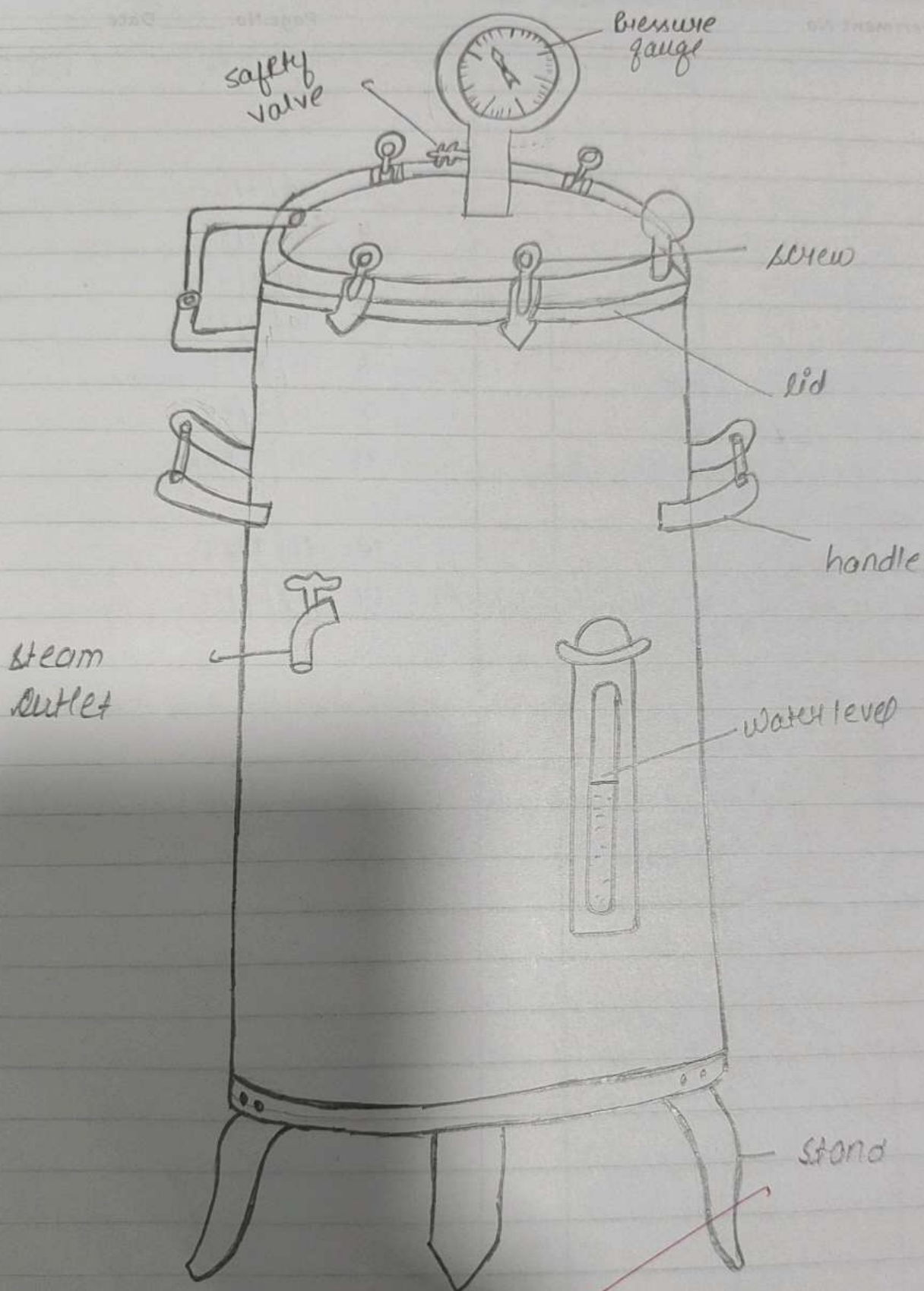


* AUTOClave:-

1. It is an equipment used for sterilizing the glassware, culture media, etc. in the laboratory.
2. It is also called steam pressure sterilizer.
3. The culture medium, glassware or any other article to be sterilized is placed in the autoclave, the door is closed, and steam under pressure is admitted.
4. For obtaining enough heat to destroy all spores and vegetative cells, it is essential to remove all the air from chamber of the autoclave just at the start of the sterilizing process.
5. The pressure most commonly used in the autoclave is 15 pounds per square inch in excess of the atmospheric pressure, which gives a temperature of 121°C [250°F] at sea level. This temperature is maintained usually for 15-20 minutes.
6. The steam valve and the discharge valve are closed at the end of the sterilizing period, and the autoclave is allowed to cool slowly.

