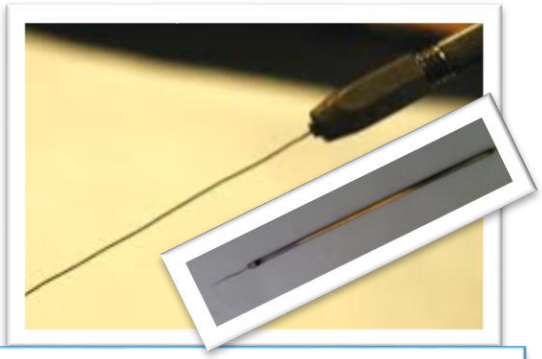
Instruments and equipments of the lab



Needle – Straight Loop (metal)

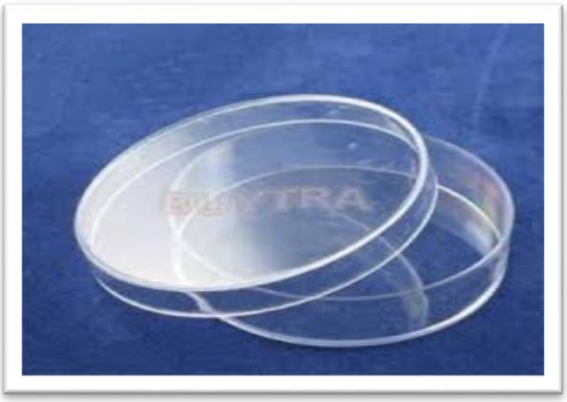
inoculation of media in tubes

wire Loop (plastic/metal)

inoculation of media in plate & tubes

Pipette (glass/plastic)

Transfer of measured volumes of solutions



Petri dish (plastic/glass)

Culturing media for bacteria

Flasks

Preparation of media

Cylinder

measure the volume of liquids

Bunsen burner

Sterilization of wire loops and metal forceps

Marker pen

Labeling Petri dishes, test tubes, flasks, bottles and microscope slides

Balance

Weight of the amount of media (powder)

Autoclave

Sterilization of media, solutions and equipment

Hot air oven

Sterilization of glass Petri dishes and pipettes and paper discs

Incubator

Incubation

Refrigerator

Storage

Sterilization and Disinfection

Decontamination

- Any procedure that reduces pathogenic microbes to a level where items are safe for handling & disposal.
- It can be achieved by cleaning, disinfection and sterilization.

Cleaning

- A process that removes foreign material (dirt, organic matter).
- Must precede disinfection and sterilization.
- Usually done with soap and water or detergent.

Disinfection: destruction of most but not necessarily all pathogenic microbes or their spores.

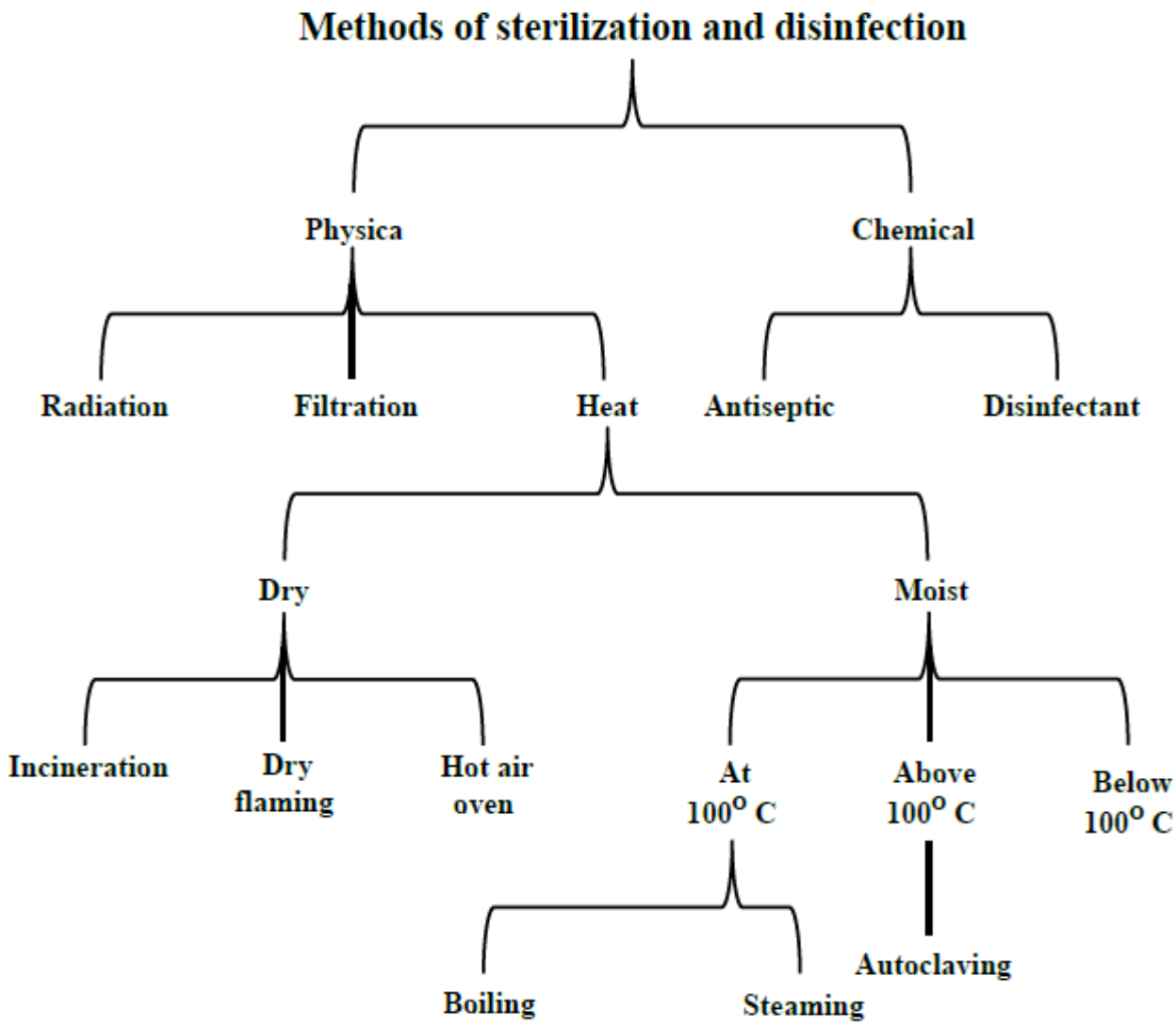
Sterilization: killing of all living forms of microbes including spores.

