Ministry of Higher Educationand Scientific Research
University of Baghdad
College of Science Department of Biology

Practical Theoretical Bacteriology

2022-2021

المرحلة الثانية/ الدراستين الصباحية والمسائية
الفصل الدراسي الثاني

تدريسيو المادة

ا.د. حارث جبار الفهد
ا.د. سمير عبد الامير علش
ا.م.د. مروى حميد الخفاجي
ا.م.د. علياء رزوقي حسن
ا.م.د. ايناس غسان سويدان
ا.م.د. لمى سعيد محمد
م.د. نهاد طة محمد
م.د. جنان عطية غافل
م. حسام محمود حسن
م. الاء رحيم كاظم
م.م. هاجر هادي عبدالامير
م.م. علي محسن علي
م.ر. علياء وائل سعدي

Lab. 1 An introduction to microbiology, aseptic technique and safety

- The teaching of such an important subject as microbiology cannot be achieved effectively without enhancing the theory with 'hands on' experience in the laboratory.
- The purpose of this laboratory is to provide you with good techniques in practical microbiology to ensure that investigations proceed safely and achieve the required educational aims successfully.

General Lab Safety

- There are many potential hazards when working with microorganisms. Potential safety hazards can be avoided with the appropriate precautions.
- When working with microbiological agents you will need to be aware of standard laboratory safety procedures, protective wear, and chemical safety.
- Safety in the lab is everyone's responsibility.
- Before starting any experiment, you should understand the entire procedure that you will be following. You need to make sure that you have the proper equipment, and that you know how to use it.

Microbiology Lab Practices and Safety Rules

1. Wear a lab coat in the lab and do not wear it to other non-lab areas.
2. Wash your hands. Use a disinfectant soap to wash your hands before and after working with microorganisms.
3. Absolutely **no food, drinks, chewing gum, or smoking is allowed in the laboratory**. Do not store food in areas where microorganisms are stored. Do not put anything in your mouth such as pencils, pens, labels, or fingers.
4. Cover any cuts on your hands with a bandage. Gloves may be worn as extra protection.
5. Avoid loose fitting items of clothing. Wear appropriate shoes (sandals are not allowed) in the laboratory.
6. Keep your workspace free of all unnecessary materials. Backpacks, purses, and coats should be placed in the cubbyholes by the front door of the lab. Place needed items on the floor near your feet, but not in the aisle.
7. Disinfect work areas before and after use. Laboratory equipment and work surfaces

should be decontaminated with an appropriate disinfectant, such as 10% bleach or 70% ethanol solution.

8. Label everything clearly. All cultures, chemicals, disinfectants, and media should be clearly and securely labeled with their names and dates.
9. Do not open Petri dishes in the lab unless absolutely necessary.
10. Inoculating loops and needles should be flame sterilized in a Bunsen burner before you lay them down.
11. Turn off Bunsen burners when not in use. Long hair must be restrained if Bunsen burners are in use.

12. When you flame sterilize with alcohol, be sure that you do not have any papers under you.
13. Treat all microorganisms (especially unknown cultures) as potential pathogens. Use appropriate care and do not take cultures out of the laboratory.
14. Wear disposable gloves when working with potentially infectious microbes or samples.
15. Sterilize equipment and materials. All materials, media, tubes, plates, loops, needles, pipettes, and other items used for culturing microorganisms should be sterilized.
16. Never pipette by mouth. Use pipette bulbs or pipetting devices for the aspiration and dispensing of liquid cultures.
17. Consider everything a biohazard. **Do not pour anything down the sink**. Autoclave liquids and broth cultures to sterilize them before discarding.
18. Autoclave or disinfect all waste material. All items to be discarded after a class, such as culture tubes, culture plates, swabs, toothpicks, wipes, disposable transfer needles, and gloves, should be placed in a biohazard autoclave bag and autoclaved **30 to 40 minutes at 121° C at 20 pounds of pressure**. If no autoclave is available and you are not working with pathogens, the materials can be covered with a **10% bleach solution and allowed to soak for at least 1 to 2 hours.**
19. Familiarize yourself with the location of safety equipment in the lab (e.g., sinks, fire extinguisher, and first aid kit).
20. Dispose of broken glass in the broken glass container.
21. Dispose of razor blades, syringe needles, and sharp metal objects in the "sharps" container.
22. Report all injuries or accidents immediately to the instructor, no matter how small they seem.
23. Report spills and accidents immediately to your instructor. Clean small spills with care. Seek help for large spills.

Biohazard waste bag

Sharp s was_te container

Broken g as cont a-ner

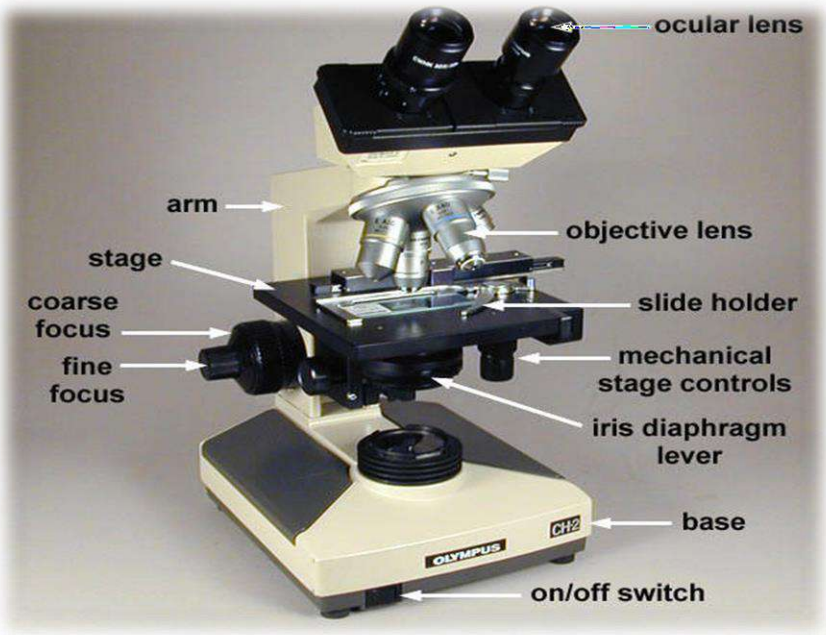
Personal Protective Equipment

Lab-2- 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 consists 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 <u>10X or 15X</u> 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

<u>4X , 10X (Low) , 40X(high dry) and 100X(Oil)</u>

2-Illumination system:

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

***Iris diaphragm**: Adjusts the amount of light that rea*

***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, <u>flat rectangles of thin glass</u>.

***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.

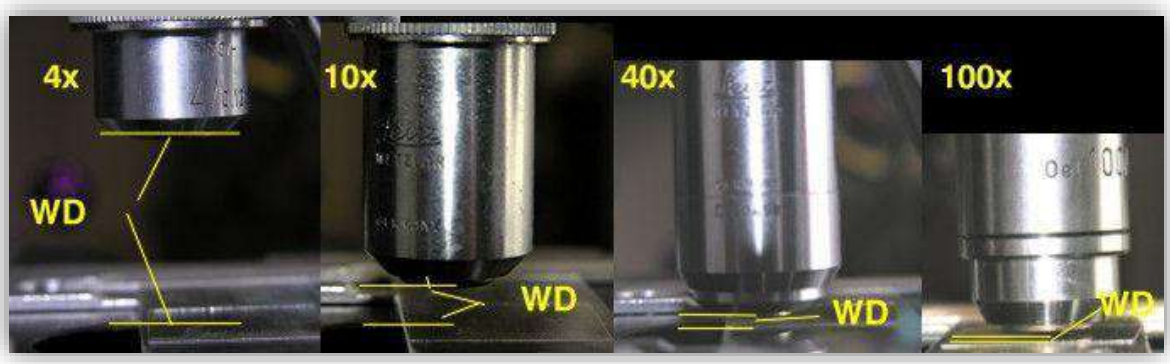
Step 6: Rotate **medium power objective** into place and repeat steps 4-5.

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 the 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*

Lab 3 Tools and Equipment

talborato egui:pment

burette

: (us also buriet)

filter pa per

gauze mat

tripod

objective

flarn@

len

s.lide

·!arnp,

rubber tubing

Bunsen bumer

mlcroscope

stand

Petri dl,s.h

crucible

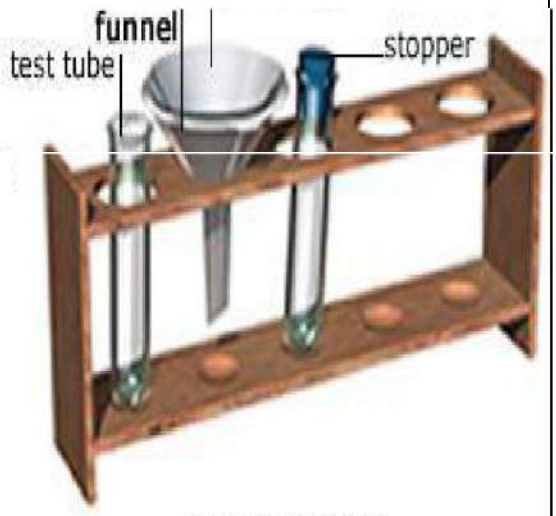
graduated
cyllnder

syringe

spatula

pipette

beaker



evaporating dish

mortar

tongs

magnet

dropper

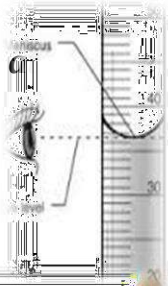
retort

Tools

1-Loop

The loop is used in the <u>cultivation of microbes</u> 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 c

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.

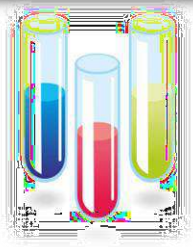
5-Petri-Dish(Petri-plate)

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

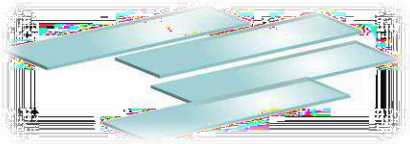


Used to place the liquid or solid or semisolid medium for stabbing or placed as slant for culture of bacteria ,it sterilized by autoclave.