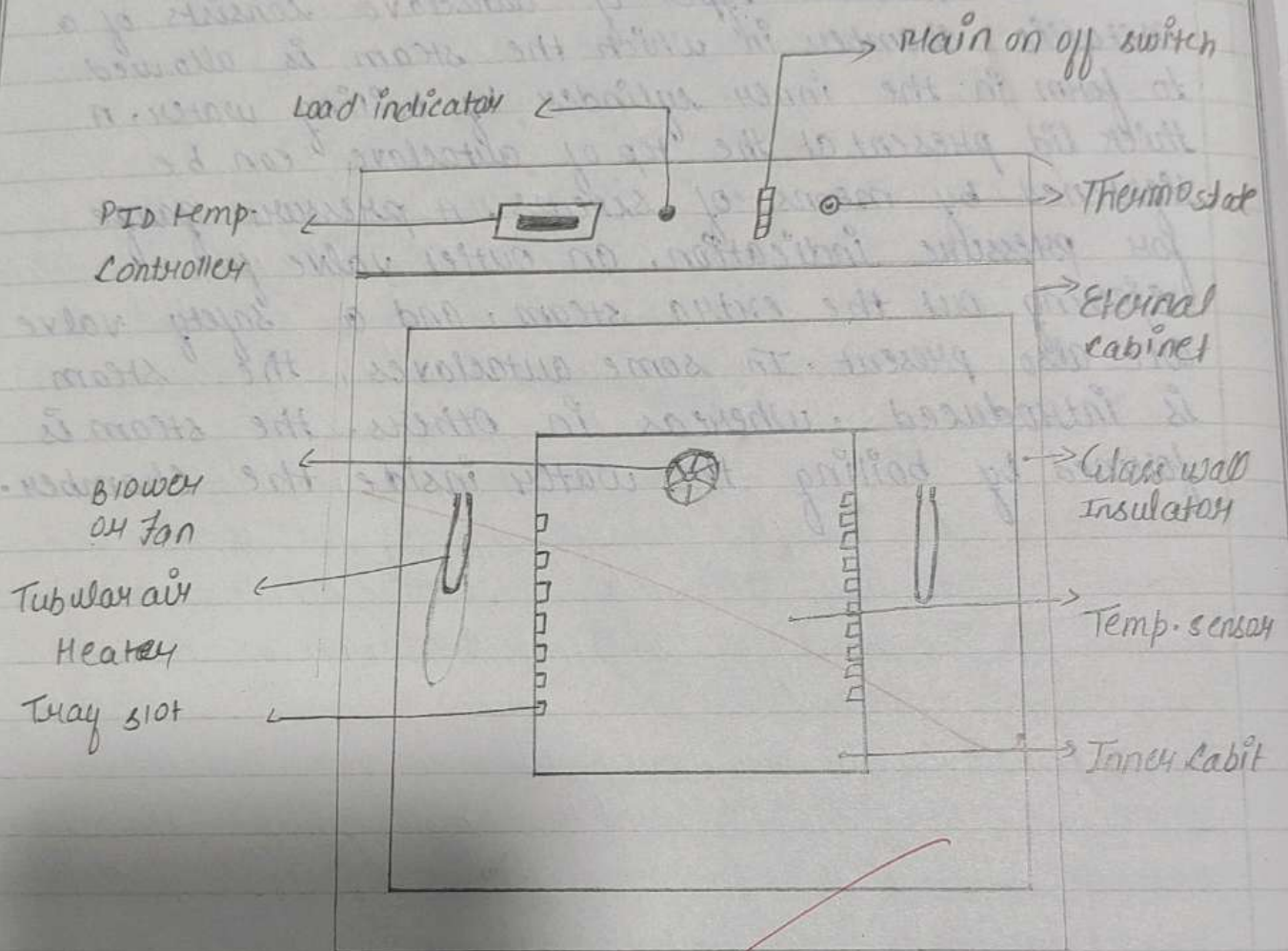


Autoclave

7. Various models of autoclaves are now available. The most common types of autoclave consists of a metallic chamber in which the steam is allowed to form in the inner cylinder by boiling water. A thick lid present at the top of autoclave can be tightened by means of screws. A pressure gauge for pressure indication, an outlet valve for ejecting out the extra steam, and a safety valve are also present. In some autoclaves, the steam is introduced, whereas in others, the steam is formed by boiling the water inside the chamber.

* HOT AIR OVEN

1. The hot air oven is most widely used equipment for sterilization by dry heat.
2. It consists of a metallic chamber made of aluminium or stainless steel. For recording temperature, a thermometer is inserted.
3. It is universally used to sterilize glassware, scissors, forceps, scalpels, glass syringes, swabs and several other products such as liquid paraffin, fats, etc.
4. A holding period of 160°C for one hour is generally used.
5. The oven is usually heated by electricity. The heating elements are present in the wall of the chamber of the oven. It must also be fitted with a fan to ensure even distribution of air.
6. The oven should not be overloaded, and the material in it should be arranged in a manner which allows free circulation of air.



HOT AIR OVEN

in between

7. Glassware should be perfectly dry before being placed in the oven
8. Usually there is no temperature, after the required temperature is reached, its switch should be put off.

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★ INCUBATOR

1. An incubator is more or less similar to an oven in outlook. However, it differs from an oven in the sense that temperature may be controlled in an incubator.
2. An automatic regulator, fitted in the incubator, controls the temperature.
3. The electric power, used in operating the incubator, increases the temperature by heating.
4. Differing from oven, an incubator operates generally at lower temperatures, i.e. from temperature to about 50°C .
5. Cultures of microorganisms, which require specific temperature for their growth and maintenance, are maintained in a incubator.