• **Antiseptics:** Chemical compounds that could be applied topically on animate surfaces.

• **Disinfectants:** chemical compounds applied for inanimate surfaces.

Importance of sterilization and disinfection

1. Safety in the laboratory.
2. The patient safety depends on using proper methods of sterilization to prepare instruments, needles, IV fluids.
3. The accuracy and validity of microbiological tests.



Disinfection

1. Heat

a. Moist heat at temperature below 100 degree *(Pasteurization).*

b. Moist heat at temperature of 100 degrees:

- Boiling
- Steaming
- Tyndallization

2. Radiation: (Ultraviolet rays)

3. Chemical: (Disinfectants)

1. Disinfection by heat

a. Moist heat at temperature below 100 degree

- Used for milk disinfection.
- Milk is heated either at 63 º C for 30 minutes or 72 º C for 20 seconds and immediately cooled to below 10 º C.

b. Moist heat at temperature 100 degree

Boiling

- Heating at 100 º C for 20 minutes.
- Used for disinfection of surgical and medical equipment in emergency.

Steaming

- Done in Koch's sterilizer.
- This sterilizer is vertical metal cylinder with removable conical lid having a small opening for escaping steam.
- The articles to be sterilized are placed on a perforated tray situated above water which is placed in the bottom of the cylinder.

Tyndallization

- Intermittent sterilization by exposure to steam at 100 º C for 20 - 45 min. for three successive days.
- Used for sterilization of sugar media which decompose at high temperatures.
- The principle is that one exposure will kill only vegetative bacteria. Between heating, the spores will vegetate to be killed during subsequent exposure.

2. Disinfection by radiation

Ultraviolet rays

- Present in sun rays or artificially produced by mercury lamp have weak penetrating power.
- Used to reduce the number of bacteria in air inside operation rooms, laboratory safety cabinet.

4. **Disinfection by chemical disinfectants**

Disinfectants may be:

a. High level disinfectants.

b. Intermediate level disinfectants.

c. Low level disinfectants.

a. High level disinfection

- large number of spores after prolonged exposure
- Vegetative bacteria
- Tubercle bacilli
- Fungi
- Viruses

b. Intermediate level disinfection

- Few number of spores
- Vegetative bacteria
- Tubercle bacilli
- Fungi
- Enveloped viruses (HBV, HIV).

c. Low level disinfection

- Mainly vegetative bacteria
- Some fungi
- Narrow range of viruses

Examples of Chemical Disinfectants

1. Alcohol (Ethyl alcohol or isopropyl alcohol 70%)

- Used as skin antiseptic.
- Intermediate level disinfectant.
- Exposure for at least 5 minutes is needed to achieve adequate disinfection.