8-Needle

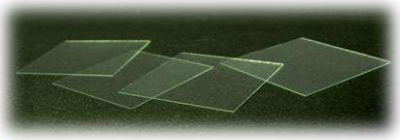
Used for transfer of bacterial cells to a solid medium or semi-solid medium by stabbing , sterilized by the flame of burner before and after use.

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.

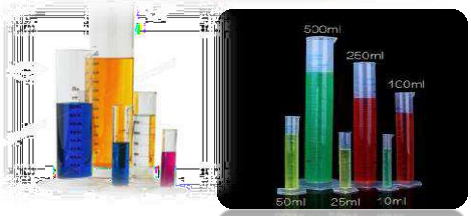
12- Cotton plugs

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

13- Beaker

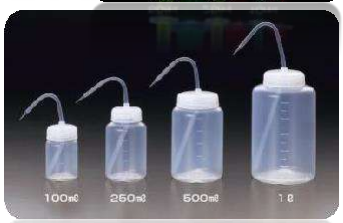
Used for graduated the volume of liquids. Sterilized by oven.

14- Cylinder(Graduated Cylinder)



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

15- Washing bottle

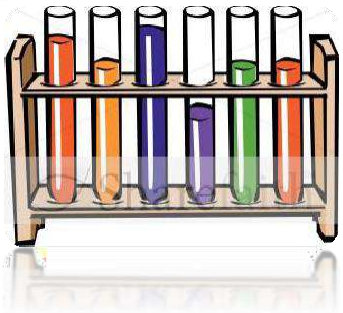


Used to fill with liquid (specially distilled water)for washing and homogenizing the glass wares and washing the slide during staining , don't need sterilization.

16- pH paper

Used to know the pH of the medium or any liquids

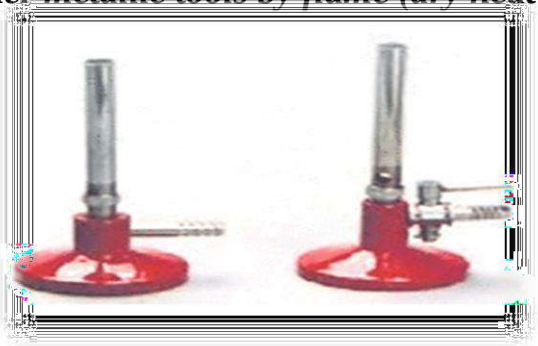
17- Rack



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

18- Burner

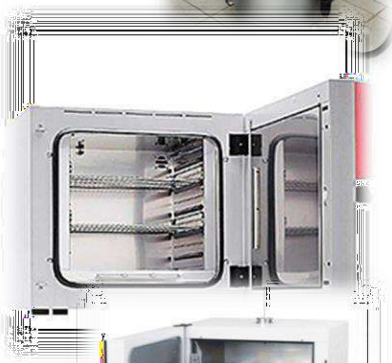
May be gaseous or alcoholic , used for sterilized the loop , needle and other metallic tools by flame (dry heat sterilization).



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

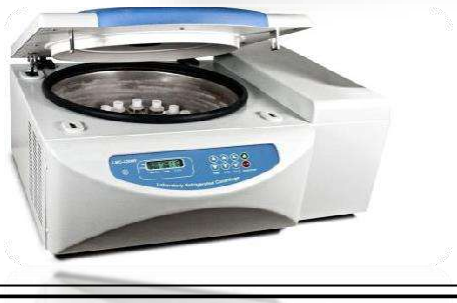
Used to maintain the sterilized media and broth when not used to avoid the contamination , and also to preserve the bacterial culture for long time by preventing the growth at 4C°.

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

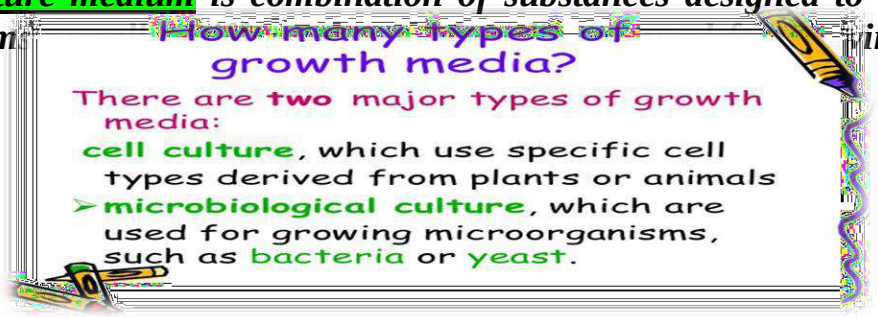


7-Centrifuge

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

Lab-4- Culture Media

Growth medium or culture medium *is combination of substances designed to support the growth of microorganism* [illegible] *ing different types of cells.*



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* i

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.

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***

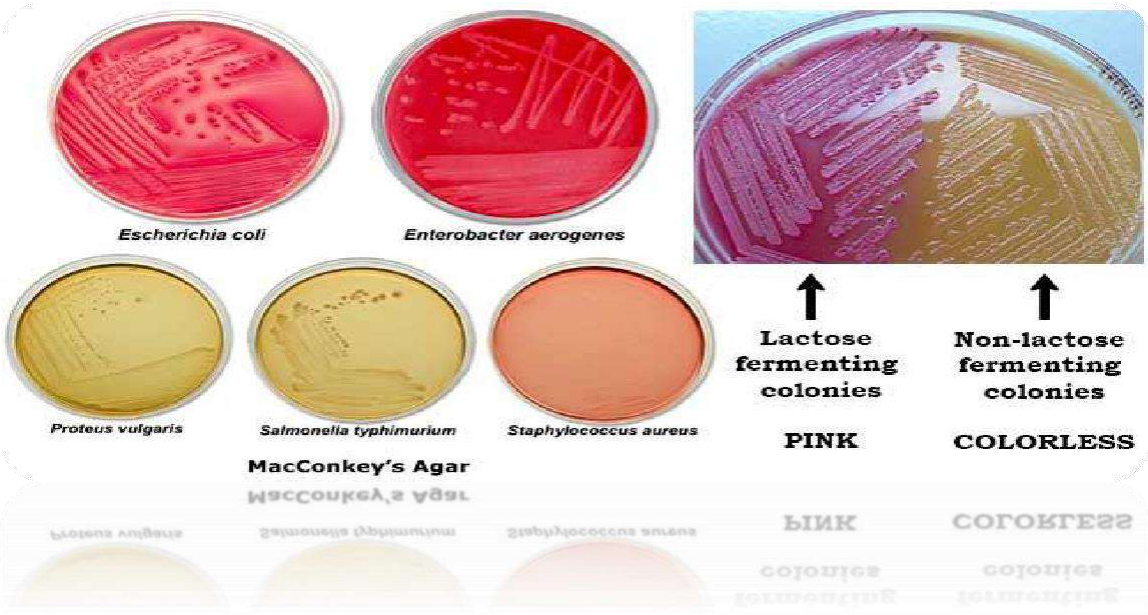
Routine Laboratory Media

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.***

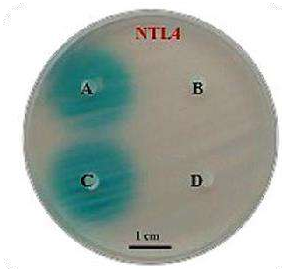
3. Selective media. These media favor the growth of a particular bacterium by inhibiting the growth of undesired bacteria and allowing growth of desirable bacteria. Examples: MacConkey agar, contain crystal violate that inhibit G^{+ve}.

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).***



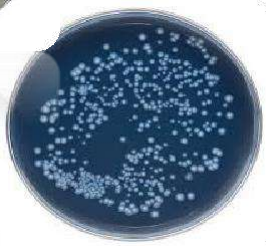
5. Transport media. *These media are used when cannot be cultured soon after collection. Examples: Cary-Blair medium, Amies medium, Stuart medium.*

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

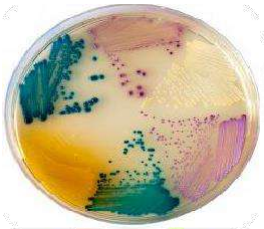


7-Assay medium *Medium used to assay the production amount of some material in bacteria*

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 usingheating 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*