shelves

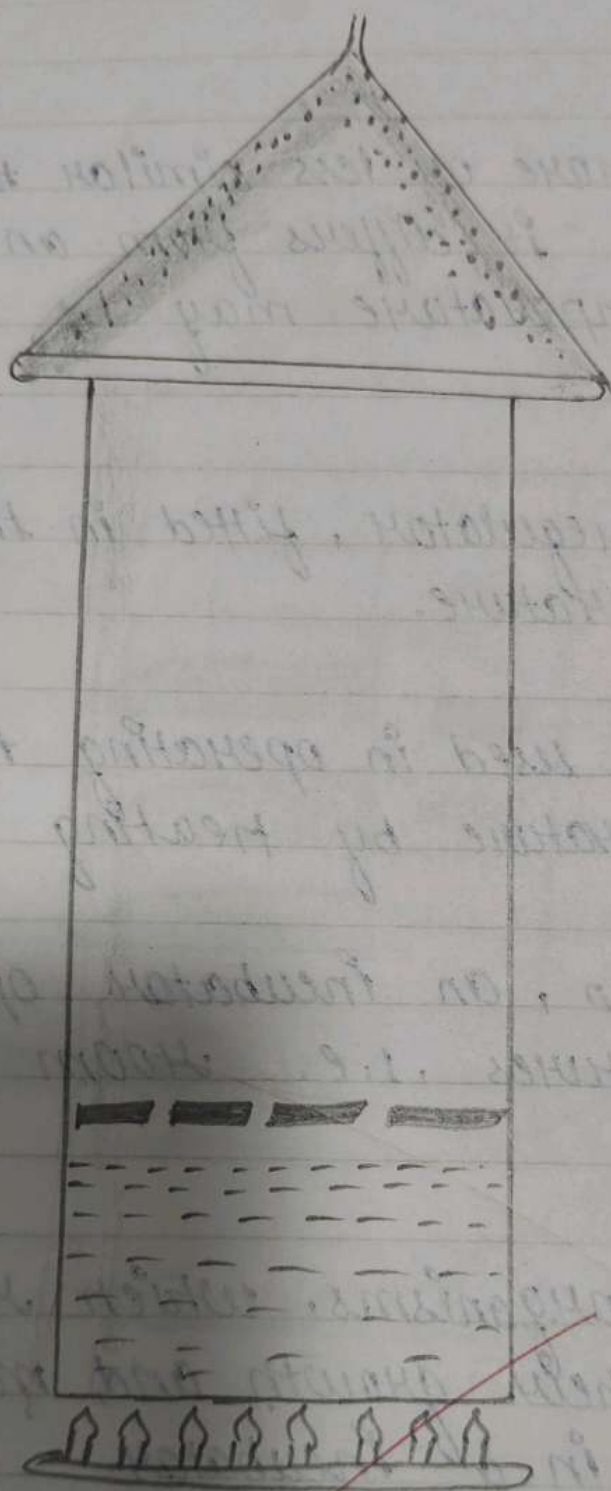
outlamp

Control button

INCUBATOR

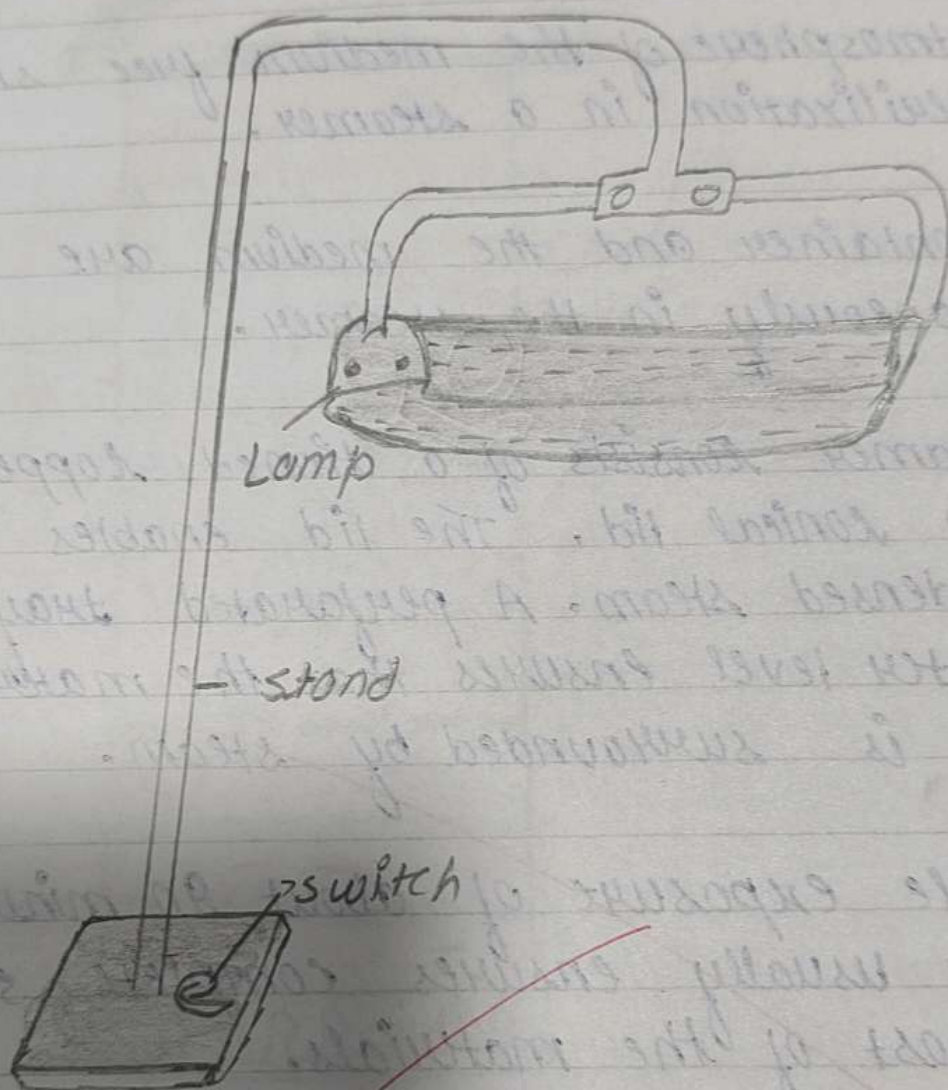
★ STEAMER

- The culture media, which may decompose at higher temperatures, are sterilized in a steamer.
2. An atmosphere of the medium free steam is used for sterilization in a steamer.
3. The container and the medium are sterilized simultaneously in the steamer.
4. A steamer consists of a tinned copper cabinet with a conical lid. The lid enables easy drainage of condensed steam. A perforated tray fitted above the water level ensures that the material placed on it is surrounded by steam.
5. A single exposure of about 90 minutes in a steamer usually ensures complete sterilization of most of the materials.



* Ultraviolet Lamp

1. This is an instrument used for reducing bacterial and mold contamination in laboratories, schools, hospitals, etc. It is also used for sterilizing the apparatuses, tools, chambers and rooms.
2. It emits ultraviolet [UV] light, and hence called UV-lamp.
3. The principle used in UV-lamp is that microorganisms are killed at certain wavelengths. Bacteria are killed by ultraviolet light with a wavelength of 2537 angstroms [Å].
4. Ultraviolet light is injurious to our eyes. The UV-lamp should, therefore, be used with great care. The ultraviolet light, however, travels only in straight line, and therefore the objects which are not exactly under the UV-lamp will not be affected at all.