2. Glutaraldehyde

- Available in 2% concentration.
- High to intermediate level disinfectant.
- Disinfection of instruments that cannot withstand heat such as endoscopes.

3. Chlorine releasing compounds

- Hypochlorite solution (such as household bleach)
- Intermediate level disinfectant.
- Widely used in homes, hospitals and laboratories to disinfect table tops, incubators, spilled cultures.
- Disinfection of water supply.

4. Iodophores

- Tincture iodine (2% iodine in ethanol)
- Betadine (10% povidone-iodine)

- Intermediate to low level disinfectant.
- Used for disinfection of surgical wounds.

5. Phenolic compounds

- Dettol, lysol (2% concentration)
- Intermediate level disinfectant.
- Used in laboratories to disinfect spilled cultures on working areas or in discard jars.

6. Quaternary ammonium compounds

- Cetavlon, savlon
- Low level disinfectant.
- Used to clean floors, walls.
- Inactivated by organic matter.
- Not affect Gram negative bacilli.

7. Hydrogen peroxide

- Available as 3% H2O2
- High to intermediate level disinfectant.
- Used as antiseptic for wounds, disinfectant for contact lenses.

8. Heavy metals

- As mercury in mercurochrome and silver in silver nitrate.

9. Chlorhexidine

- Safe antiseptic used for hygienic and surgical hand washing and for oral hygiene.

Sterilization

1. Chemical methods (Cold sterilization)

2. Heat

- Dry heat (Red heat, hot air oven, incineration)
- Moist heat at temperature above 100 degree (Autoclave)

3. Radiation: (Ionizing radiation & infrared rays)

4. Filtration: (Fluid filters & air filters)

5. Gaseous sterilization: (Ethylene oxide + gas plasma)

1. Chemical sterilization

- Glutaraldehyde (contact time 10 hours).
- Liquid peracetic acid.
- H_2O_2 6%.

2. Sterilization by heat

a- Dry heat

- Less effective than the moist heat.
- Examples of dry heat are:

1) Red heat

- Sterilization of the bacteriological loop by heating in the Bunsen flame or electric incinerator till becomes hot red.

2) Hot air oven

- Temperature of 160 º C for 2 h. or 170 º C for 1 h.
- For glass ware, and metallic instruments.

3) Incineration

- Destruction of contaminated materials in the incinerator.

b- Moist heat (Autoclave)

Autoclaving is highly efficient because:

- High temperature.
- High penetrating power of the steam under pressure.
- When steam condenses on the articles, it liberates latent heat to the articles to be sterilized.
- Non toxic
- Not time consuming.

3. Sterilization by radiation

- Ionizing radiation:
- Such as gamma rays emitted from radioactive cobalt 60 or beta rays emitted from electron accelerators.
- High penetrating power.
- Used for plastic syringes, catheters, gloves, surgical sutures.

4. Sterilization by filtration

1. Fluid filters

- Used for sterilization of biological fluids destroyed by heat such as serum, plasma, vitamins, hormones.

2. Air filters

- Air is filtered by HEPA filters.
- HEPA means high efficiency particulate air way arresters.
- Air filtration is needed in operation rooms, safety cabinets.

5. Sterilization by gases

1. Ethylene oxide gas

- Used for plastic and rubber articles.
- Ethylene oxide gas is toxic, explosive and carcinogenic to laboratory animals.

2. Plasma gas sterilizer

- Plasma means any gas which is formed of ions, electrons, neutral particles.
- Used for surgical instruments mainly those with narrow lumen such as arthroscopes & laparoscopes.
- Non toxic

The bacterial culture media

What's meaning Media?

Its meaning: nutrient environment for micro-organisms growth.

Media = plural

Medium = singular

The necessary factors for bacterial growth:

1. Suitable temperature:

a. Thermophilic bacteria: the bacteria that like high temperature.

b. Mesophilic bacteria: the bacteria that like mild temperature.

c. Psychrophilic bacteria: the bacteria that like low temperature.

2. Suitable pH: most pathogenic bacteria live in mild pH and some of them live in acidic environment as milk bacteria.

3. Suitable aerobic condition:

a. Aerobic bacteria (Aerobic obligate bacteria).

b. Anaerobic bacteria (Anaerobic obligate bacteria).

c. Facultative bacteria (It can live in aerobic or anaerobic conditions)

4. Suitable humidity.

5. Suitable nutrient substances: such as carbohydrates, protein, nitrogen, carbon, fats, mineral salts, enzymes, water …

Types of media:

We can divide the media into two groups:

i. According to stature:

1. Liquid (broth) media: the media without agar or gelatin.

2. Solid media: the media with agar.

3. Semi-solid (semi-liquid) media: the media with gelatin or insufficient agar.

ii. According to chemical composition:

1. Simple ordinary media: it consists of simple materials, such as Nutrient agar and Nutrient broth.

2. Enrichment media: it's simple ordinary media with enriched nutrient substances eg. blood, serum, plant extracts or animal extracts, such as Blood agar, Serum agar, and Milk agar.