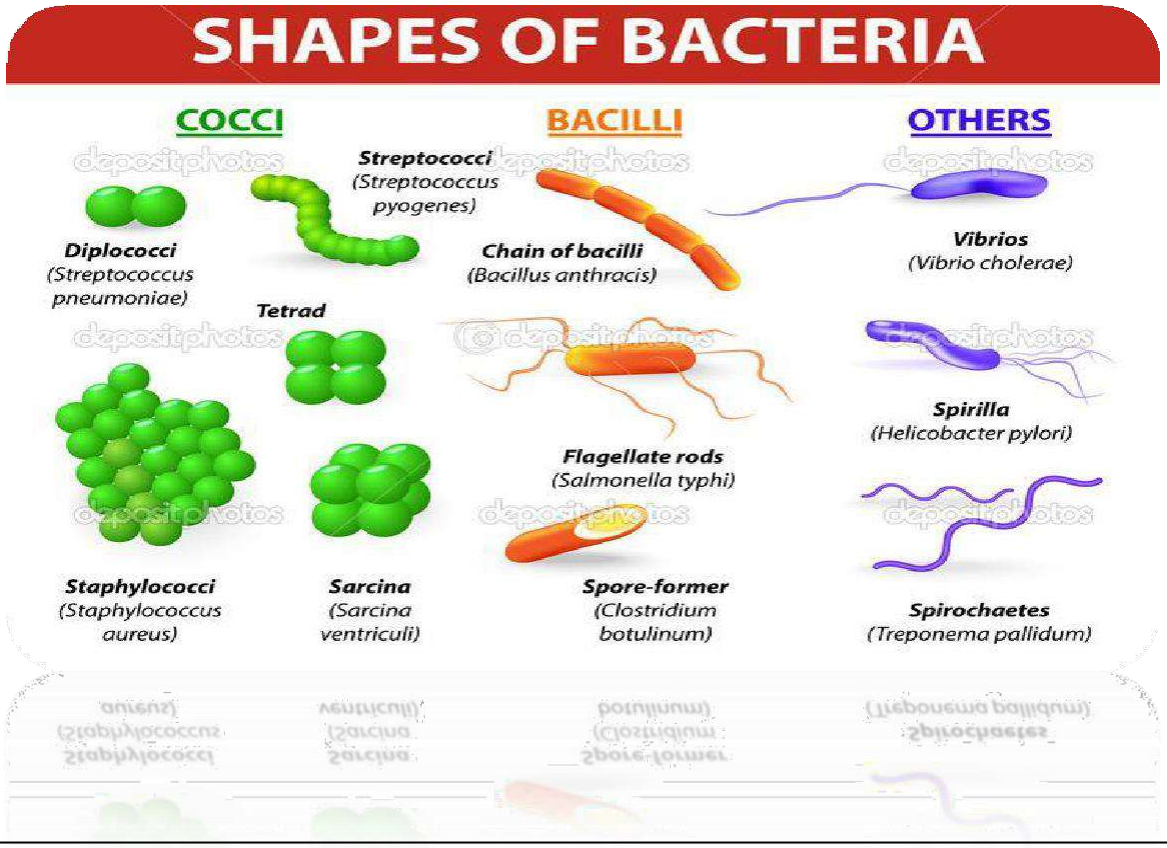
x=8 * 500/1000 = 4 gm of media dissolve in little amount of D.W. then complete the volume to 500 ml then autoclaved and poured in plates

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



Lab -5- Bacterial Staining

Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image .Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes.

A dye is a colored substance that has an affinity to the substrate to which it is being applied. The dye is generally applied in an aqueous solution

The bacteria are stained for the following reasons:

1- ***To study their shape.***
2- ***To differentiate the bacterial species by using differential stain.***
3- ***To study the internal components of the bacterial cell.***

The stains divide into 2 groups (according to their affinity to cell components):

1-Indirect stain (Negative stain):

It is can penetrate the cell envelope therefore the cell become very obvious by making counter dark (stain the slide but not the cell):India ink, Congo red

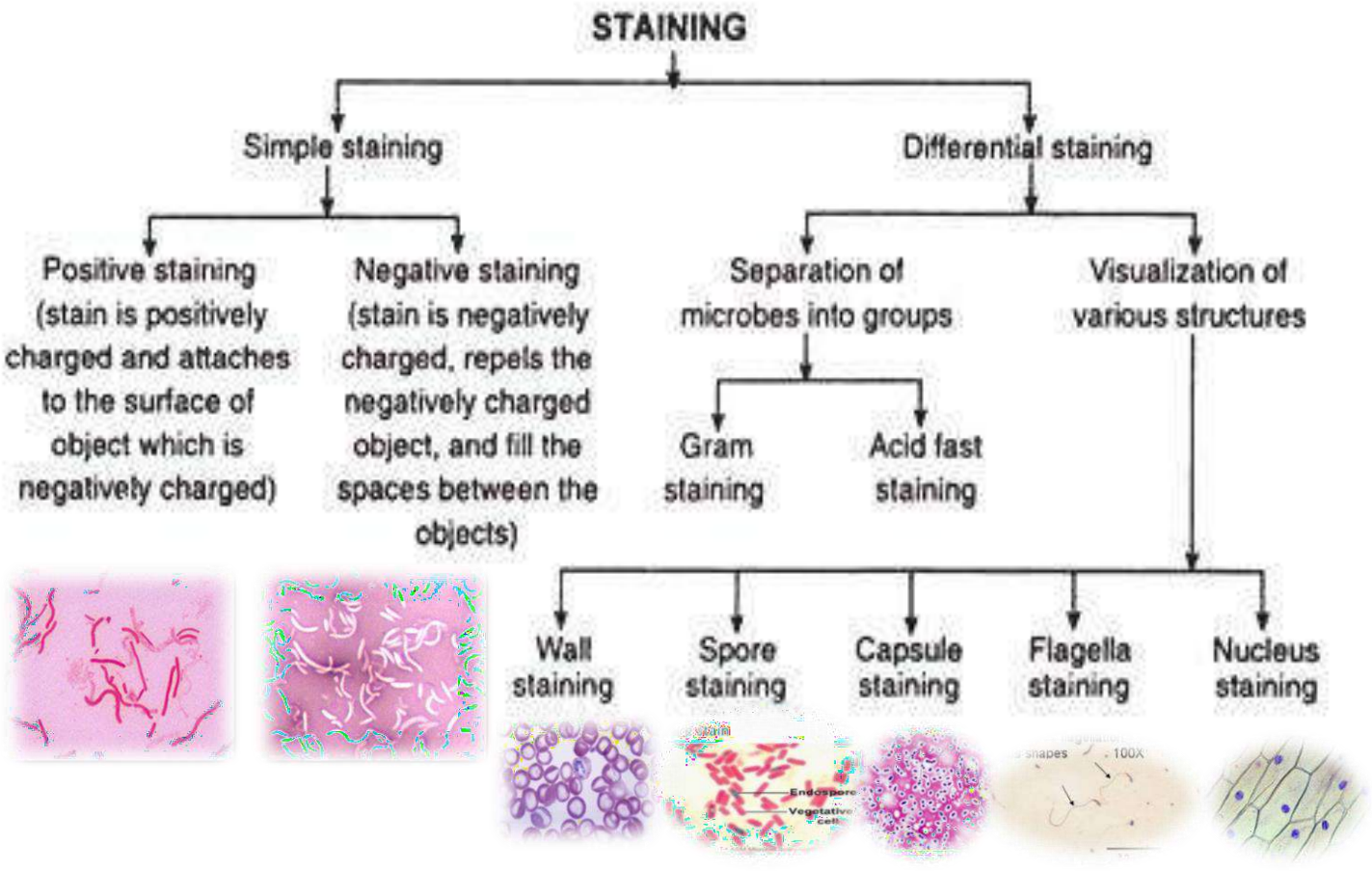
2-Direct stain(Positive stain)

It has strong affinity to one or more of cell components

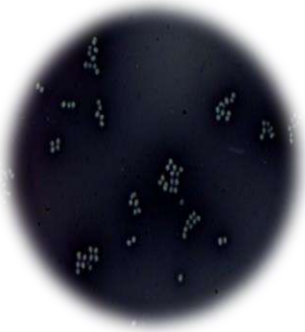
a-Simple stain: ***Acidic (Safranine) ,Basic (Methylene blue, Crystal violet)***

b-Differential stain: ***Gram stain , Acid fast stain(Ziehl Neelson stain)***

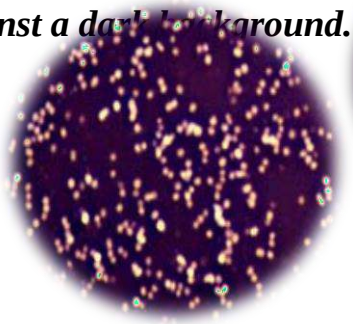
c-Selective stain:Spore stain , Capsule stain , Flagella stain , Cell wall stain , Nucleic acid stain

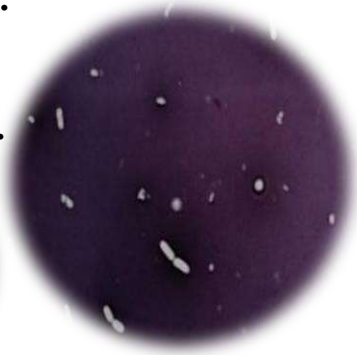


Indirect stain(Negative stain)



