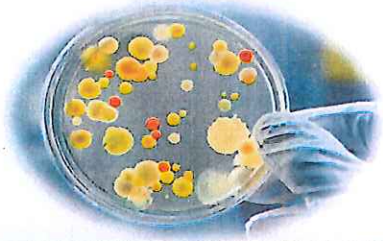
How many types of growth media?

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**.

Pure culture *:culture medium containing the growth of single species of bacteria and we can preserve it by*
1-Cooling *2-Freezing* *3-Lyophilization(Freeze drying)*

Mixed culture : *culture medium containing the growth of two or more species of bacteria*

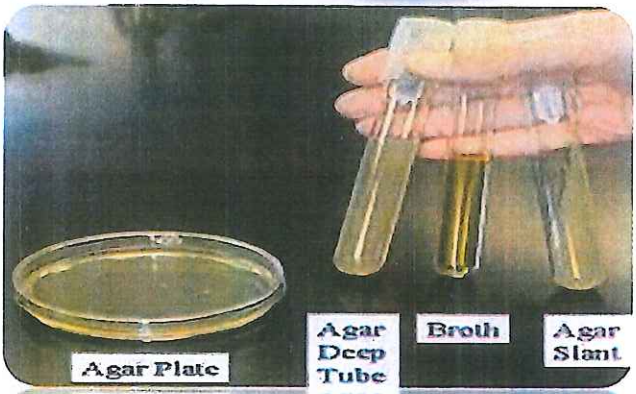


Kinds of culture media

Culture media can be divided according to
1-Their consistency
a-Solid media 2% agar

b-Semisolid media 1% agar

c-Liquid media 0% agar



Agar

Is a complex carbohydrate extracted from sea algae called Gelidium ,used in preparing culture media as solidifying agent because of its characteristics which are :
1-Its melting properties, melt at 90-100C° and solidify at 42C°.
2-It has no nutritive value for majority of bacteria.

2-Their uses and contents

Natural media(non-synthetic)

Media contain natural material rich with vitamins and their structure and concentration are not defined such milk and blood

Defined media (synthetic media)

Medium contain chemical materials their structure and concentration exactly defined

Semi –synthetic media

Media contain natural material as well as chemical materials

Living media

medium contain living tissue used to culturing viruses and cancer cell

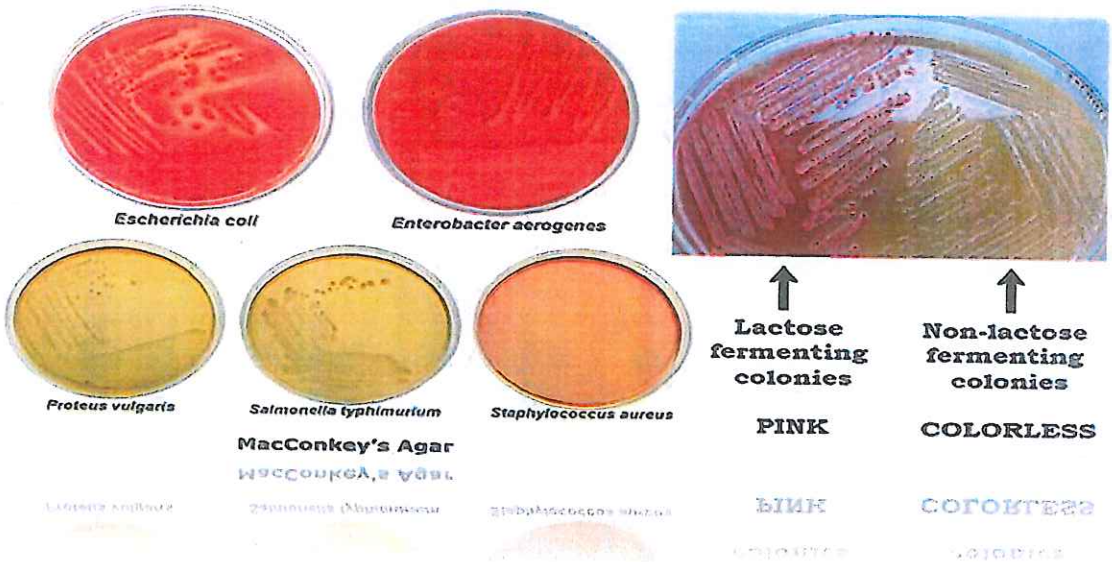
Routine Laboratory Media

1. **Basal media.** *Basal media are those that may be used for growth (culture) of bacteria that do not need enrichment of the media. Examples: Nutrient broth, nutrient agar and peptone water. Staphylococcus and Enterobacteriaceae grow in these media.*

2. **Enriched media** *The media are enriched usually by adding blood, serum or egg. Examples: Enriched media are blood agar and Lowenstein-Jensen media. Streptococci grow in blood agar media.*

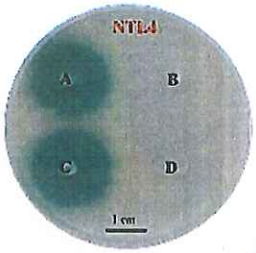
3. **Selective media.** *These media favor the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: MacConkey agar, contain crystal violate that inhibit G^{+ve}.*

4. **Differential media (Indicator).** *An indicator is included in the medium. A particular organism causes change in the indicator, e.g. MacConkey agar are differential media(contain lactose sugar and neutral red).*



5. *Transport media.* These media are used when cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium.