3. Selective media: it contains chemical substances with specific concentration which led to growth group of bacteria and prevents growth another group, eg.

Crystal violet and bile salts, such as MacConkey agar, S.S. agar (**S**almonella **S**higella agar)

4. Differential media: these media led to growth two or more of bacterial types, such as Blood agar, where we can define haemolytic bacteria from non-haemolytic bacteria. Also MacConkey agar which contains bile salts that kill all Gram-positive bacteria and and we can determine lactose fermentation Gram-negative bacteria from non- lactose fermentation.

5. Special media: these media contain specific substances which led to growth one genus of bacteria, such as Cooked meat medium, this medium leads to growth anaerobic bacteria eg. *Clostridium* spp.

6. Biochemical media: these media use to define species of bacteria by change colors of media, such as Simmon's Citrate agar, **K**liglar **I**ron **a**gar (KIA).

Preparation of cultural media (1)

Procedure:

1. Weigh powder of medium as instructions on bottle of medium.

2. Add the powder to specific quantity of distilled water inside flask.

3. Adjust the pH degree by pH paper or pH system.

4. Distribute the solution into test tubes and cover them. (If the medium is broth)

5. Put the flask or test tubes inside autoclave at 121 ^{0}C for 15 minutes under 1.5 bar.

6. Out them from autoclave and wait until cool slightly, then pour it in sterilized petri dishes (if the medium is solid) in atmosphere without air streams on sterilized table beside Bunsen burner.

7. Leave the petri dishes until solid and put them inside refrigerator until use.

Example:

Nutrient agar 28 grams per 1000 ml of distilled water, when we want to prepare 250 ml of medium:

Powder of medium Distilled water

28 g ⟶ 1000 ml

X ⟶ 250 ml

$$X = \frac{28 \times 250}{1000} = 7 \text{ g.}$$

Preparation of Nutrient agar:

Suspend 28 g of nutrient agar powder in 1 litre of distilled water. Bring to the boil to dissolve completely. Dispense as required and sterilize.

Preparation of MacConkey agar:

Suspend 50 g of the medium in one liter of distilled or deionized water. Mix well and heat with frequent agitation. Boil for one minute. Sterilize in an autoclave at 121^{o} C (15 lbs) for 15 minutes. Cool to 45 - 50°C and pour into Petri dishes, 20 ml in each dish. Allow to solidify and later invert the dishes to avoid excess moisture on the surface of the medium.

Preparation of Blood agar:

Blood Agar consists of a base medium which is Nutrient agar containing 5% sheep blood (5 ml of blood per 100 of Nutrient agar).

Procedure for the preparation of Blood Agar

1. Prepare the Blood Agar base as instructed by the manufacturer. Sterilize by autoclaving at 121$_o$C for 15 minutes.
2. Transfer thus prepared Blood Agar base to a 50^{o}C water bath.
3. When the agar base is cooled to 50^{o}C, add sterile blood agar aseptically and mix well gently. Avoid formation of air bubbles. You must have warmed the Blood to room temperature at the time of dispensing to molten agar base.
4. Dispense 15 ml amounts to sterile petri plates aseptically
5. Label the medium with the date of preparation and give it a batch number (if necessary).
6. Store the plates at 2-8 ^{o} C, preferably in sealed plastic bags to prevent loss of moisture. The shelf life of thus prepared Blood Agar is up to four weeks.

Preparation of Chocolate agar:

- Chocolate Agar is the lysed blood agar. The name is itself derived from the fact that Red blood cell lysis gives the medium a chocolate-brown color.
- The composition of Chocolate agar and the Blood Agar is same and the only difference is while preparing Chocolate agar, the red blood cells are lysed.

- The lysis of RBC releases intracellular nutrients such as hemoglobin, hemin (Factor X) and the coenzyme Nicotinamide adenine dinucleotide (NAD or V Factor) in to the agar for utilization by fastidious bacteria.