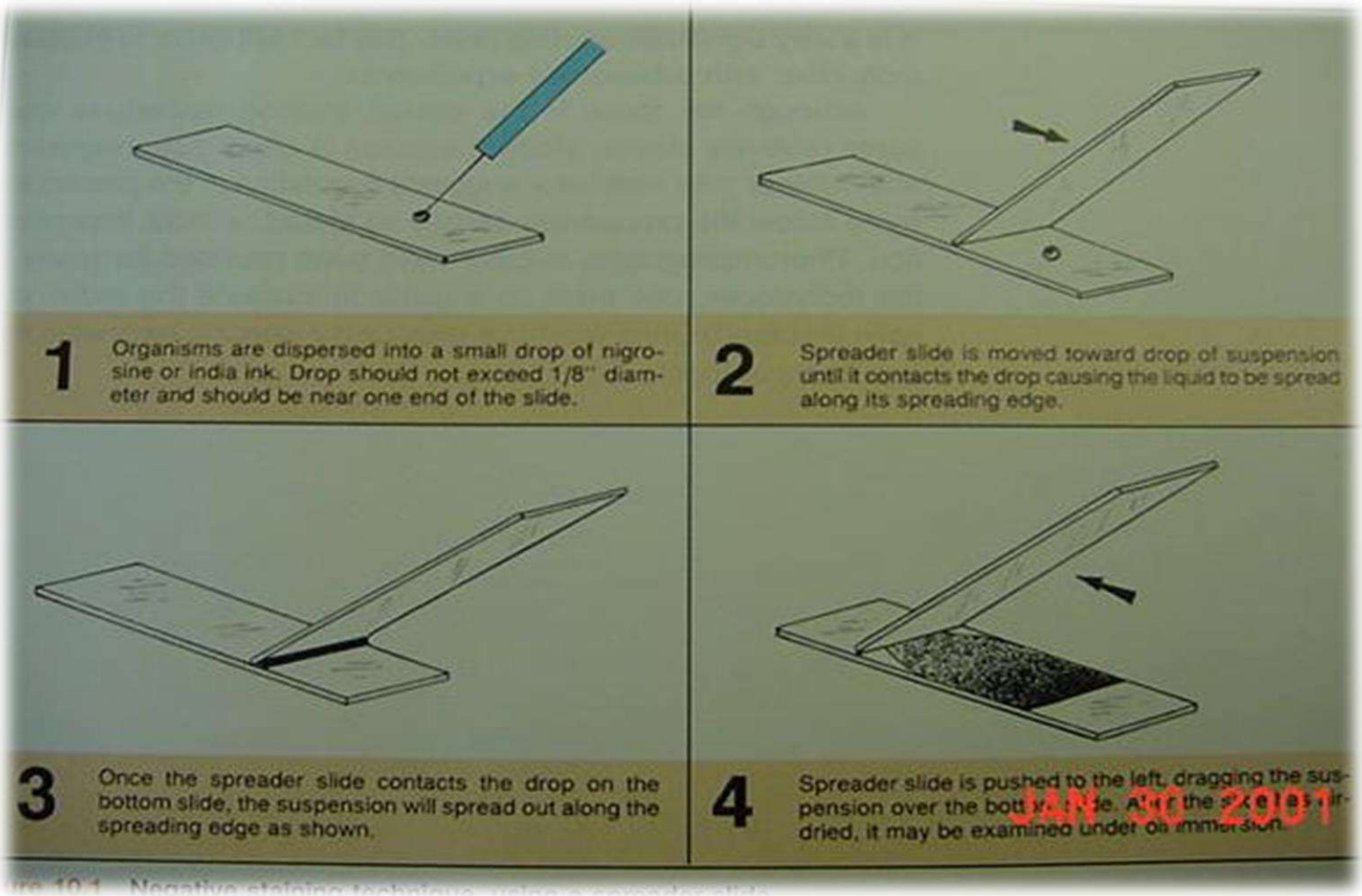
The main purpose of Negative staining is to study the morphological shape, size and arrangement of the bacteria cells that is difficult to stain. Negative staining requires an acidic dye such as India Ink or Nigrosin. India Ink or Nigrosin is an acidic stain. Since the surface of most bacterial cells is negatively charged, the cell surface repels the stain. The glass of the slide will stain, but the bacterial cells will not. The bacteria will show up as clear spots against a dark background.





Procedure:

Mix one drop of India ink with culture by sterile loop on slide
Used another slide for dispensing the mixture
Air dry the smear(do not use heat fixation)



Lab -6 Simple Stain *(using one dye –one step for staining)*

It used to reveal the morphological features of most bacterial cell including relative size , shape and arrangement of group of cells.
, it consist from :

1-Colored ion (chromophore)
Which could be either positive charged and have affinity to stain negative charged component of bacterial cell or negative charged and have affinity to stain positive charged component of bacterial cell.
2-Counter ion
To balance the charge of dye.
The bacteria are colorless therefore we must stain them , the use of stains that chemically react with cell material enhance the contrast between the cell and the background, to study the shape , size , and arrangement.

There are two groups of simple stain
1-Acidic stain:
has negative charge it is stain the basic compounds (protein)of the cell with a positive charge(safranine , acid fuchsin)

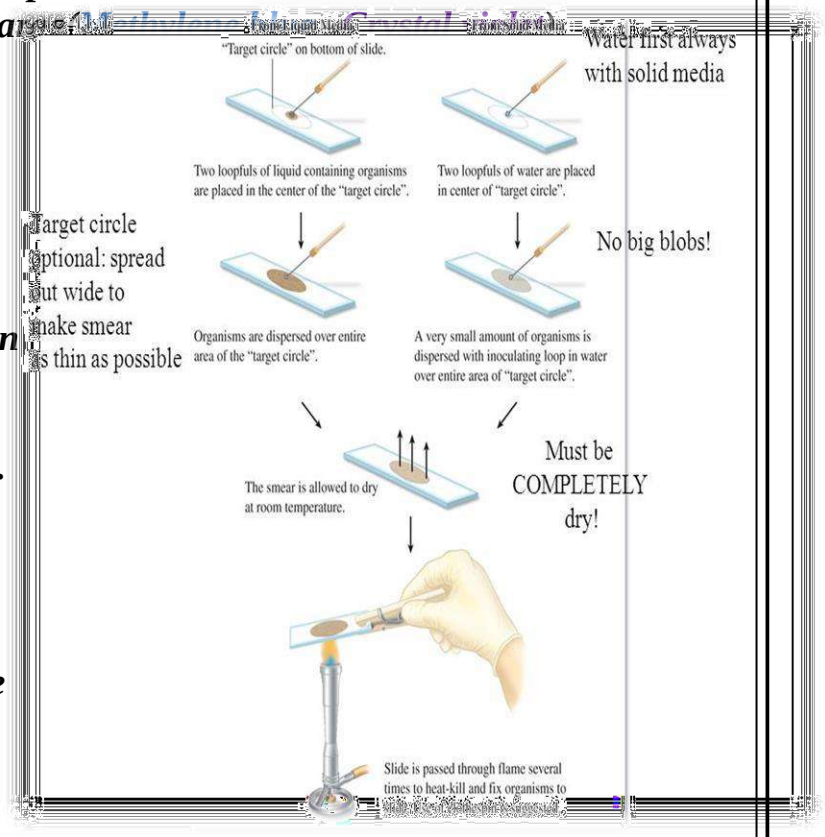
2-Basic or Alkaline stain:
has positive charge it is stain the acidic compounds (RNA,DNA))of the cell with a negative charge

Steps of work in general

1-Smear preparation
2-Fixation
3-Staining with stain

Smear preparation
From broth: one loopful directly from broth on the slideFrom solid culture:put one drop of water in the center of the slide the take one touch from colony and mix well with the drop.

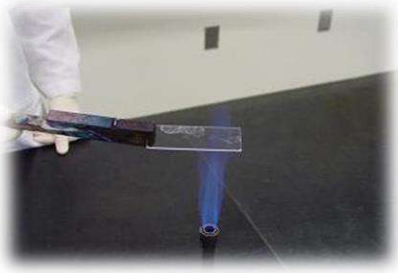
After air drying
The fixation will done by Passing the slide rapidly across the flame three times the slide should be warm not hot , why?hot will change the shape of bacterial cell.



The fixation will

1-Kill the bacteria so the stain will penetrate without serious destruction of cell structure.

2-Fix the cell on the slid (by coagulation of their protein) So they are not removed during staining.



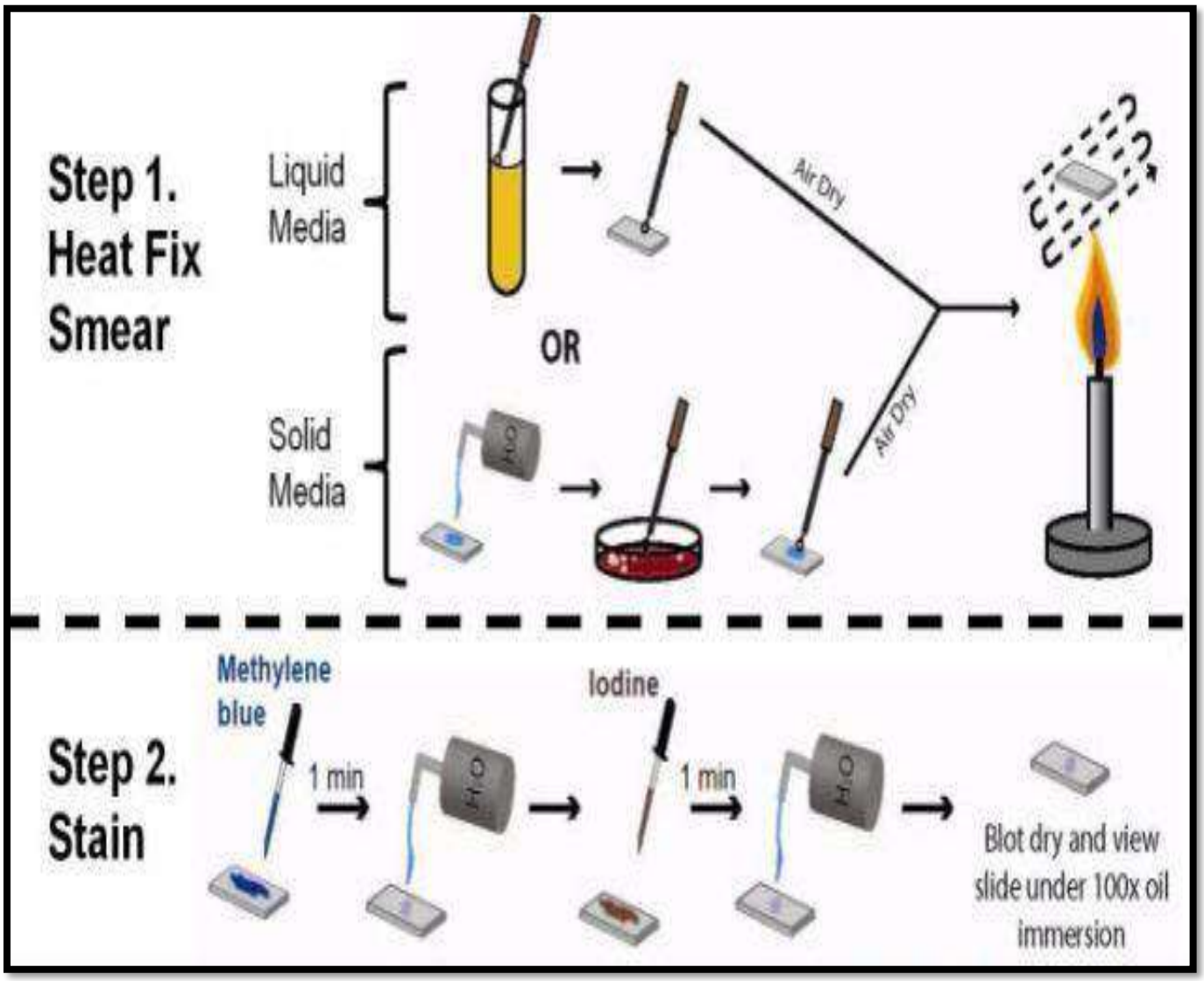
Staining with dye:

Put the slide on the staining rack and flood the smear with simple stain (Crystal violet or Safranine)for 1 min.