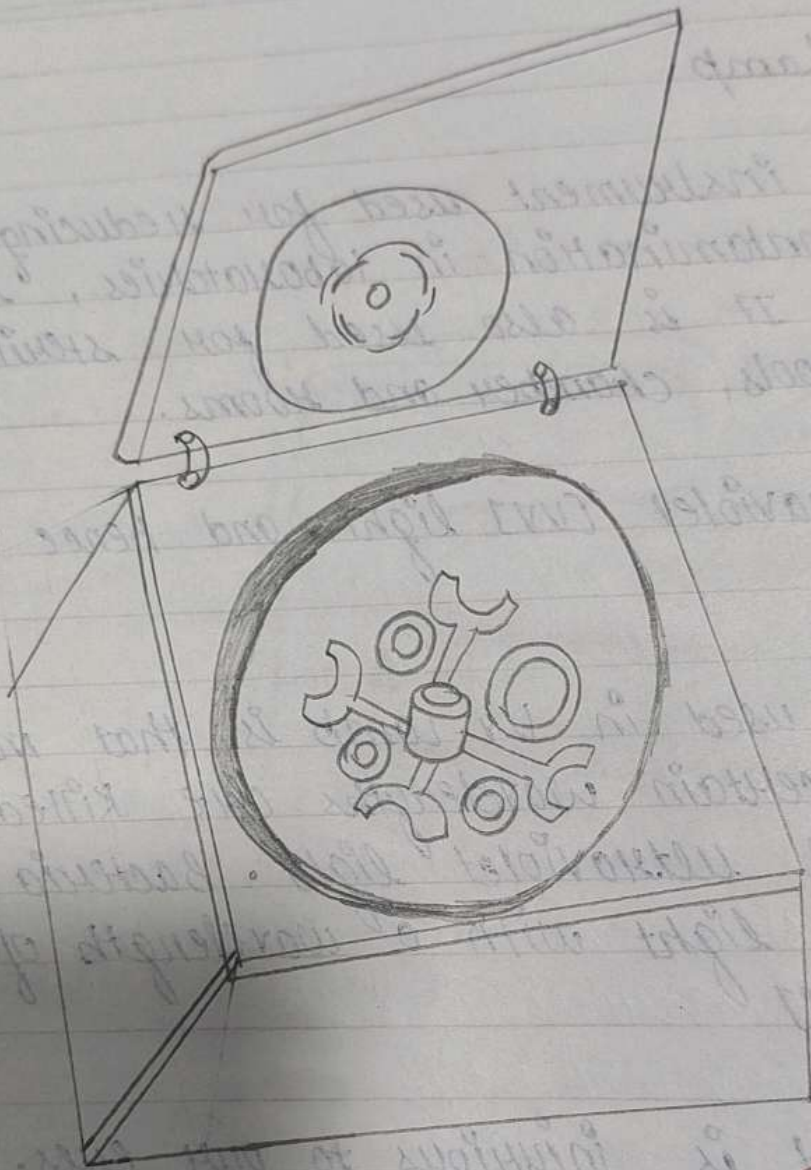


* Centrifuge

1. It is an apparatus that rotates at high speed and separates particles substances on the basis of mass and density by means of centrifugal force.
2. The centrifugal force is noted in revolutions rpm (revolutions per minute)
3. The centrifuge consists of a head which is rapidly revolved by an upright motor
4. usually, 4 cups or containers of metal remain attached to the head for holding tubes or other containers of the material, from which the particulate matter be separated.
5. In the process of centrifugalization, the liquid containing particulate matter settled at the tubes and runs at a particular speed
6. At the completion of centrifugalization process, the particulate matter settled at the bottom of the tubes.
7. Different types of centrifuges commonly used are low-speed or clinical centrifuge, high speed

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ultracentrifuge or superspeed centrifuge

8. Low-speed centrifuges can revolve at 5000 rpm. High speed centrifuges can revolve at 18000 rpm while ultracentrifuges have as much as 20000 to 60000 rpm.

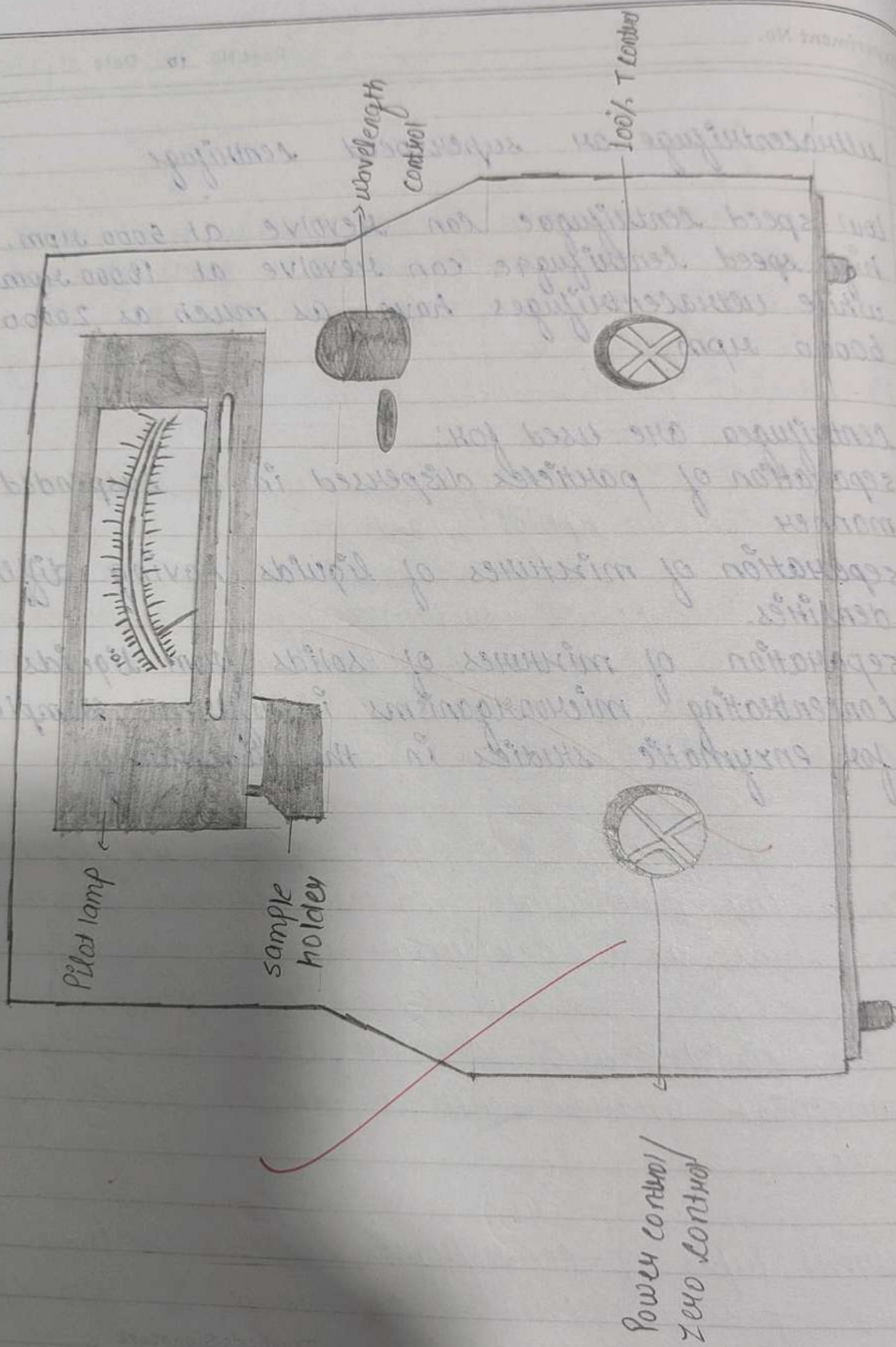
9. Centrifuges are used for:

- (i) separation of particles dispersed in a suspended manner
- (ii) separation of mixtures of liquids having different densities.
- (iii) separation of mixtures of solids from liquids
- (iv) Concentrating microorganisms in different samples for enzymatic studies in the laboratory.