Procedure for the preparation of Chocolate agar

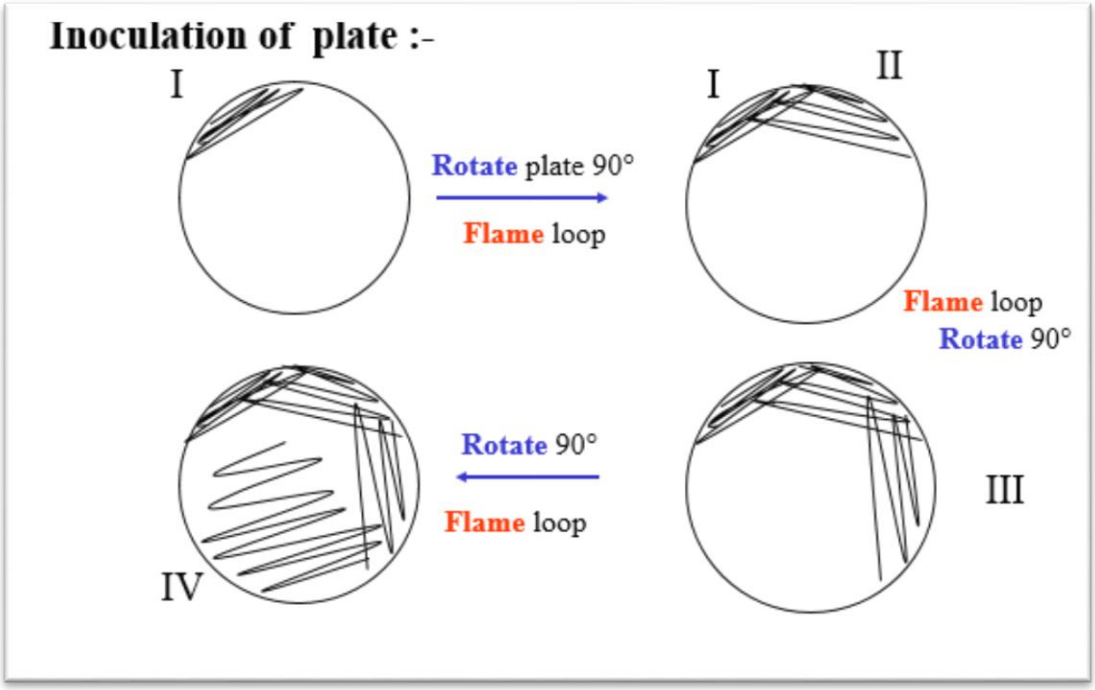
1. Heat-lyse a volume of horse or sheep blood that is 5% of the total volume of media being prepared very slowly to 56°C in a water bath.
2. Dispense 20 ml into 15x100 mm Petri dishes. Allow the media to solidify and condensation to dry.
3. Place the plates in sterile plastic bags and store at 4ºC until use.

Isolation of bacteria

Streak plate (loop dilution) method:

This is better way to obtain single colony.

Method:

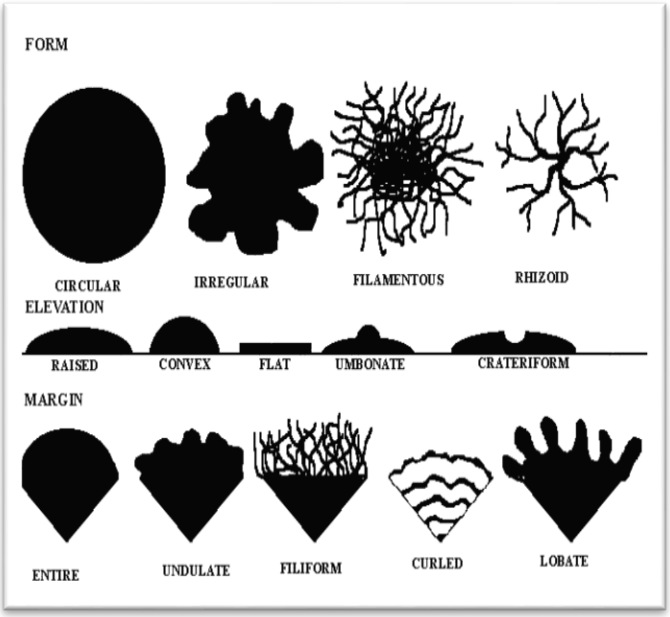


Identification of bacteria

You can identify bacteria by following steps:

1- Colonial morphology: You will see individual colonies and describe using the following criteria:

- Shape: round, irregular, punctiform.
- Elevation :(convex ,flat , raised …
- Color: white, yellow.
- Edge: entire , filamentous , undulate..
- Opacity: Transparent, Opaque …
- Surface: smooth, Glistening, Rough.
- Consistency: Viscous, Brittle, Dry…



2- Microscopically:

Staining

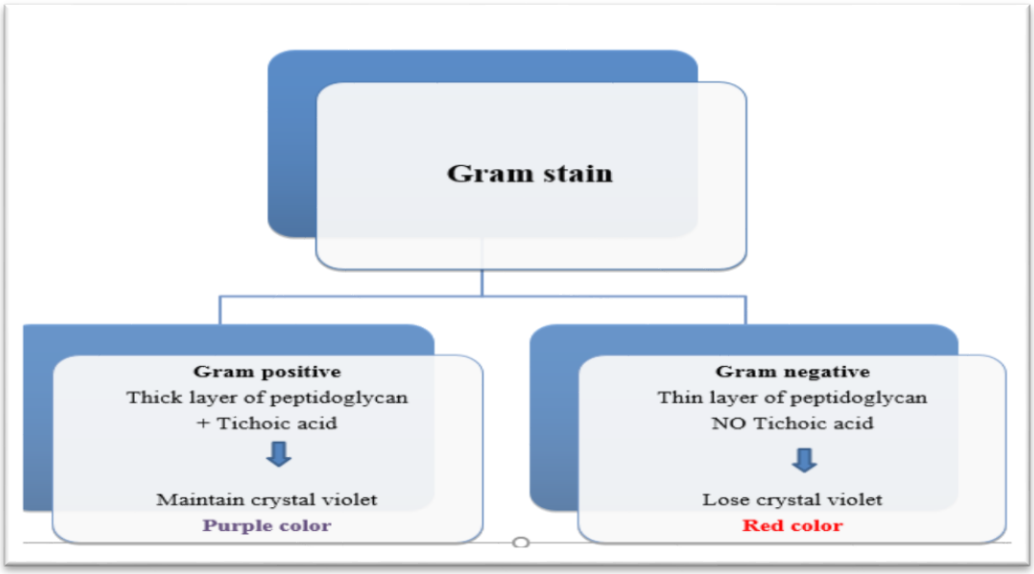
Unstained cells are difficult to see staining cells makes them easier to see

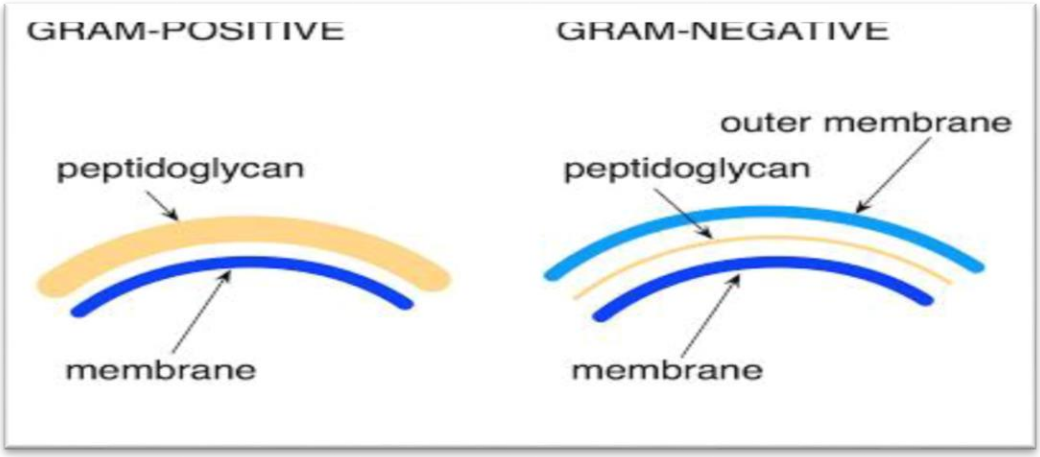
Types of stains:

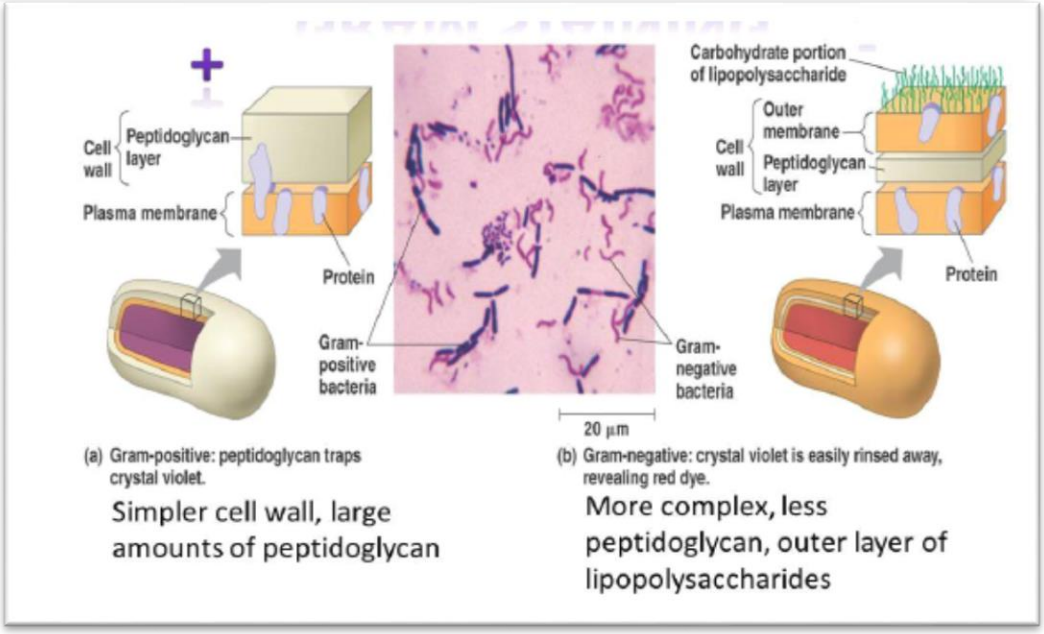
- Simple stains: Use of a single stain e.g, Methylene blue
- Differential stains: react differently with various organisms, can differentiate one from another reaction based on the chemical composition of organisms. e.g, Gram stain and Acid fast stain
- Special stains: e.g, Albert's stain; used to demonstrate metachromatic granules found in Corynebacterium diphtheriae.