Wash the slide with tap water gently and drain off excess water

Air dry

Examine with microscope under oil –immersion lens.



Lab -7- Differential Stain

Acid fast stain

The Ziehl–Neelsen stain, also known as the acid-fast stain, It is a special bacteriological stain used to identify acid-fast organisms, mainly Mycobacteria. Mycobacterium tuberculosis (tuberculosis) (TB). Other important Mycobacterium species involved in human disease are Mycobacterium leprae, (leprosy). Acid fast organisms like Mycobacterium contain large amounts of lipid substances within their cell walls called mycolic acids. These acids resist staining by ordinary methods such as a Gram stain. it can also be used to stain a few other bacteria, such as Nocardia.

Reagents

Ziehl–Neelsen carbol fuchsin, acid alcohol, methylene blue.

Acid-fast bacilli will be bright red after staining.

A typical AFB stain procedure involves

1- Dropping the cells in suspension onto a slide, then air drying the liquid and heat fixing the cells.

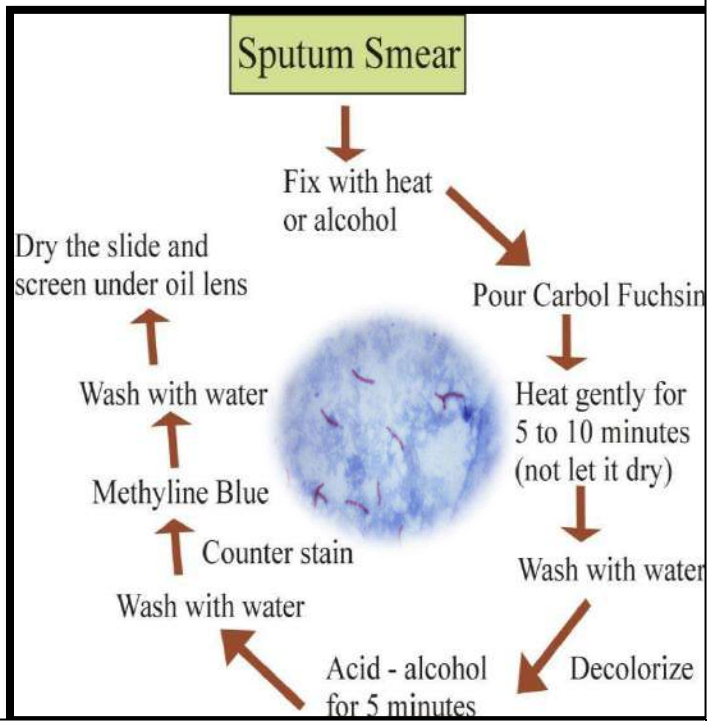
2- The slide is flooded with Carbol Fuchsin, which is then heated to dry and rinsed off in tap water.

3- The slide is then flooded with a 1% solution of hydrochloric acid in isopropyl alcohol (or methanol) to remove the carbol fuchsin, thus removing the stain from cells that are unprotected by a waxy lipid layer.

4- The cells are stained in methylene blue and viewed on a microscope under oil immersion.

Mechanism

Initially, Carbol Fuchsin stains every cell. When they are destained with acid-alcohol, only non-acid-fast bacteria get destained since they do not have a thick, waxy lipid layer like acid-fast bacteria. When counter stain is applied, non-acid-fast bacteria pick it up and become blue when viewed under the microscope. Acid-fast bacteria retain Carbol Fuchsin so they appear red.



Differential Stain

Gram stain

Gram staining is a common technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The Gram stain procedure distinguishes between Gram positive and Gram negative groups

by coloring these cells red or violet. Gram positive

bacteria stain violet due to the presence of a thick

layer of peptidoglycan in their cell walls, which

retains the crystal violet these cells are stained with. Alternatively, Gram negative bacteria stain red, which is attributed to a thinner peptidoglycan wall, which does not retain the crystal violet during the decoloring process.

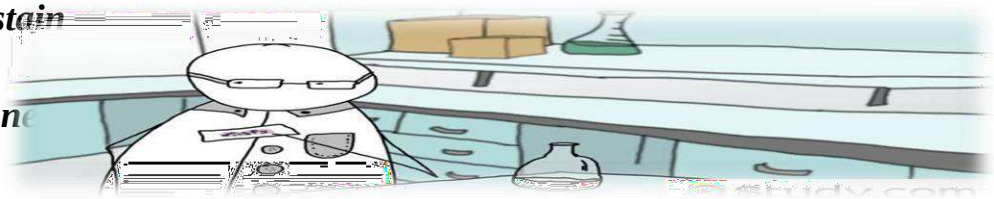
Principle of Gram Staining

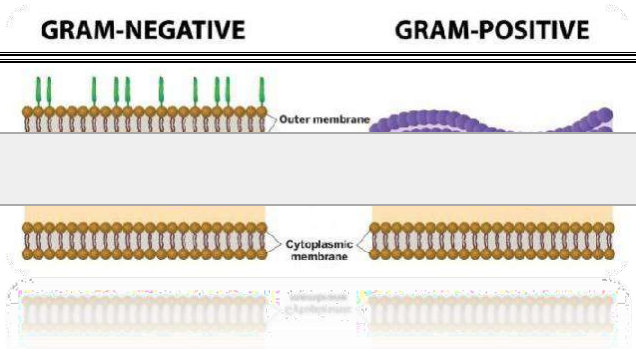
When the bacteria is stained with primary stain Crystal Violet and fixed by the mordant, some of the bacteria are able to retain the primary stain and some are decolorized by alcohol. The cell walls of gram positive bacteria have a thick layer of protein-sugar complexes called peptidoglycan and lipid content is low. Decolorizing the cell causes this thick cell wall to dehydrate and shrink, which closes the pores in the cell wall and prevents the stain from exiting the cell. So the ethanol cannot remove the Crystal Violet-Iodine complex that is bound to the thick layer of peptidoglycan of gram positive bacteria and appears blue or purple in color.

In case of gram negative bacteria, cell wall also takes up the CV-Iodine complex but due to the thin layer of peptidoglycan and thick outer layer which is formed of lipids, CV-Iodine complex gets washed off. When they are exposed to alcohol, decolorizer dissolves the lipids in the cell walls, which allows the crystal violet-iodine complex to leach out of the cells. Then when again stained with safranin, they take the stain and appears red in color.

Reagents Used in Gram Staining

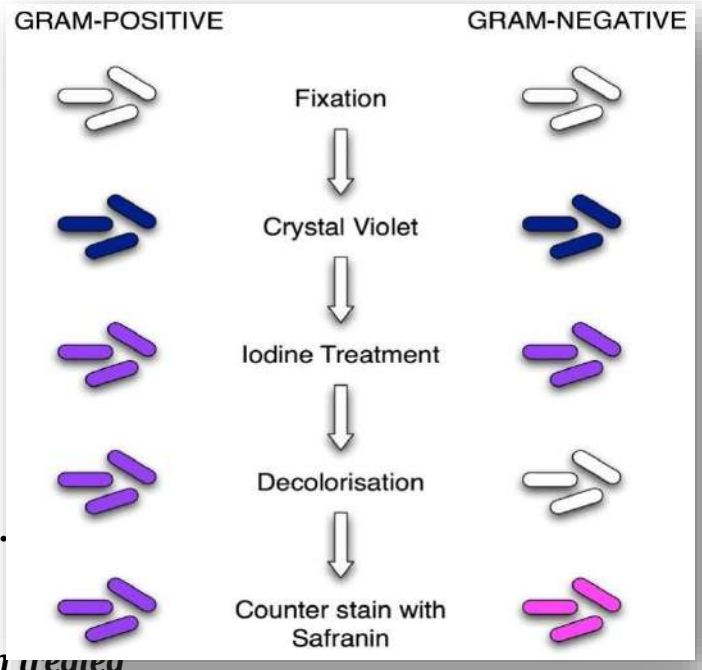
- ***Crystal Violet, the primary stain***
- ***Iodine, the mordant***
- ***A decolorizer made of acetone***
- ***Safranin, the counterstain***





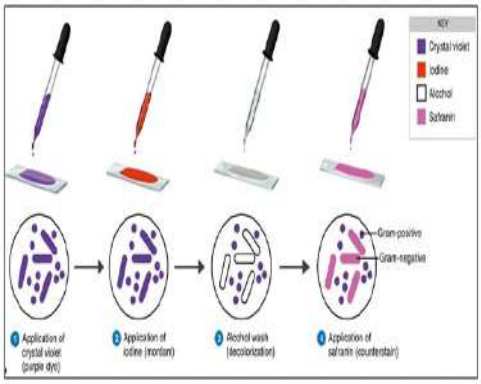
Procedure of Gram Staining

1. *Take a clean, grease free slide.*
2. *Prepare the smear of suspension on the clean slide with a loopful of sample.*
3. *Air dry and heat fix*
4. *Crystal Violet was poured and kept for about 30 seconds to 1 minutes and rinse with water.*
5. *Flood the gram's iodine for 1 minute and wash with water.*
6. *Then ,wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water.*
7. *Add safranin for about 1 minute and wash with water.*
8. *Air dry, Blot dry and Observe under Microscope.*



-Some bacteria do not lose the primary stain when treated with decolorization agent(G^{+ve},appear purple in color)

-Other bacteria(G^{-ve}) ,lose the primary stain after decolorization , and after counterstaining with safranine they appear pink.



If G+ve cell are not treated with iodine mordant after primary staining , they will lose their primary stain at decolorizing step and appear as G-ve .