* Spectrophotometer or Colarimeter

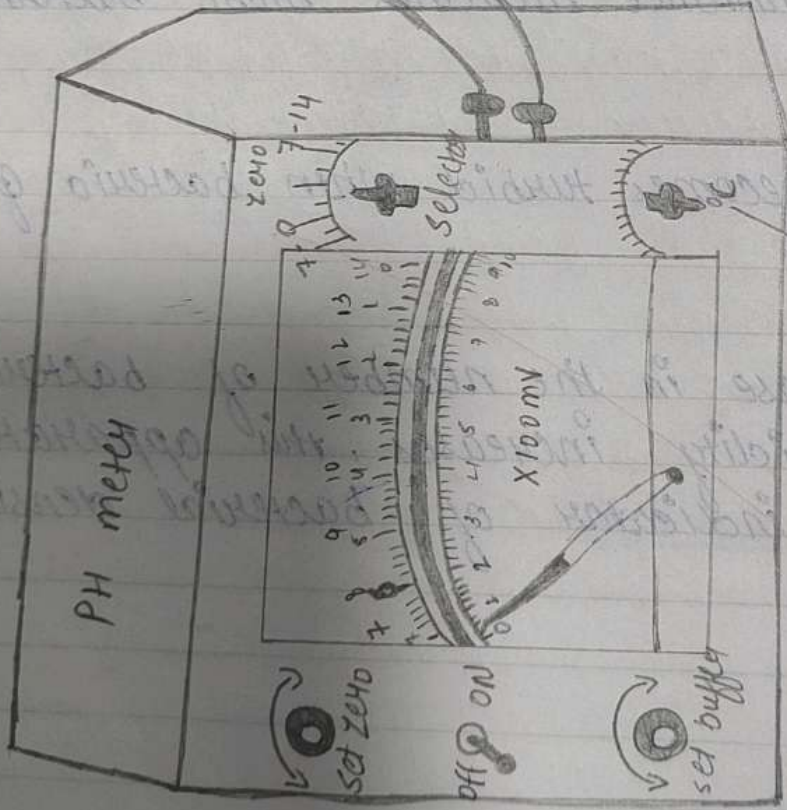
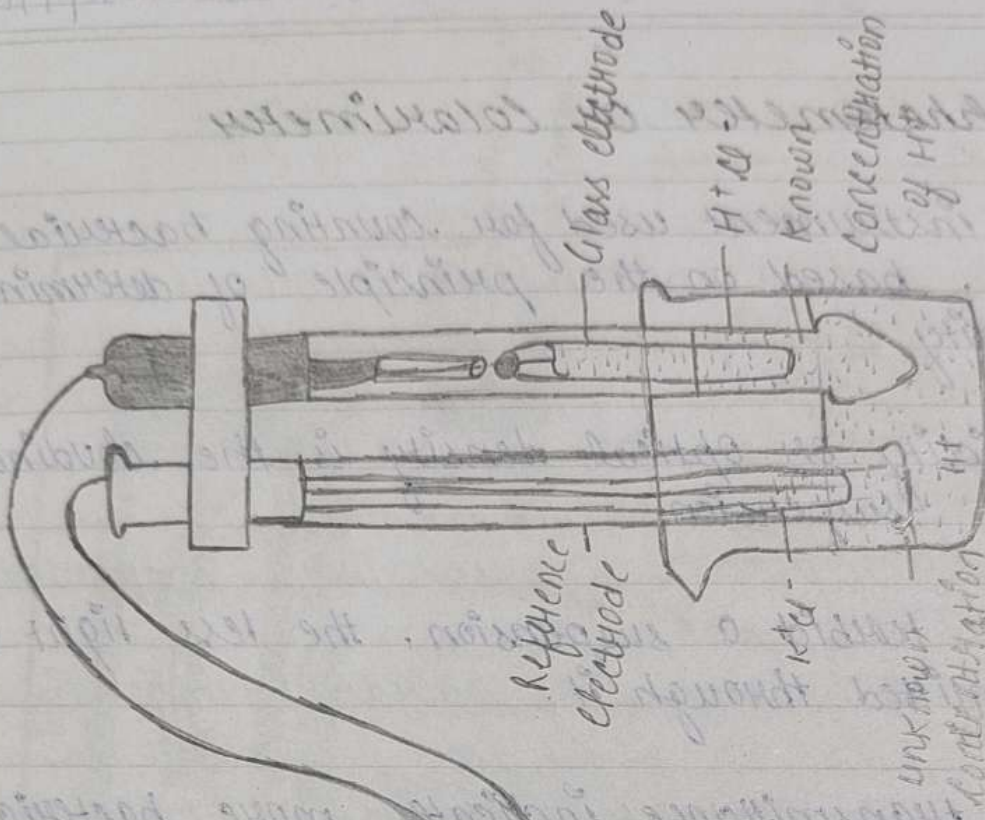
1. It is an instrument used for counting bacterial population, based on the principle of determination of turbidity.
2. The turbidity or optical density is the cloudiness of any suspension.
3. The more turbid a suspension, the less light will be transmitted through it.
4. Less light transmittance indicates more bacteria in the sample.
5. A clear broth becomes turbid when bacteria grow in this broth.
6. Due to the increase in the number of bacterial cells the turbidity increases, this apparatus is used as an indicator of bacterial density in the broth.