Gram stain

Principle:







Components of Gram Stain:

- Primary stain (**Crystal violet**, methyl violet or Gentian violet)
- Mordant (**Gram's Iodine**)
- Decolourizer (**ethyl alcohol**, acetone or 1:1 ethanol-acetone mixture)
- Counterstain (Dilute carbol fuchsin, **safranin** or neutral red)

Method

Preparation of smear

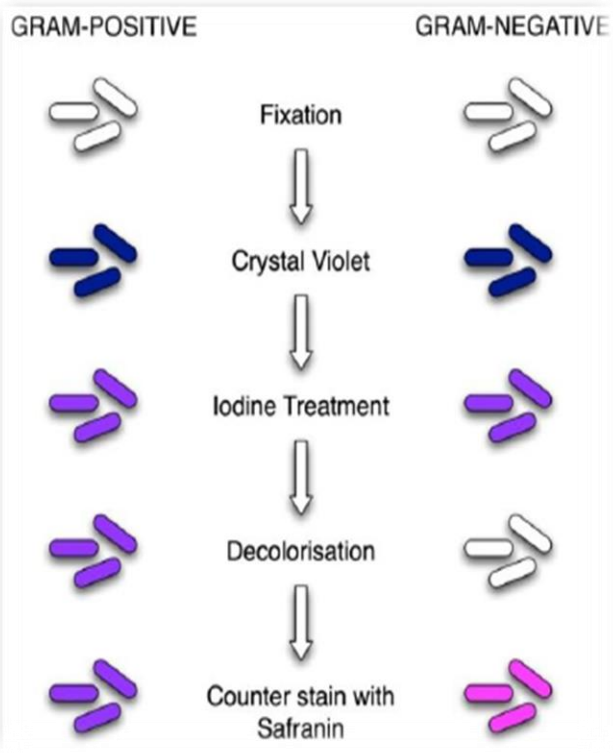
- Put one drop of water on slide + little amount of bacteria

Fixation of smear

- By using flam
- To kill the microorganism & fix them to the slide

Staining

1. The slide is flooded with crystal violet stain, for 1 min
2. Pour off the stain, wash in water.
3. Apply iodine solution, for 1min.
4. Pour off iodine, wash in water.
5. Decolorize with a few drops of ethanol, for not more than 4 seconds.
6. Wash thoroughly in water.
7. Counter stain with safranin for 1min.
8. Wash and dry it.



Result: gram positive look like a dark blue /purple color, and gram negative look red.

Ziehl Neelsen's Method

This stain use for acid-fast bacteria like *Mycobacterium* spp. which has a high concentration of lipids in its cell wall.

The reagents:

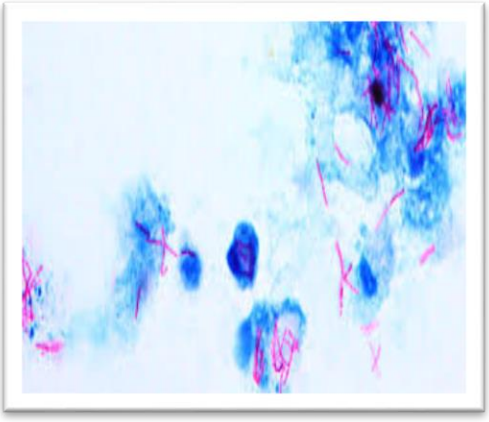
1- carbol fuchsin,

2- acid alcohol.