Several factors affect the results of the gram stain procedure , these include:-

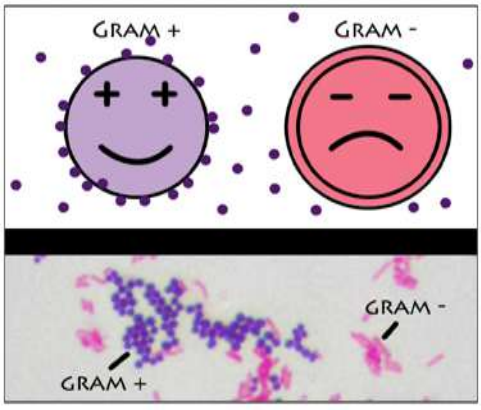
1-The smear preparation

If the smear is too thick proper decolorizing will not be possible, if the smear is over heated during heat fixation the cell will may rupture.

2-Concentration and freshness of reagent may effect quality of staining.

3-Wash and drying the smear between steps should be consistent , excess water left on the slide will dilute reagents particularly Gram iodine.

4-Gram stains are effect only on cells from cultures that are dur of growth. Older culture contain more ruptured and dead cells , cultures may stain pink even if the bacteria are Gram positive.



Lab -8- Selective Stain

Capsule stain

Capsules are formed by organisms such as Klebsiella pneumoniae . Most capsules are composed of polysaccharides, but some are composed of polypeptides. The capsule differs from the slime layer that most bacterial cells produce in that it is a 1-thick 2-detectable 3- discrete layer outside the cell wall.

Some capsules have well-defined boundaries, and some have fuzzy, trailing edges.

Capsules functions

1-Protect bacteria from the phagocytic action of leukocytes

2-Allow pathogens to invade the body. If a pathogen loses its ability to form capsules, it can become avirulent.

Capsule formation affect by:

1-The medium in which the culture is grown

2-The temperature at which it is grown

3-The age of the culture will .Older cultures are more likely to exhibit capsule production.

Bacterial capsules are non-ionic, so neither acidic nor basic stains will adhere to their surfaces. Therefore, the best way to visualize them is to stain the background using an acidic stain and to stain the cell itself using a basic stain. We use India ink and Gram crystal violet. This leaves the capsule as a clear halo surrounding a purple cell in a field of black

Procedure of Capsule Staining

1-Place a small drop of a negative stain (India Ink, Congo Red, Nigrosin, or Eosin) on the slide.

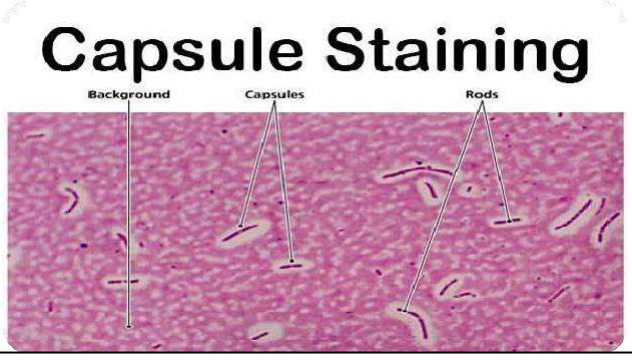
2-Using sterile technique, add a loopful of bacterial culture to slide, smearing it in the dye.

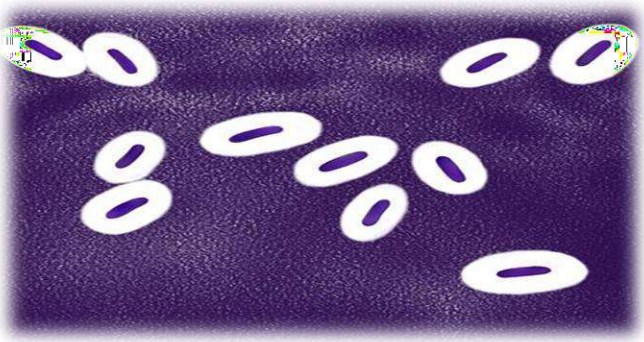
3-Use the other slide to drag the ink-cell mixture into a thin film along the first slide and let stand for 5-7 minutes.

4-Allow to air dry (do not heat fix).

5-Flood the smear with crystal violet stain (this will stain the cells but not the capsules) for about 1 minutes. Drain the crystal violet by tilting the slide at a 45 degree angle and let stain run off until it air dries .

6-Examine the smear microscopically (100X) for the presence of encapsulated cells asindicated by clear zones surrounding the cells.





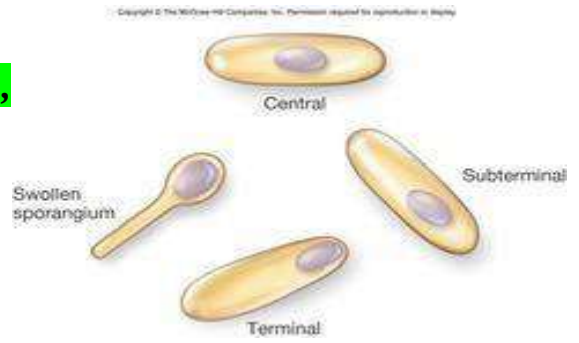
Spore stain

Endospore staining is a technique used in bacteriology to identify the presence of endospores in a bacterial sample, which can be useful for classifying bacteria.

Within bacteria, endospores are quite protective structures used to survive extreme conditions, but this protective nature makes them difficult to stain using normal techniques.

Special techniques for endospore staining include the Schaeffer–Fulton stain and the Moeller stain. A good stain to use for spore staining is malachite green. It takes a long time for the spores to stain due to their density, so time acts as the mordant when doing this differential stain; the slide with the bacterium (Bacillus subtilis 72 hr aged) should be soaked in malachite green for at least 30 minutes. Water acts as the decolorizer. A counter stain to differentiate the vegetative cells is 0.5% safranin.

Types of endospores that could be identified are Free endospores central endospores, terminal , swollen endospores and subterminal endospores.



Procedure and Reagents used for Endospore Staining

1-Preparing smear of Bacillus subtilis(72hr age)

2-Dry over low gas flame and fix, heat is used to provides stain penetration into spore wall

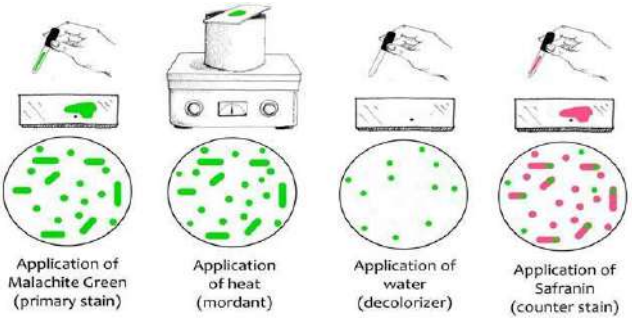
3-Cover the smear with Primary Stain: Malachite green (5%)for 30-60 sec+ heating until it steam for 30 sec

4-Wash with tap water or Distilled Water for 30 sec

5-Counter Stain: Cover the smear with counterstain Safranin (0.5%)for 60 sec to give another color for vegetative cell

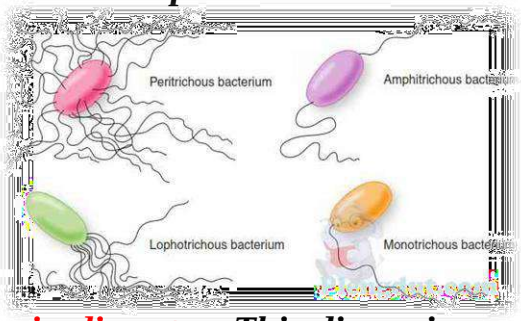
6-Wash with tap water ,drain, blot and air dry

7-The spore stains green while the reminder of the cell red



Flagella stain

Many bacterial species are motile , most by means of long thin (fine thread like organelles)flagella that project from the cell surface in response to nutrient concentrations.



The mode of attachment of these flagella and their point of emergence on the cell surface are characteristic of taxonomic significance.

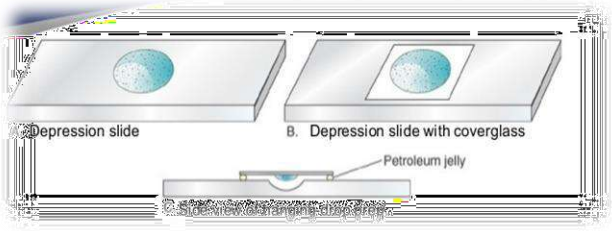
The basic difficulty in visualizing bacterial flagella with ordinary light microscope is that each flagellum consists of a single protein filament measuring only 15 nanometer in diameter .This dimension places the flagellum well below the resolving power of the light microscope.

To some extent, the problem of flagella thickness can be solved by precipitating a mordant (ex: tannic acid and potassium aluminum sulphate)on the filament to increase its diameter so that it is within the limits of resolution of the light microscope , and then staining the coated filament as in Difco spot test flagella stain which employs an alcoholic solution of crystal violet as primary stain and tannic acid and potassium aluminum sulphate as a mordant.

We can also notice the bacterial motility with their flagella by two method:

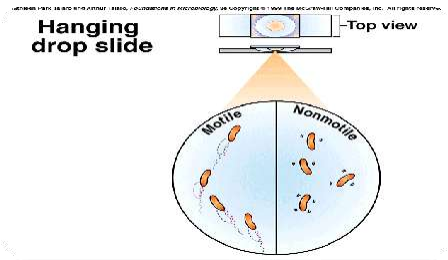
1-Hanging drop 2-Motility test

Hanging drop



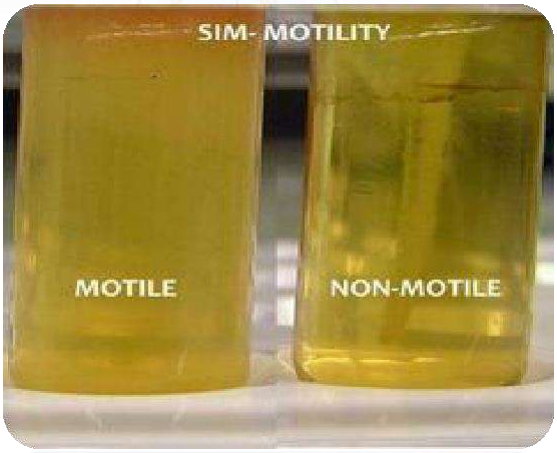
This method is used to see the motility of living cell without staining by using a depression slide:

1-Put a drop full of bacterial broth on cover slip
2-Use a depression slide, put 4 small drops of water around the depression.
3-Turn the cover slip over the depression slide.
4-Examine under oil-immersion lens.



