

Cell & Molecular Biology Practical Manual For M.Sc (Zoology)

Semester – III

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General Laboratory Safety

Safety in the Laboratory Should always be in your mind. Throughout this manual Safety recommendations are given, below are some general considerations that anyone in a laboratory should know.

Laboratory safety precautions

1. Follow all instructions carefully. Use special care when you see the word CAUTION in your laboratory instructions. Follow the safety instructions given by your teacher.
2. Determine the location of Fire Extinguishers, Chemical safety showers and Eye washers, Chemical Spill Kits and alternative exit routes for lab evacuation.
3. Remember that smoking, eating, or drinking in the lab room is totally prohibited.
4. Wear lab aprons when working with chemicals, hot material, or preserved specimens.
5. Wear safety goggles when using dangerous chemicals, hot liquids, or burners.
6. Any chemicals spilled on the hands or other parts of the skin should be washed off immediately with a plenty of running water. If you have an open skin wound, be sure that it is covered with a water proof bandage.
7. Never work alone in the laboratory.
8. Keep your work area clean & dry.
9. Turn off all electrical equipment, water, and gas when it is not in use, especially at the end of the laboratory period.

Special precautions for working with heat or fire:

1. Never leave a lighted Bunsen burner or hot object unattended. When an object is removed from the heat & left to cool, it should be placed where it is shielded from contact.
2. Inflammable liquid bottles should not be left open, not dispensed near a naked flame, hot electric element or electric motor.
3. Use test tube holders to handle hot laboratory equipments.
4. When you are heating something in a container such as a test tube, always point the open end of the container away from yourself & others.

5. Use only Pyrex glassware's for heating.
6. Allow hot materials to cool before moving them from your lab station.
7. Make sure that Bunsen burner hoses fit tightly.

Special precautions for working with chemicals

1. Never taste or touch substances in the laboratory without specific instructions.
2. Never smell substances in the laboratory without specific instructions.
3. Use materials only from containers that are properly labeled.
4. Wash your hand after working with chemicals.
5. Do not add water to acid. Instead, dilute the acid by adding it to water.
6. Mix heat generating chemicals slowly.

Special precautions for working with electrical equipment.

1. Make sure the area under & around the electrical equipment is dry.
2. Never touch electrical equipment with wet hands.
3. Make sure the area surrounding the electrical equipment is free of flammable materials.
4. Turn off all power switches before plugging an appliance into an outlet.

Special Precaution for working with Glassware's and other laboratory equipments.

1. Become familiar with the names and appearance of all the laboratory equipments you will use.
2. Never use broken or chipped glassware.
3. Make sure that all glassware's are clean before you using it.
4. Do not pick up broken glass with your bare hands. Use a pan and a brush.
5. If a Mercury thermometer breaks, do not touch the mercury. Notify your teacher immediately.
6. Do not aim the mirror of your microscope directly at the sun. Direct sun light can damage the eyes.
7. Use care handling all sharp equipments, such as scalpels and dissecting needles.

Special precautions for working with live or preserved specimens.

1. If live animals are used treat them gently. Follow instructions for their proper care.
2. Always wash your hands after working with live or preserved organisms.
3. Specimens for dissection should be properly mounted and supported. Do not try to cut a specimen while holding it in the air.
4. Do not open Petri dishes containing live cultures unless you are directed to do so.
5. Detergents (dettol 5 – 10%) should be used to sterilize and clean benches, glassware and equipment.
6. Safety cabinet should be used while working with microbes.
7. Lab coats should be worn during the work in the lab.
8. Disposable items should be collected and autoclaved.

First Aid

1. Injuries: bleeding should be reduced using bandages; the wound should be cleaned with iodine alcohol mixture, and wrapped with sterile bandage.
 2. Acid and fire burns: body burns must be washed immediately with tap water. Eye burns must be washed using eye washer, special cream for burns can be used.
 3. Poisoning: if any toxic chemical is swallowed, the mouth must be rinsed with water, in case of acid, milk is drunk, in case of alkaline, diluted acetic acid (vinegar) can be used.
 4. Skin contamination requires washing with water and removal of contaminated clothing, if the contaminant is insoluble in water remove with soap and water.
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The Microscope

Highlights:

This Exercise focuses on how to develop a working knowledge of the Microscope and its use. Students should identify the different parts of the Microscope. List and follow recommended procedures in using and caring for the Microscope.