3- malachite green or methylene blue

Method of staining:

1. Prepare the film and fix it.
2. Apply carbol fuchsin to cover the film then heat the slide until the stain evaporation .and repeat the heating for 4 minutes.
3. Cool and rinse with water.
4. Decolorize with acid alcohol until the film become dark color.
5. Rinse with water.
6. Counter stain with Methylene blue for about 2 min.
7. Dry the slide, then examine it.

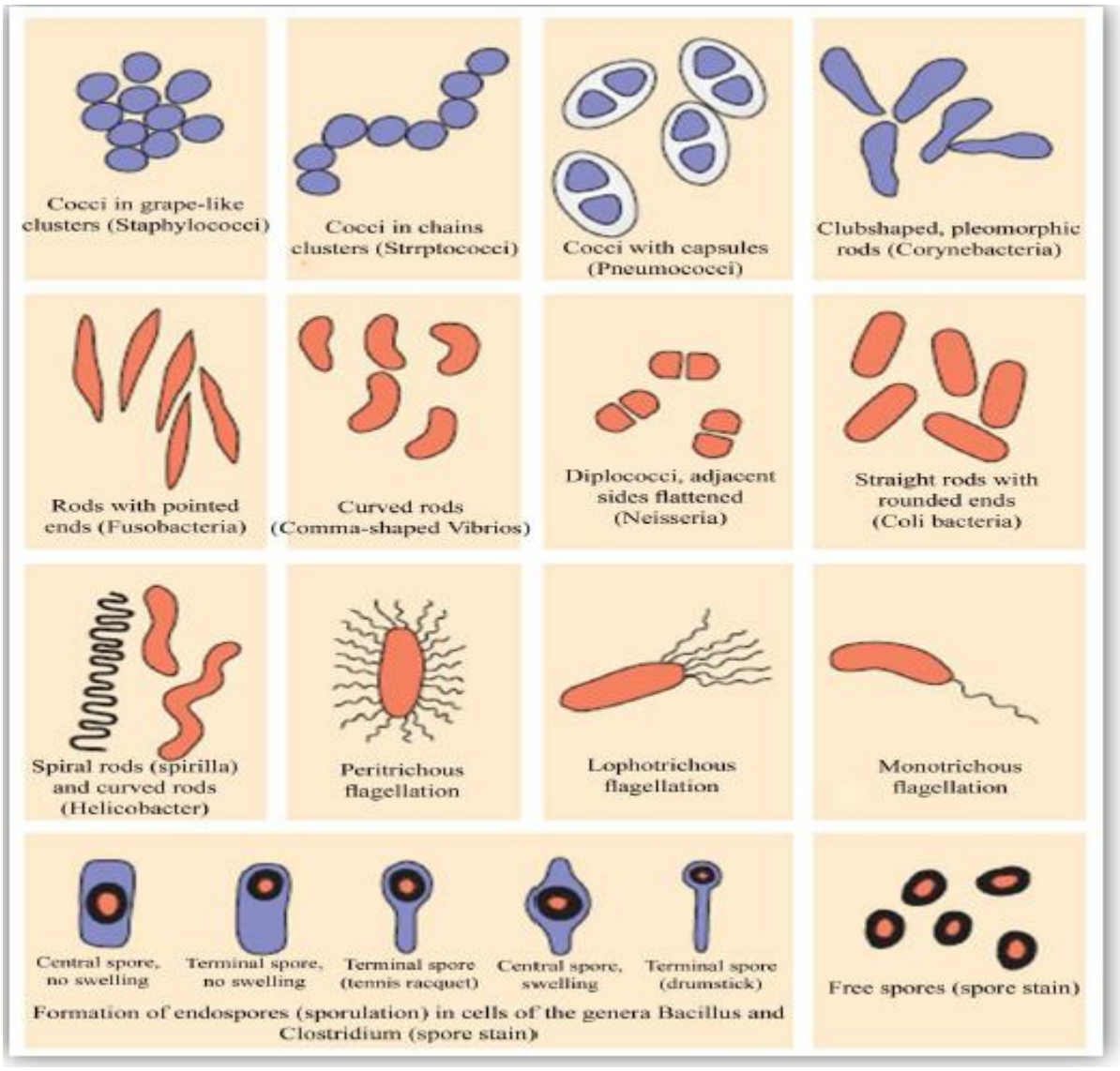


<u>The Result:</u>

- Acid-fast bacteria appear with red color
- Non- Acid-fast bacteria appear with blue color
- The background will be blue color.

Shape of Bacteria:

1. Cooci (monococci, diplococcic, streptococci, staphylococci)
2. Bacilli.
3. Coccobacilli
4. Vibrios
5. Spirills
6. Spirochetes.
7. Fusiform bacilli



Shapes of Bacteria

Good luck