Motility test

1-Prepare test tube filled with media containing 0.5% agar(semi-solid media).
2-Stab the semi-solid media deep with inoculated needle.
3-Incubate for 24hr. at 37C˚.
4-Examine the culture for motile activity .
positive test seen as cloudy area around the stabbing.



Lab -9- Bacterial Count

In order to observe microbial reproduction, it is necessary to determine numbers of M.O. the estimation of microbial population or count could be:

Direct count of cell number:

Under the microscope, used in food microbiology to assess the sanitation level of a product and its also used for performing blood cell counts in hematology.

Indirect count:

The growing of M.O. on a suitable media and use the no. of the development M.O. as colonies to determine the no. of cells since each microbial cell multiply and from one colony , thus the number of colonies should give the no. of live bacteria that can grow under the incubation condition employed.

Also increased turbidity in culture is another index of bacterial growth and no. by spectrophotometer.

There are different methods that can employ which include:

Total count (counting living and non-living cells)

1-Breed method
2-Haemocytometer (counting chamber)
3-Optical density (O.D.) by spectrophotometer

Viable plate count

1-Dilution to extinction
2-Pour plate method
3-Most probable number (MPN)
4-Spread plate method
5-Membrane filter method

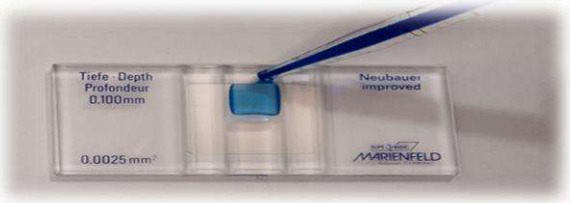
Total count

1-Breed method

1-Draw a square (1 cm^2) by wax pen on the slide .
2-Put 0.01 ml (one loop full)from broth culture (Bacillus or Staph aureus)on the back of the square.
3-Make a smear inside the lines of the square.
4-Fix on the flame
5-Wash with tap water.
7-Examine under oil-immersion objective lenses.
8-Count the cells in 10 fields. Then find the range of this count and use the following formula:-

No. of cell/ 1 ml= the average no .of cells in 10 field x 5000 x 100 x inverse of dilution if used

2-Haemocytometer (counting chamber)



1-Put the cover on the counting chamber

2-Put 0.01 ml (one loop full) of culture near the edge of the cover , the drop will spread (under the cover)

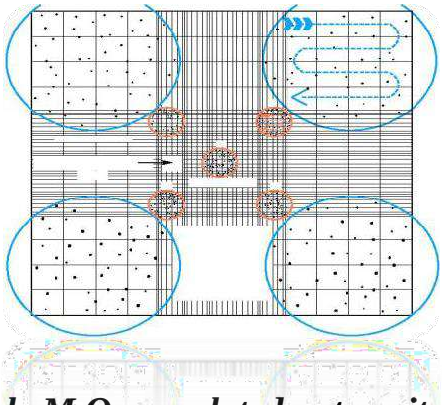
3-Count the cells as the following.

Count the cells in squares (4 corners and one in the middle)if the chamber with 25 squares , used the following formula :

No. of cells/1 ml=no. of cell in 5 squares x 5 x 10 x 1000 x inverse of dilution if used

Count the cells in 4 squares (4 corners only) if the chamber with 16 squares , and used the formula:

No. of cells/1 ml=no. of cell in 4 squares x 4 x 10 x 1000 x inverse of dilution if used

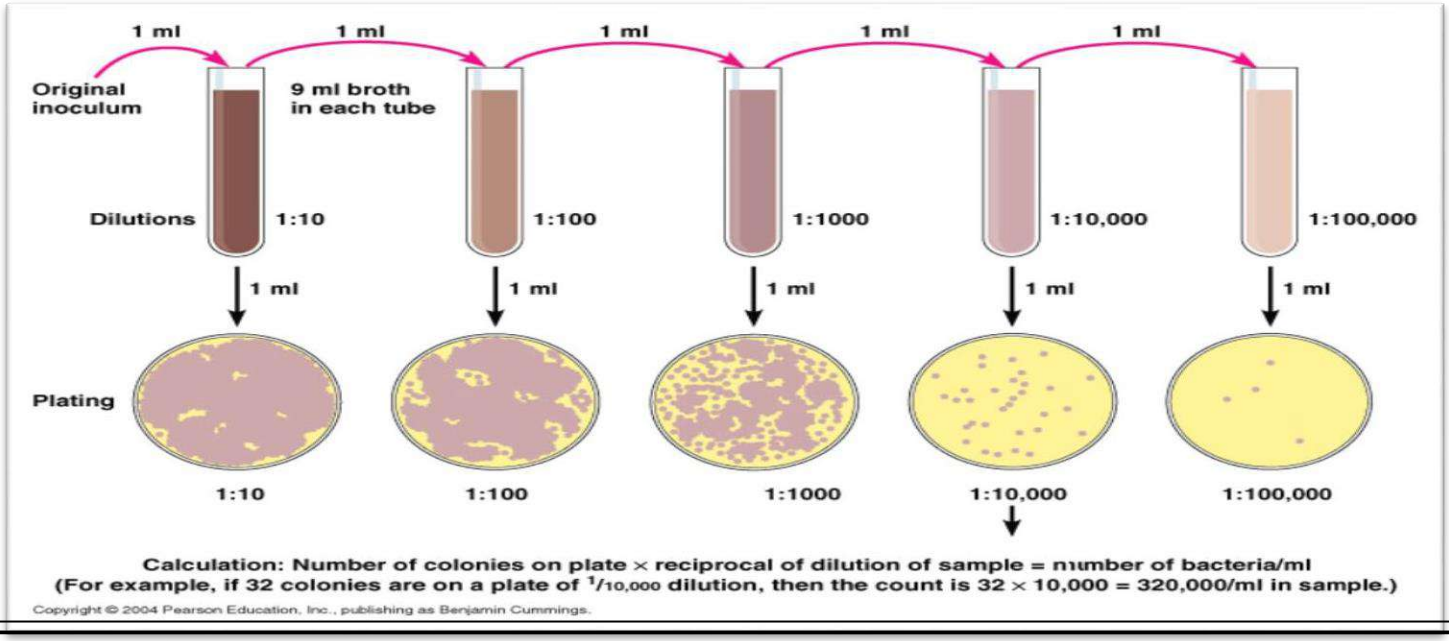


Viable plate count

In this methods , serial dilutions of sample containing viable M.O. are plated onto suitable growth medium. This method is used to determine the viable population in sample containing bacteria.

After the culturing , the plates are then incubated under conditions that permit microbial reproduction. It is assumed that (each bacterial colony arises from individual cell that has undergone cell division)

Thus , by counting the no. of colonies and counting for the original dilution factor, the number of bacteria in the original sample can be calculated , which is a way to obtain pure culture , but the major disadvantage of this method that is selective because there is no universal set of conditions that permits the growth of all M.O.



Dilution to extinction

Dilution are achieved by adding an aliquot of the specimen to sterile water tube

1-if 1 ml of sample is added to 9 ml of sterile water,the dilution is 10^{-1} or adding 0.1 ml of sample to 9.9 ml sterile water , the dilution is 10^{-2}

2-greater dilutions are achieved by sequentially diluting the sample in series.

Or

Adding 0.1 ml from the first dilution(10^{-2}) to 9.9 ml of sterile water , so the total dilution is 1:10000= 10^{-4}

The formula use in dilution is:

Dilution = Part(the transfer volume)/ All(transfer volume+ diluents volume)