Material:

Compound Microscope
Clean Microscope Slides
Cover Slips
Lens papers
Sharp razor blades
Medicine droppers
Scissors
Distilled water
Xylene

Introduction:

Since an unaided eye cannot detect anything smaller than 0.1 mm in diameter, cells, tissues, and many small organisms are beyond our visual capability, so we need equipment to magnified objects which is too small to be seen with unaided eye. There are several types of microscopes but the only one used in this laboratory is the compound light microscope. The compound microscope (sometimes called the student microscope or light microscope); these microscopes are known as compound microscope because there are two magnifying lenses in the microscope.

One magnifying lens is in the ocular or eyepiece, which further magnifies the image formed by the objective lens, and one, is in the objective. Each contributes to the magnification of the object on the stage. The total magnification of any set of lenses is determined by multiplying the magnification of the objective by the magnification of the ocular. The nose piece rotates the magnification of the microscope. Generally compound microscope magnifies from 40 x to 100 x.

A binocular compound microscope

Parts of a microscope:

The compound microscope is a delicate instrument composed of many parts that are accurately filled together in (Figure)

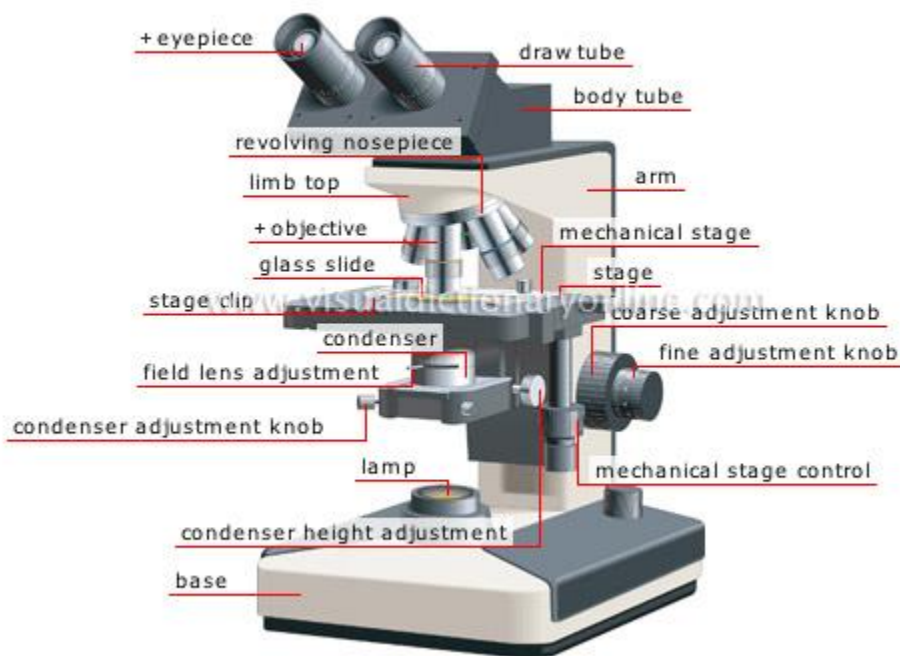


Figure 1: Binocular Microscope

1. Ocular of eyepiece lens.

The ocular lens is the lens you look through, it is inserted at the top of the body tube. If your microscope has one ocular, it is a monocular microscope, if it has two, it is binocular. Its magnification is written on it.

2. Body tube.

Body tube is the optical housing for the objective lenses.

3. Objective lenses.

The objective lenses are a set of three to four lenses mounted on a rotating turret at the bottom of the body tube. The four objective lenses of your microscope and their magnifications are:

Scanning lens	4X magnification
Low power lens	10X magnification
High power lens	40-45X magnification
Oil immersion lens	100X magnification

The magnification of the objective lens is written on the lens.

Note: with the exception of the oil immersion lens all the objective lens is used dry.

The magnification of oil immersion lens requires using the lens with special immersion oil for proper resolution.

4. Stage

The horizontal surface on which the slide is placed is called the stage. It may be equipped with simple clips for holding the slide in place or with a mechanical stage, a geared device for precisely moving the slide. Two knobs, either on top of or under the stage, move the mechanical stage.

5. Condenser lens.

Condenser lens system, located immediately under the stage, contains a system of lenses that focuses light on your specimen. The condenser may be raised or lowered using the condenser knob. An older microscope may have a concave mirror instead.

6. Iris diaphragm

Iris diaphragm is located below the condenser or immediately below the stage in microscopes without a condenser. It functions in regulating the light intensity passing through to the stage. More light is required at higher magnification.