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* pH Meter

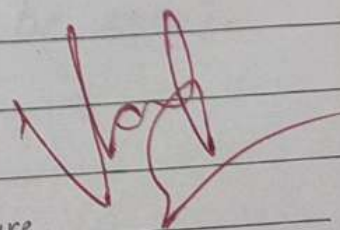
1. pH meter is an instrument used:
 - (i) To determine pH of solutions of unknown pH, and
 - (ii) For setting of pH of various culture media used for the cultivation and kinetic biochemical activity of microorganisms.
2. pH is a measure of how acidic or basic a solution is. It is measured on a scale of 0-14 [where 0 = very acidic, 14 = very basic, and 7 = neutral]
3. In pH, the number [0-14] is an expression of the concentration of hydrogen ions $[H^+]$ in the solution. i.e., higher the concentration the more acidic is the solution. ~~pure~~
4. Pure water has a pH of 7 i.e. neutral
5. The specific range of pH for bacteria is between 4 to 9 with the optimum being 6.5 to 7.5 while that for fungi the optimum being 4 to 6 [i.e. fungi prefer acidic environment]
6. Glass electrodes are used in pH meter



Temperature compensation

7. A glass electrode is made up of an internal sealed tube with a metallic tip. This tip is usually made of silver chloride. There is also present an external tube that contains a standard solution.

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* Observing the milk bacteria:

* Requirements :- Slides, Milk sample, methylene blue, tap water, glycerine, coverslips, microscope, needle.

* Method:

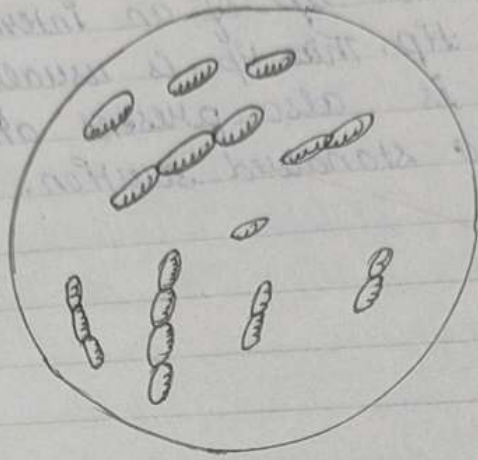
1. Put a drop of milk on a clean and dry slide and spread it to make a thin smear.
2. Allow the smear to dry and stain it for two minutes with methylene blue.
3. Drain off the excess of stain and allow it to dry.
4. Wash with tap water - off the excess of water and make it dry.
5. Put a drop of glycerine and observe it under high power on oil immersion lens of microscope.

* Observations and Result: Following types of bacteria are seen.

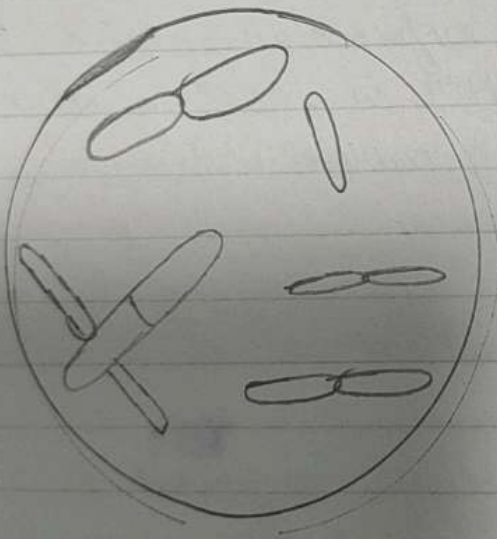
- (a) Generally, long and slender rods; non-motile; Gram positive; Ferments carbohydrates to lactic acid; Generally anaerobic. *Lactobacillus casei*.
Some other species such as *L. brevis*.

L. plantarum and *L. fermentum* may also be seen.

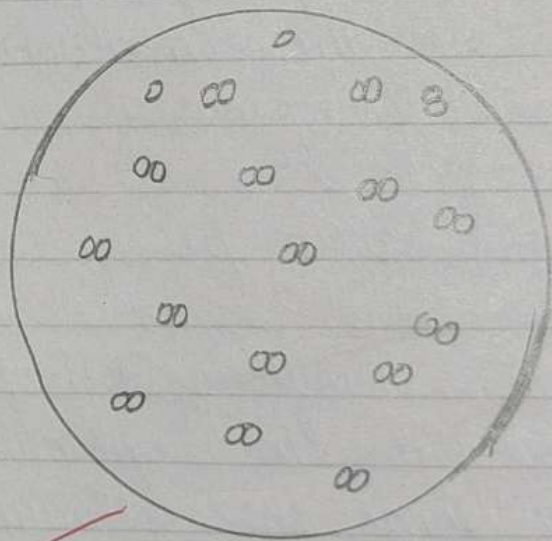
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Streptococcus lactis



Lactobacillus
lactis



Micrococcus

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- (b) spherical cells seen in irregular masses; Gram positive motile or non-motile; aerobic; some cells with yellow, red orange pigment ... *Micobacterium luteus*
- (c) ovoid or ellipsoid small cells, in pairs or in short chains, non-motile; Gram positive; some cells bear capsules; ferments lactose to lactic acid ... *Streptococcus lactis*

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* Object: To demonstrate the technique for pouring a petri-dish.

* Requirements:- Culture tube with medium and plug, burner, petri-dish

* Method:

1. Remove cotton plug from the tube containing medium and pass the mouth of this tube through the flame of burner.
2. Raise one edge of the cover of petri-dish and pour medium into the lower plate as shown in Fig.

Result: Pouring process of petri-dish is complete.