6. *Storage media.* Media used for storing the bacteria for a long period of time. Examples: Egg saline medium, chalk cooked meat broth.



7-Assay medium Medium used to assay the production amount of some material in bacteria

8-Enumeration media that used to calculate the number of bacteria in water ,soil and food sample

9-Characterization media that used to characterize and recognize type of bacteria

Preparation of culture media

1-Weightining the medium ingredients according to the direction written on its container.

2-Dissolve with little amount of D.W. then complete the volume to the volume you want and may be need using heating and stirrer for complete dissolving.

3-Check pH .

4-Dispensing the medium in to test tube by pipette.

5-Sterilization by autoclave.

6-Dispensed agar medium into petri dish when the heat reach to 45.

EX :prepare 500ml of N.A. medium if the direction on container wrote 8gm/liter

gm	ml
8	1000
x	500

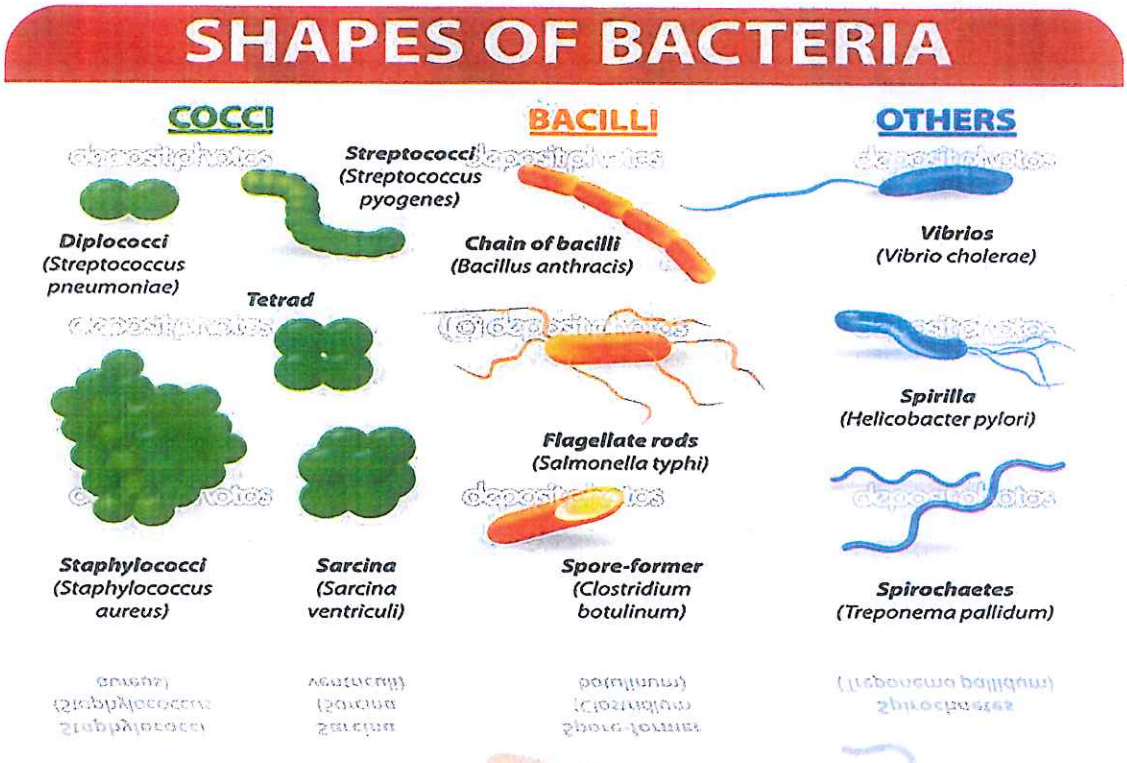
*x=8 * 500/1000 = 4 gm of media dissolve in little amount of D.W. then complete the volume to 500 ml then autoclaved and poured in plates*

Method of pouring the media in plate

The sterile plates should be on the table near the burner then ***Cooling*** *the solid medium to 45C° to avoid solidify it and to avoid forming of drop on the cover of plates*
Remove *the cover (or cotton plug) and sterile the upper part by burner*
Remove *the cover of plate near the burner and pouring the medium and close the cover of plate*
Moving *the plate on table 5 times in two direction to distribute the media equally in plate.*

Sterility test

This test mean putting the flasks tubes and plates which contain sterile media before using in incubator at 37C for 24 hr. to ensure that there is no contamination while preparing and pouring the media



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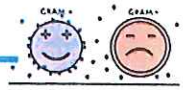

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