7. Light source

The light source has an (ON/Off) switch & may have adjustable lamp intensities & color filters.

8. Base

Base – also called the supporting stand, rests on the bench.

9. Body Arm

The body arm is used when carrying the instrument.

10. Nose piece

Nosepiece is the mounting for the objective lenses which rotates to bring the desired objective into position.

11. Coarse adjustment

Coarse adjustment knob is a large knob located at either side of the microscope which functions in controlling the distance between the objectives and the stage. Use the coarse adjustment only with the scanning (4X) & low- power (10X) objectives. So coarse adjustment is used for rapid focusing of the specimen until the specimen is roughly in focus & then left alone, in which the fine adjustment knob controls precise focusing of the object.

12. Fine adjustment

Fine adjustment is a small knob located at either side of the microscope. This is used for the control of the object, precise focusing you should use just the fine adjustment knob with the higher magnification objective lenses; Because using the coarse adjustment knob with the higher objective lenses may damage the lens &/or the slide you are observing.

Magnification:

Compound microscopes consist of two lens system: the objective lens, which magnifies, & projects a “virtual image” into the body tube and the ocular lens, which magnifies the image further and projects the enlarged image into the eye. The total magnification of a microscope is the product of the magnification of the objective and the ocular. If the objective lens has a magnification of 5X and the ocular 12X, then the image produced by these two lenses is 60 times larger than the specimen.

Microscope safety cautions:

1. Always carry the microscope in an upright position using both hands.
2. Keep the microscope away from the edge of the table.

3. Always examine a slide first with the low-or medium power objective, never use the high – power objective to view thick specimens.
4. Remove slide only after low-power objective has been rotated into viewing position, never when high – power objective is in position.
5. Keep the stage dry at all times. A wet stage will prevent the slide from being accurately positioned.
6. When returning your microscope to its proper place in the cabinet always:
 - Remove the slide from mechanical stage.
 - Clean all lens surface and the stage.
 - Rotate the nosepiece that the scanning lens is in place.

Steps Used in viewing a slide:

1. Obtain a slide.
2. Check that the ocular and all objective lenses as well as the slide clean.
3. Use the coarse adjustment knob to obtain maximum working distance.
4. Place the slide on the stage, the slide should fit into the slide holder. Use the stage adjustment knob to move the slide over the hole in the stage.
5. Rotate the lower objective in place.
6. Use the coarse adjustment knob to obtain the minimum working distance.
7. Look through the ocular. Adjust the light with the iris diaphragm lever if necessary. Slowly turn the coarse adjustment knob until something comes into focus. Use the fine adjustment knob to sharpen the focus.
8. Using the stage adjustment knob move the slide around until you find an area you wish to examine more closely. Move the slide until the object you wish to examine is in the center of the field.
9. Rotate the high power objective in to place. Use the fine adjustment knob to sharpen the focus. Do not use the coarse adjustment knob. Adjust the light using the iris diaphragm lever if necessary.
10. Rotate the high power object halfway to the next position, place a drop of immersion oil on the slide, and then rotate the oil immersion objective into place. The objective should be immersed in the oil on the slide.
11. Use the fine adjustment knob to sharpen the focus. Adjust the light using the iris diaphragm lever if necessary.
12. When finished viewing the slide use the coarse adjustment knob to maximize the working distance and remove the slide from the stage. If you are finished with the microscope clean the microscope and return it to storage.

Procedure for cleaning a microscope:

1. Turn off the light.
2. Using the coarse adjustment knob to obtain maximum working distance and remove the slide from the stage.

3. Using lens paper cleans all the lenses starting with the cleanest first ocular, and objectives lens.
4. Clean any oil off of the stage using paper towels.
5. Rotate the scanning objective into place. Use the coarse adjustment knob to obtain minimum working distance.
6. Return the microscope to the appropriate storage area.

Procedure for cleaning a microscope slide:

Before placing a specimen on a slide, it must be clean, as any small foreign body might mislead the observation. If your slide is not clean, do the following:

1. Hold the slide from its ends by fingers of one hand.
 2. Using a detergent liquid, rub the slide with one finger of the other hand.
 3. Wash the slide under running tap water; rub again, until no trace of the detergent is left.
 4. Rinse the slide with distilled water to remove the tap water.
 5. Either blot dries the slide by placing it between two towel papers, or place in alcohol solution & keep until used.
 6. Never touch the slide from the middle, clean slide always holds it from its ends.
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Mitosis in Onion Root-Tip Cells

Introduction:

In this lab, you will observe the various stages of mitosis in onion root tip cells. Cell division is especially rapid in the growing root tip; therefore, it is easier to observe each stage of mitosis than in slowly growing tissues. You will harvest the young tips, fix them, digest them in acid, treat them with a reagent which stains chromosomes, and view them under a microscope. You will then estimate the proportion of time that cells in actively dividing tissues, such as the root tip, actually spend in M-phase and cytokinesis.