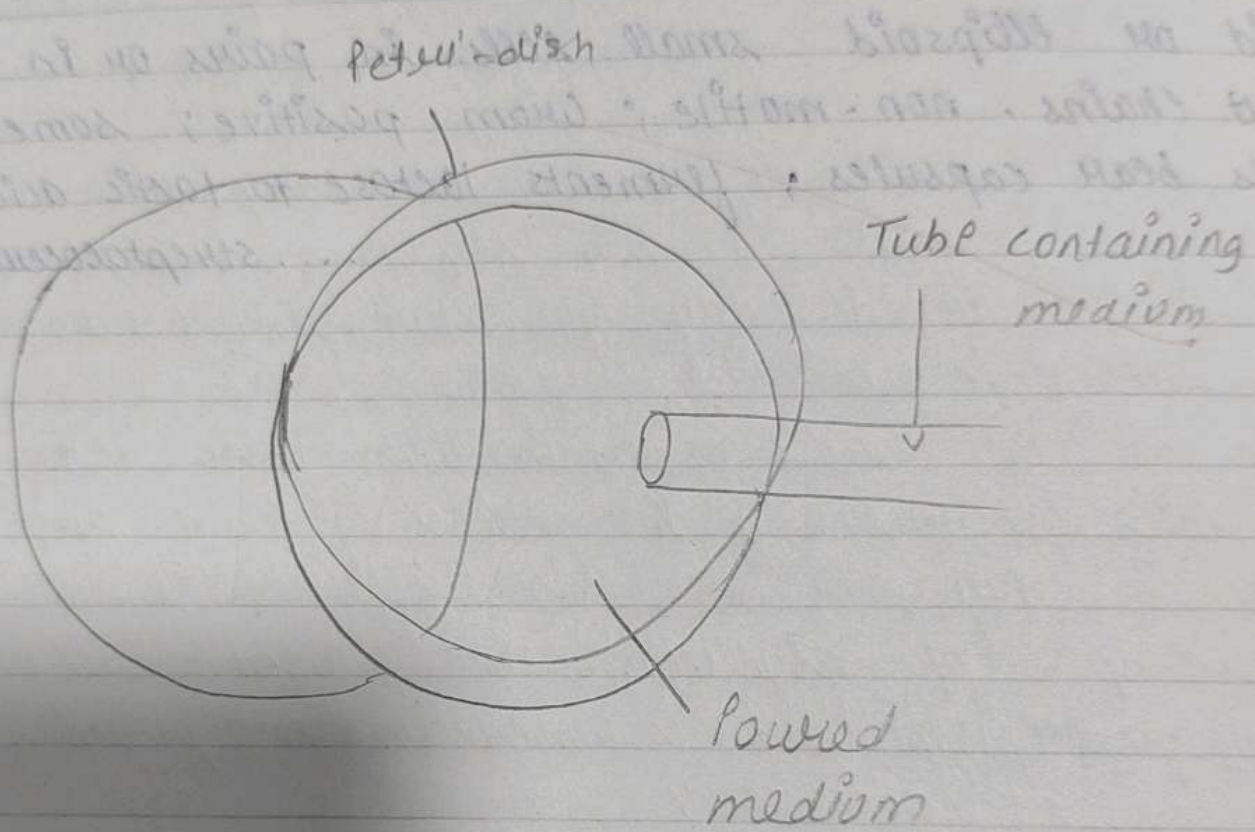
* Technique of Dilution and counting the no. of Microorganisms.

1.1 Gram-staining of Bacteria

Gram-staining technique of bacteria is differential stain technique developed by Hans Christian Gram in 1884

It is extremely popular technique to differentiate bacteria species into Gram-positive and Gram-negative. The basis of Gram's staining technique is the ability of a bacterial cell stained with crystal violet to retain the colour when treated with a differentiating agent [usually alcohol]. Bacteria that retain the

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Technique for pouring a petri-dish

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violet/purple are called Gram-positive, because crystal violet is more extensively trapped in thick peptidoglycan layer, while those loose colour when reacting with alcohol [decolourizing agent] are called Gram-negative. In Gram-negative, peptidoglycan layer is very thin.

1.1* Requirement

1. 24 hours old culture of *E. coli* and *Staphylococcus aureus*
2. Gram staining reagents:
 - Crystal violet [Primary stain]
 - Gram's Iodine solution [Mordant]
 - 95% Alcohol [Decolourizing agent]
 - Safranin [Counter stain]
3. Staining tray
4. Inoculation loop
5. Glass slides
6. Microscope
7. Bunsen burner or spirit lamp
8. Blotting paper / absorbing paper
9. Dropper
10. Wash bottle of distilled water

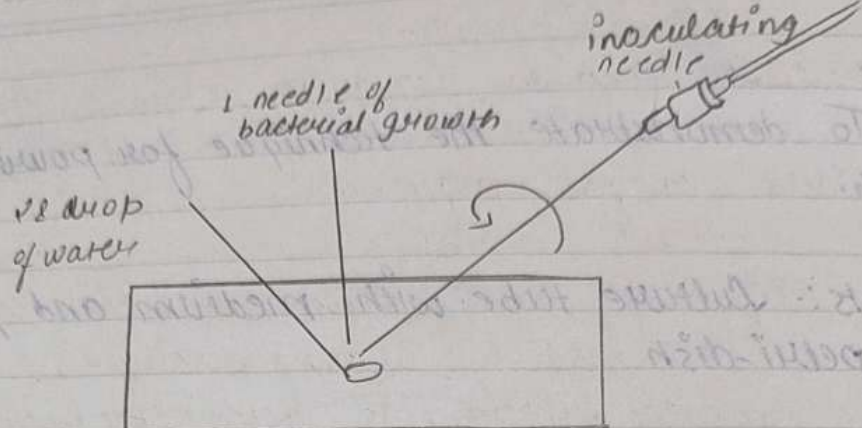
*1.3 Bacterial smear preparation by Heat Fixation

- Unless fixed on the glass slide, the bacterial smear will wash away during the staining procedure. This

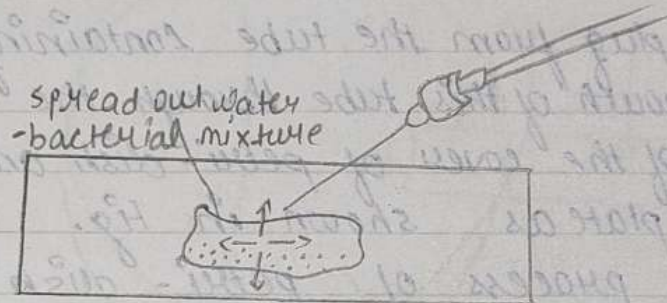
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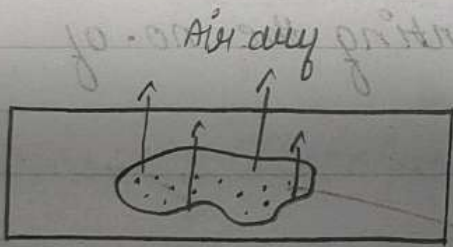
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(a)



(b)



(c)

