JHARKHAND RAI UNIVERSITY
RANCHI

LAB MANUAL

PHYSIOLOGY –I

BPT I

LIST OF EXPERIMENTS

Physiology –I (23A102P)

Experiment No.1

Aim: To study the compound microscope and its parts.

Objective: To study the various parts and draw the compound microscope.

Requirement:

Compound microscope

Histology slides

Theory:

Microscope is a device used for the magnification of the sample. It is commonly used in laboratory for studying the specimen.

Object which are not visible by naked eyes are seen with microscope. Generally an object smaller than 0.1 mm cannot be seen by our eyes Therefore to observe an object smaller than this compound microscope is very helpful.

Parts of compound microscope:

The parts of compound microscope can be divided into different ports-

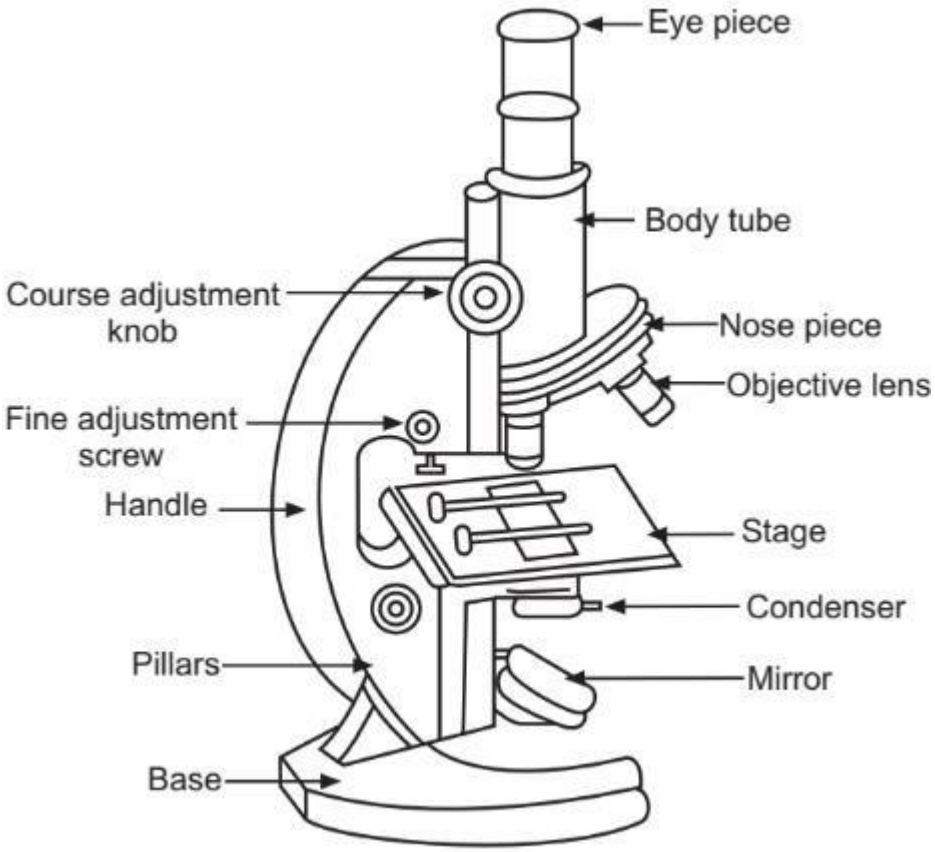
1. Base-provide support
2. Arm-handle to carry.
3. Fine adjustment knob-adjusts focus on an object
4. Stage-Place to keep the specimen
5. Clips-Fixes the specimen slide
6. Body tube- Tube going eye piece and objective lens

- Objective lens- View the specimen
- Eye piece: Position to place viewer's eye
- Reflector-light source

7. 3 objective lens are as follows:-

- Low power (10 x)
- High power (45x)
- Oil immersion (100x)

Figure 1: Parts of microscope



Procedure:

i. View into the eye piece, rearrange the mirror, such that adequate light passes into the microscope
ii. The mirror, lens, stage and slide should be clear of dust.
iii. Place the slide in the middle of the stage
iv. The eye piece adjusted in such a way that low power Objective is aligned with the object of focus placed.
v. The coarse adjustment knob can be shifted upward downward such that the slide is well under.
vi. Turn the fine adjustment knob by moving upward and downward for a clear and sharp image.