Principle:

Cell division in flowering plants takes place in particular regions of the plant called meristems. Cells in meristems are not specialized for any particular function and divide repeatedly by mitosis. Some of the daughter cells remain meristematic others cease dividing and become differentiated into appropriate cell types depending on their position. The root tip meristem is usually a denser white and more rounded than the cut end. Chromosomes in root tip tissue are made visible with the stain. Dividing cells (if present) will show up clearly with chromosomes in

different forms according to the stage of mitosis. Individual chromosomes (as tightly-coiled threads) are visible during anaphase. The links between the cellulose walls of plant cells are broken down by the treatment with hydrochloric acid. This ensures that the stain can penetrate the cells and allows the tissue to be squashed out one cell thick.

Materials:

Carnoy's fixative
1N HCl
Feulgen stain
45% Acetic Acid
Freshly sprouted onion, about 2-3 cm long root tip

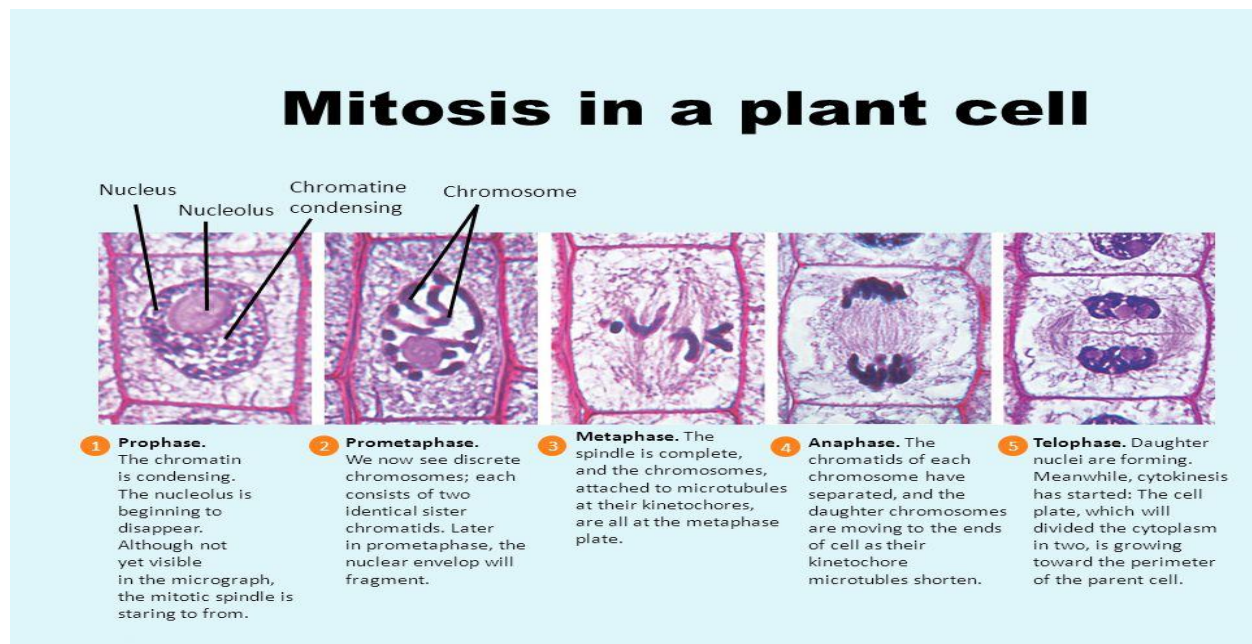
Equipment:

Eppendorfs
Pasteur pipettes
60 °C Water bath
Microscope slides
Razor blades
Cover slips

Protocol:

1. Cut off the last 6 mm (1/4 inch) of root tip from sprouting onions. Place 5 of them in the labeled Eppendorf tube.
2. Add 1 ml Carnoy's fixative and make sure that all tips are immersed.
3. Close tube and incubate for 24 hours.
4. Remove your root tips from the Carnoy's fixative and immerse in a new tube filled with 1 ml 1N HCl. Incubate for 12 minutes at 60C.
5. Remove the HCl with a Pasteur pipette and discard in the drain with running cold tap water.
6. Add 0.5 ml Feulgen stain. (Watch out: this stain does not look brightly colored, but stains strongly – keep it away from your clothes, books, etc)
7. Let the root tip stain in Feulgen for about 10 minutes, or until the very tip of the root shows distinct dark coloring.
8. Put one drop of 45% Acetic Acid on the slide.
9. Place the root tip in the Acetic Acid on the slide. With a scalpel or razor blade, remove all but the red-stained very tip of the root.
10. Add the cover slip on top of the root tip.
11. Place the slide on a white piece of paper on your bench. Tap gently and straight down with the eraser of a pencil until the stained tip is spread out to a faint purple monolayer. Do not smear the cover slip sideways – this will shear the chromosomes.
12. Examine your spread under the microscope at low power to ensure that the cells are spread to a monolayer. If not, squish the cover slip some more.
13. Once you have spread your cells into a nice monolayer, switch to oil immersion.

14. Spend some time identifying the different stages of the cell cycle visible in your root section squashes. Illustrate examples of each mitotic stage (prophase, metaphase, anaphase, and telophase).



Meiotic cell division in grasshopper testis

Aim: To perform Meiotic cell division in the given sample (testis follicles)

Materials Required:

Acetocarmine
Glass slide
Cover slip
Lab needle
Sample (Grasshopper testis follicles)

Miscellaneous:

Eppendorfs
Pasteur pipettes
Watch glass
Razor blades
Dissection box

Methodology:

1. Identify the male grass hopper, which has lean and lengthy tail whereas, female has short and blunt tail.
2. Turn the dorsal side of grasshopper and count the 7th segment from the tail which is abdominal portion of the grasshopper.
3. Using sharp scalpel cut at the 7th segment and press gently to release a yellow fluid clumps (which is genital organ covered with yellow fat bodies).
4. Transfer the clump carefully to the vessel containing saline for washing.
5. Shake gently with lab needle so the fat bodies and follicles (white fibers in elongated shape) get separated.
6. Transfer this follicle bunch to a Petri dish and separate it into individual follicles.
7. Take a pre-cleaned slide and transfer a follicle to the centre of the slide.
8. Add a drop of acetocarmine incubate it for 5minutes.
9. If it is dehydrated add another drop of acetocarmine and cover with cover slip.
10. Using your forefinger press gently and prepare squash carefully.
11. Remove the excess stain by blotting technique.
12. Focus at 10x and examine at 40x for sharp image using Light microscope.

Result:

Different stages of meiotic cell division could be visible.

Interphase	Condensed chromosome
Prophase	Thread like chromosome structure'
Metaphase	Spindle fibers and distinct chromosome at center of the cell
Anaphase	Stretching of chromatids towards opposite poles
Telophase	cell undergoes equal division



Blood Smear Preparation

Aim:

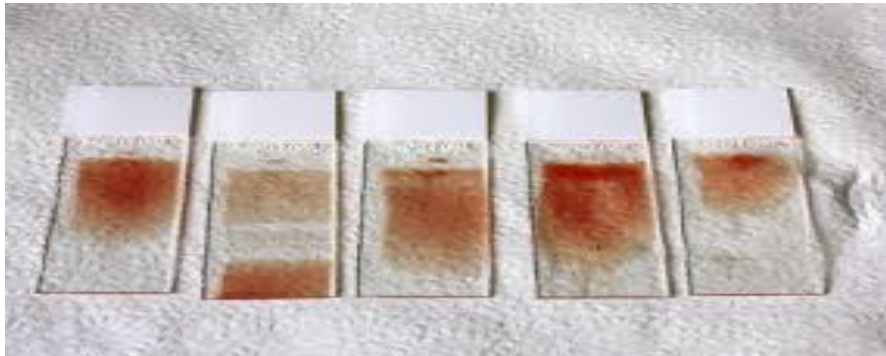
To identify the blood cell types in human blood smear.