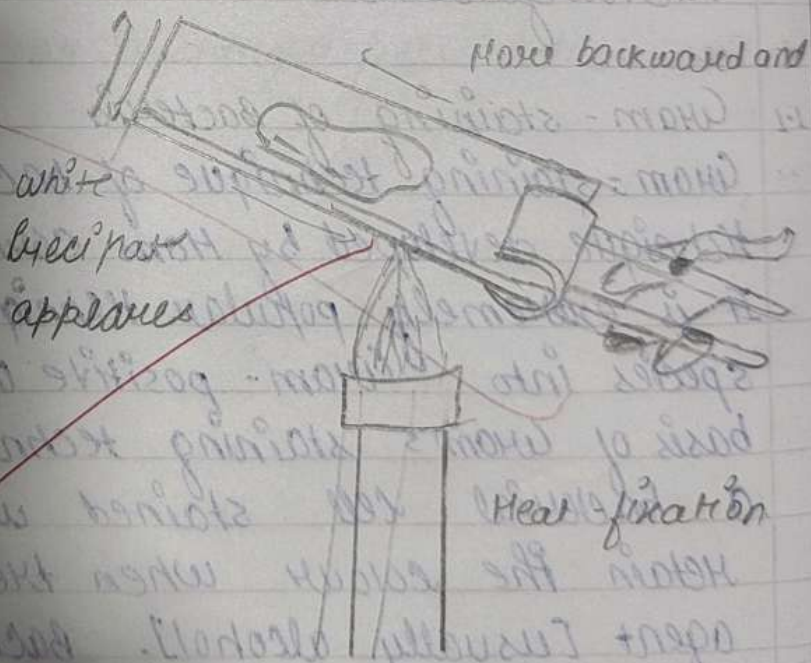


Fig. 3 Bacterial smear preparation

is avoided by heat fixation, during which the bacterial proteins are coagulated and fixed to the glass surface. Heat fixation is performed by the rapid passage of the air dried smear 2-3 times over the flame of the Bunsen burner.

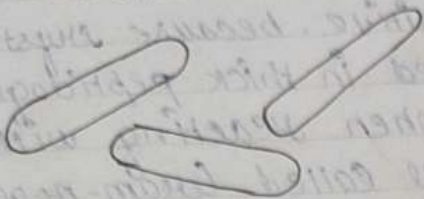
* 1.4 Making smear

1. Take two clean sterilized glass slides and label with marker 'A' and 'B' transfer.
2. With the help of flame sterilized loop, two loops full of distilled water on slide
3. Flame the loop and allow to cool.
4. Aseptically transfer a part of single colony from mother culture of both bacteria on agar plate into distilled water
5. Make a suspension of the culture in the water on slide and thoroughly, gently and evenly spread over the slide in a 2cm area.
6. Now dry the suspension on by warming gently on spirit lamp flame and then fix it by quickly passing through the flame a few times.
7. This heat fixed smear is now ready for staining.

1.5 Gram-staining procedure.

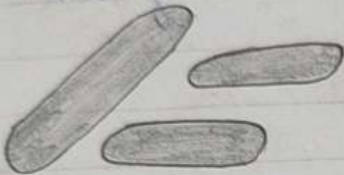
1. Take both heat fixed slide 'A' and 'B'
2. stain separately with crystal violet for 1 minute

Gram (+)



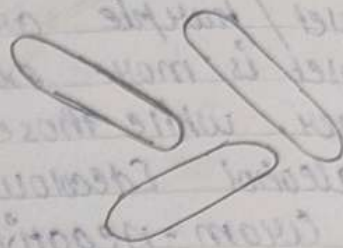
Smeared on slides

Primary stain, crystal violet



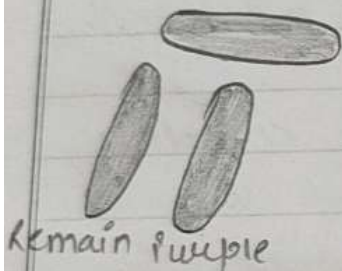
stain purple

Gram (-)

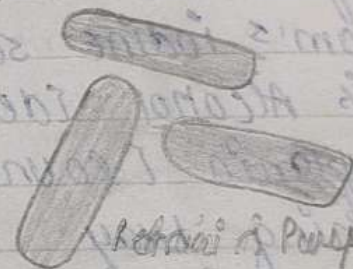


stain purple

Mordant (Gram's iodine) increases affinity of primary stain for cell

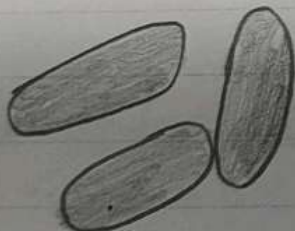


Remain purple

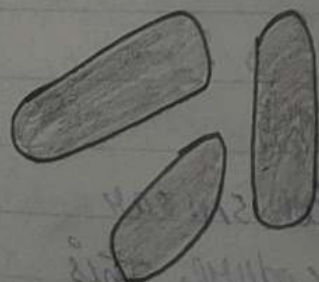
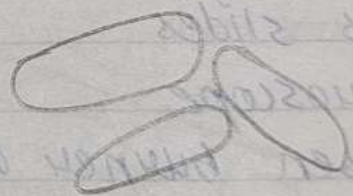


Remain purple

Decolorizer alcohol

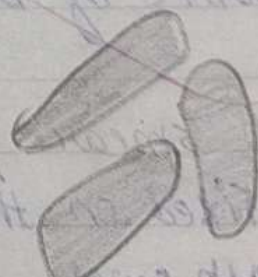


Remain purple



Remain purple

Counterstain safranin



stain pink

3. Rinse with water using wash bottle
4. Now treat the smear with iodine solution for 30 seconds. Iodine acts as mordant, that helps the stain to adhere to the specimen, a crystal violet-iodine complex is formed and smear look black.
5. Decolorize the iodine solution with 95% ethyl alcohol with dropper, until the washing appears pale violet
6. Rinse immediately with water and drain
7. Counter stain with safranin both the slides for 30 seconds.
8. Wash with water and blot dry with absorbent paper.
9. Let the slides dry
10. Examine the slide under compound microscope

*1.6 Observation

1. Bacterial cells appearing violet/purple = Gram-positive bacteria [*S. aureus*]
2. Bacterial cells appearing red/pink = Gram-negative bacteria [*E. coli*]

1.7* Precautions

1. Use only fresh culture
2. Prepare smear by gentle heating. avoid excessive heating

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3. smear should be thin and evenly spread
4. Avoid using excessive alcohol.

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