Precautions:

i. Handle with care.
ii. Always cover the microscope when not in use.
iii. Do not touch the lens with fingers.

Conclusion:

The compound microscope is a vital tool for magnifying small specimens that are not visible to the naked eye. By understanding its parts—such as the objective lenses, eyepiece, and adjustment knobs—accurate observation of specimens can be achieved. Proper handling and maintenance ensure clear and focused images for effective laboratory work.

Experiment No.2

Aim: To determine the bleeding time of the given Sample by Duke's method.

Objective: To determine the bleeding time of a sample using Duke's method and assess platelet function in haemostasis.

Requirements:

i. Filter paper
ii. Stop watch
iii. Lancet
iv. Cotton
v. Spirit

Procedure:

a. Select the appropriate finger, usually ring finger and clean the tip of the finger using 70% alcohol or any other suitable antiseptic.
b. Prick the finger using sterile disposable needle (26G) to obtain free flowing blood with minimum pain
c. Immediately start the stopwatch and note the time
d. Absorb or remove the blood drops every 30 seconds by touching puncture site on the piece of filter paper without pressing or squeezing the finger.
e. Note the time when bleeding stops i.e. when there is no trace of blood spot on filter paper
f. Count the total number of spots and note down number at which blood as endpoint.
g. Express the result in minutes & seconds (NOTE – The normal bleeding time is 1-5 minutes)

Result: The bleeding time is ….. Minutes.

Conclusion: The Duke's method provides a simple and effective assessment of bleeding time, reflecting the functional status of platelets and the integrity of capillary haemostasis.

Experiment No.3

Aim:

To determine the clotting time of blood by Capillary method.

Objective: To determine the clotting time of blood by the capillary method and evaluate the efficiency of the coagulation process.

Requirements:

i. Sterile disposable needle (261)
ii. 10-12cm long clean capillary tube with uniform base diameter of 1-2 mm
iii. stopwatch
iv. cotton swab
v. 70% alcohol or any other suitable marketed antiseptic.

Theory:

The process occurs in three stages-

• Formation of prothrombin activator or prothrombinase (combination of activated Clotting factors V & X) by intrinsic & extrinsic pathway (an injury to blood vessels and tissues respectively).

• Conversion of prothrombin (clotting factor II) into the enzyme thrombin by prothrombinase.

• Conversion of soluble fibrinogen (clotting factor I) into insoluble fibrin by thrombin; fibrin forms the threads of the clot.

Clotting time is interval between entry of blood into glass capillary tube and formation of thin fibrin thread.The clotting time is commonly determined by: 1 Capillary blood clotting time method (wright's method).

Procedure:

i. Select the appropriate finger, usually sing finger and clean the tip of the finger using 70% alcohol or any other suitable antiseptic.
ii. Prick the finger using sterile disposable needle (26 G) to obtain free flowing blood with minimum pain.
iii. Immediately start the stopwatch & note the time.
iv. Dip one end of capillary tube in blood, the blood will rise in tube by capillary action which can be enhanced by keeping its open end at a lower level.
v. Note the time when blood starts to enter the capillary tube as zero time.
vi. Hold the capillary between the palms of your hands to keep the blood near body temperature.

vii. Gently break off approximately 1 cm bits of capillary tube from one end at intervals of 30 seconds and observe for formation of fibrin thread of minimum 5 mm length of between the broken ends of capillary tube. This is called as rope formation. Note the time.

Result: The clotting time is ....Minutes.

Conclusion: The capillary method for determining clotting time provides a simple and effective means of evaluating the efficiency of the coagulation process. By observing the formation of fibrin threads, this test offers insight into the functionality of intrinsic clotting pathways and aids in the early detection of coagulation disorders.

Experiment No.4

Aim: To determine own blood group by using ABO system.

Objective: To determine the blood group of an individual using the ABO and Rh system by observing agglutination reactions with specific antisera.

Requirements:

i. Sterile disposable needle (26 G)
ii. Clean & dry glass Slides
iii. Glass droppers and anti-sera kit
iv. Cotton swab
v. 70% alcohol or any other suitable marketed antiseptic.