Requirements:

Microscope
Glass slides
Spirit lamp
Sterilized needle
A drop of blood
Giemsa stain
Distilled water

Preparation of Blood smears:

1. Obtain 2 clean microscope slides, alcohol wipes, and lancet
2. Clean a finger with an alcohol and puncture with lancet
3. Place a small drop of blood at the end of one slide.
4. Use the second slide to make a thin blood film as directed below:
 - a. Place the second slide at a 30 degree angle and touch the slide with the blood drop
 - b. Move the spreader slide to touch the blood drop allowing the drop to spread by capillary action along the edge of the slide
 - c. Immediately pull/push the slide away from the blood drop, making a thin smear that should dry quickly as you move away from the drop.
 - d. A perfect smear will have a “feathered” edge and separated RBCs when you view it with the microscope.



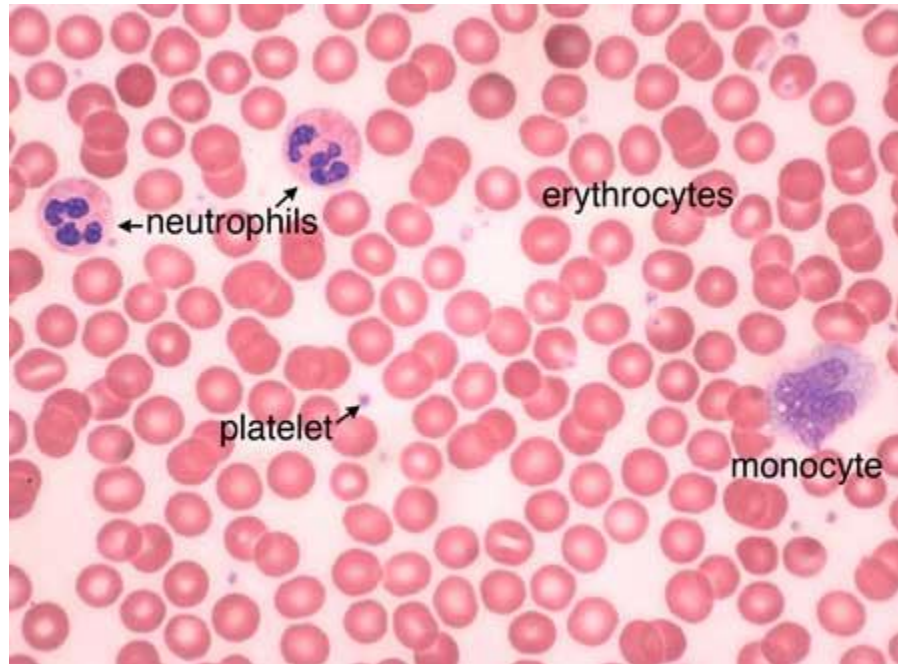
Staining the blood smear (Horizontal staining procedure):

1. Place thoroughly dried smear on horizontal staining rack
2. Flood smear with Fixative for 10 seconds, (fixes cells to slide/prepares cells for dyes) drain
3. Flood smear with Dye for 10 seconds, drain
4. Rinse the smear with distilled water for 1 minute
5. Air dry and examine under the microscope, using low power first, then high power.

6. Observe as many different types of blood cells as possible. Pay close attention to size, frequency, and nuclear features.

Observation:

Different types of blood cells are visible (Neutrophil, Basophil, eosinophil and monocytes).



Buccal smear – Identification of Barr Body

Introduction:

In human being the sex can be identified by observing the nucleus of their resting cells (the interphase nucleus) in the cells of female. A darkly stained chromatin matrix is observed on the slide in the interphase nucleus. This is known as sex chromatin or Barr body after the name of its discoverer Murray Barr in 1940.

Aim:

To identify the presence of Barr body in the female Buccal cavity.

Principle:

Buccal epithelial cells especially have Barr body structure, which are considered to play a major role for sex determination. This small round Barr body is located either in the border of nuclear membrane or sometimes inside of nucleus. This Barr body may be single or more in number in some cases. These structures are present only in the female sex.

Requirements required:

Pre-cleaned slides
Methylene blue
Microscope
Epithelial cells (sample)

Methodology:

1. Wash your mouth with sterile water to prepare mucous.
2. Take a sterilized slide and scrap epithelial cells superficially from the inner side skin of the mouth.
3. Keep the sample on the center of the pre sterilized glass slides and dry it for few minutes.
4. Then add few drops of Leishman stain on the smear and incubate for 5-10 minutes.
5. After incubation, wash it or remove the excess stain using water by keeping the in slant position.
6. Eventually, blot the water using tissue paper.
7. The smear is now ready for microscopic observation.

Observation:

In female epithelial cells dark stained heterochromatin (Barr body) is observed usually periphery of the nucleus, clearly under high power microscope.