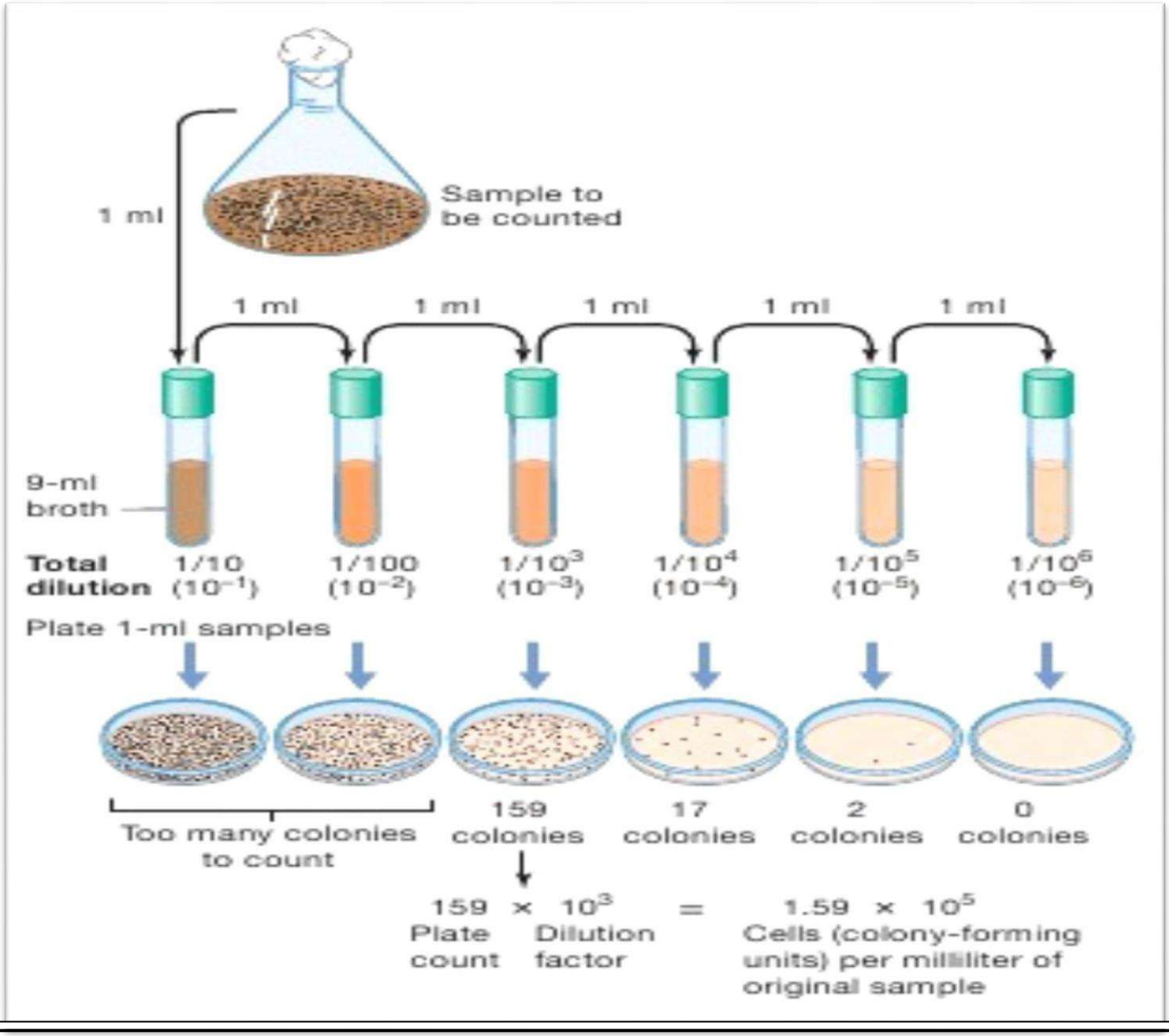
The types of liquids are used as diluents

1-Saline(NaCl=0.85%)

2-Sterile distilled water

3-Peptone water (0.1%)

4-Nutrient broth (N.B.)



Lab -10- Methods of Culturing

Methods of culturing used to:

1-Isolation of M.O. from sample.

2-For culturing M.O. in the sample.

3-Obtain pure culture.

The methods of culturing are :-

1-Pipetting by pipette (in broth medium) 2-Inoculation by loop (in broth medium) 3-Streaking by loop (on solid medium)

4-Stabbing by needle (on solid or semi-solid medium)

5-Swabbing by swab (on solid medium)

6-Pouring plate method (on solid medium)

7-Spreading by spreader (on solid medium)

1-Pipetting by pipette or micropipette

This method was suitable in the dilution to extinction laboratory, the purpose from this method is to count the M.O, (viable plate count).

2-Inoculation by loop

This method was used to increase the number of M.O. in liquid media

Method

1-Hold the tube that contain the broth culture in your hand .

2-Mix the tube that contain the culture to spread the bacteria through the broth

3-Hold the other tube that contain the sterile broth in the same hand that carrying the broth culture .

4-Sterile the loop.

5-Remove the two cotton plugs of the tubes by thehand that carrying the loop.

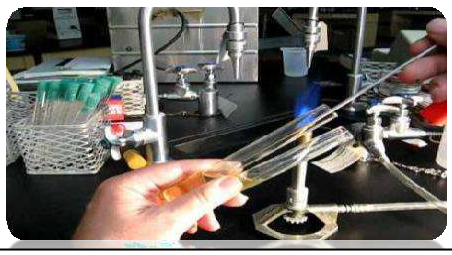
6-Sterile the upper of the tubes by flame.

7-By the sterile loop , transfer drop from broth culture to sterile broth.

8-sterile the upper of the tubes and close them .

9- Sterile the contaminated loop with flame.

10-Put the new cultured tube in the incubator at 37C° for 24 hr.



3-Streaking by loop

Streaking on agar plate in order to isolate bacteria , this will enable you to isolate pure cultures of bacteria by obtaining isolated pure colony.

A-Streaking on plates

-ABCD method:

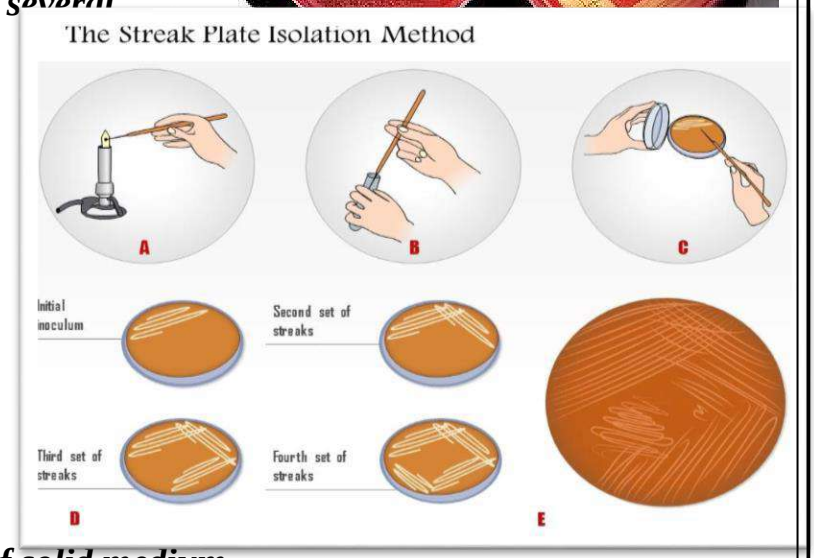
1-Transfer by sterile loop colony from solid medium or drop from broth medium to solid medium in petri dish near the edge , spread it to become point A

2-Sterile the loop and cool it in the edge of solid medium.

3-Rotate the dish , then touch the A area by sterile loop ,vertically draw several parallel lines to form in their end point B .

4-Repeat step 2.

5- Rotate the dish in the same direction, and touch the B point by sterile loop , vertically draw several parallel lines to form in their end point C .



6-Repeat step 2.

7- Rotate the dish in the same direction, and touch the C point by sterile loop , vertically draw several parallel lines to form in their end point D .

8- From D draw one zigzag line to the middle of dish.

9-Repeat step 2.

10-Put petri dish in the incubator at 37 C˚ for 24hr.

-Continuous streaking

1- Sterile the loop and cool it in the edge of solid medium.

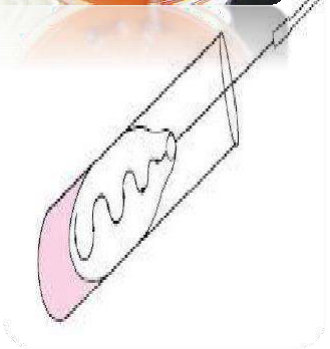
2-transfer the culture to the solid media

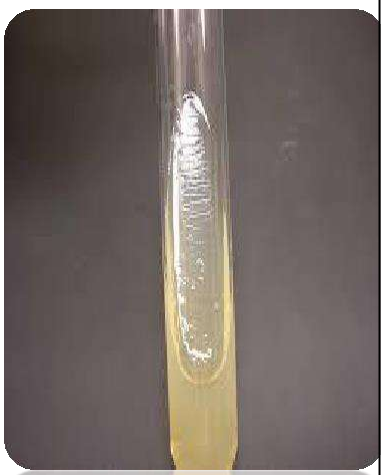
3-spraed the culture as continuous parallel lines to the end of the dish.

4-Sterile the loop.

5-Incubate at 37C˚ for 24 hr as inverted dish.

B-Streaking on slant





1-By sterile loop , take a colony from solid medium or drop from cultured broth medium.

2-Remove the cover of slant tube and sterile the upper part.

3-cultivate on the slant medium by streaking , then sterile the tube and close it.

4-sterile the loop by flame and then put the slant in the incubator at 37C˚ for 24 hr.

4-Stabbing by needle

This method is used to study the ability of bacteria to:-

1-Grow with presence O_2 or not.
2-Motility.
3-Production of some chemical compounds during the metabolism.

Method

1-Sterile the loop by flame .
2-Hold one colony or part from bacterial growth from solid medium by needle.
3-Open the tube that contain sterile semisolid medium and sterile the upper of tube.
4-Stab the needle that contain bacteria in the medium vertically ,then hold it directly.
5-Sterile the upper of the tube again and close it.
6-Sterile the needle by flame.
7-Incubate the tube at 37C˚ for 24 hr

5-Swabbing by swab

Used to obtain heavy growth for some biochemical tests such as sensitivity test and for colony counting

Method

1-Submerge the sterile cotton swab in bacterial broth culture.
2-Swab on the sterile solid medium parallel lines over the medium.
3-Rotate the plate and repeat step 2
4-Put the swab on alcohol (used for one time).
5-Incubate the tube at 37C˚ for 24 hr

6-Pouring plate method

This method is used for counting the colonies

Method

1-Prepare serial dilutions from 10^{-1} to 10^{-5} of sample
2-Transfer by sterile pipette 0.1 ml of each dilution to sterile petri-plate
3-Prepare sterile solid culture medium and cooled it to 45-50 C˚ in water bath
4-Aseptically pour agar into each petri-plate , swirl the plate to mix the sample with the agar.
5-Allow the agar to cool and solidify.
6-Incubate the inverted plate at 37C˚ for 24 hr

Spreading by spreader

Used to spread the bacteria on the surface of the solid medium for colony counting Method

1-Prepare 3 dilutions (10^{-1} , 10^{-2} , 10^{-3}) from sample.

2-Prepare nutrient agar plates and label them.

3-Using sterile pipette , transfer 0.1 ml from each of dilution tubes and the original sample to agar plates

4-Sterilize the glass spreader by putting it in alcohol and burn it by burner.

5-After the cooling of spreader , spread the sample over the surface of the plate.

6-Incubate inverted plates at 37C˚ for 24 hr