Theory:

As per ABO blood grouping system human blood is mainly divided into four groups This groups are made based upon presence of absence of antigens (also called agglutinogens) present on the surface of RBCs & antibodies(also called agglutinins) present in the plasma. Agglutinogens & agglutinins are of two types viz A & B and Anti A & Anti-B respectively.

The process of clumping followed by haemolysis of RBC occurs due to reaction between specific agglutinogens & agglutinins. This reaction is also a called as agglutination.

In addition, there may be presence of one more antigen called D-antigen (Rh-factor) on the surface of RBCs which constitutes the Rh System. Presence or absence of D-antigen designates the blood as Rh^+ or Rh^- respectively.

The ABO blood grouping system is shown in the following table:

Sl.No	Blood Group	Agglutinogen	Agglutinin
1	A	A	Anti B
2	B	B	Anti A
3	AB	A & B	-
4	O	-	Anti A & Anti B
5	Rh^+	D	-
6	Rh^-	-	-

For determination of blood group commercially, available kit is widely used which includes three agglutinin containing Solutions viz antisera A, antisera B, and antisera D. The RBCs that is present in blood sample are made to react with these antiseras.The slide is then observed for agglutination which occurs as a result of reaction between similar agglutinin agglutinogen.

Procedure:

i. Divide the glass slide in three parts & mark them as anti-A, Anti-B & anti-D
ii. Prick the finger under aseptic Conditions and add two drops of blood in each divided part of slide.
iii. Put one drop of each anti-serum at respective part of slide and mix it with blood.
iv. Wait for 8-10 minutes & observe the slide for agglutination reaction i.e. clumping & haemolysis of red cells

Observation Table:

Sl No.	Antisera	Agglutination status (+/-)	Blood group
1	A		
2	B		
3	D		

Result:

The blood group was found to be...

Conclusion: The ABO and Rh blood grouping test is a fundamental procedure in haematology that determines an individual's blood type based on the presence or absence of specific antigens on red blood cells. Through agglutination reactions with antisera, this practical method enables accurate identification of blood groups, which is essential for safe blood transfusion, organ transplantation.

Experiment No.5

Aim: To determine the Haemoglobin content of blood (sahli's method its indirect method).

Objective: To determine the haemoglobin (Hb) concentration in a blood sample using Sahli's acid hematin method by converting Hb to acid hematin and comparing the resulting colour with a standard.

Requirements:

i. Sahil's hemoglobinometer
ii. N/10 HCl
iii. Distilled water.
iv. Two droppers
v. Materials for sterile finger prick

Theory:

The amount of Hb can be estimated by the conversion of known volume of blood into acid hematin by the addition of dilute N/10 HCl and subsequent calorimetric comparison with Suitable standard.

Acid Hematin method.

Principle: Haemoglobin is converted to acid hematin by 0.1N HCl and the resulting brown colour is composed with standard brown glass reference blocks

The intensity of the brown colour depends on the amount of acid hematin produced. & this is directly proportional to the amount of haemoglobin in the blood sample

Advantage of Acid Hematin method:

- Acid Hematin method (indirect method) is easy to perform and convenient.
- The cost is minimal
- It is not time consuming (maximum fifteen minutes)

Disadvantage of Acid Hematin method:

- The colour of the standard may not be reliable, especially with old apparatus.
- The acid hematin is not true solution. Some degree of precipitation may be present at times which may interfere with colour matching.

Procedure:

i. Place 0.1 N Hcl into the Haemoglobin tube up to the lowest mark.
ii. Deliver 0.02ml of blood from a Haemoglobin pipette into the tube thereafter.
iii. Stir with a glass rod/stirrer & wait for 10 minutes to allow Colour development.
iv. Add distilled water drop by drop & stir till colour matches with the comparator because 95% of Haemoglobin is converted at the end of 10 minutes & others much later.
v. Take the reading at upper meniscus.

Result: Hb:…. g/dl

Conclusion: The Sahli's method offers a reliable and practical approach for estimating haemoglobin concentration by converting it to acid hematin and comparing the colour intensity with a standard.