AmbujaNeotia

THE NEOTIA UNIVERSITY

ज्ञानम् आत्म प्रदीपाय

I SEMESTER (1st year B.Pharm)

HUMAN ANATOMY AND PHYSIOLOGY I

PRACTICAL LAB MANUAL

Semester – I HAP practical

EXPERIMENT NO- 1

Study of Compound Microscope

Objects which are ordinarily not visible by naked eye 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. Hand lens (magnifying lens) is also a type of microscope but its magnifying capacity is very low. Dissecting microscope is also used to visualize tiny things, but it has only one lens. Compound microscope is generally used in the laboratories. Therefore, description, use and maintenance of ordinary compound microscope are mentioned here.

Parts of a Compound Microscope

Take out the microscope from the box holding the arm by one hand and supporting the base by another hand. Place carefully on the table and study the names and functions of the parts as mentioned in the figure. The parts of a compound microscope can be divided into 4 main parts:

(a) Base : This is U-shaped lower portion of the microscope on which the other parts of the microscope lie. Above the U-shaped portion, there is a perpendicular portion known as the pillar. On the top of this, another arm is fixed. This is known as inclination joint. This can be used to tilt the microscope at a desired angle.

(b) Arm : It supports the body tube and base of the microscope. This portion is used to hold or carry the microscope. On the base of this, stage is fixed. On the top of the arm body tube of the microscope is fixed and two knobs are fitted. One is for the coarse adjustment and the other for the fine adjustment. These are used for focussing the body tube.

(c) Body Tube : This is attached to the knob of the arm. It has one lens on the upper end known as eye piece. This lens can be changed according to the required magnification. On the bottom of this tube there is a nose piece. Two to four lenses can be fitted in this nose piece. Because the lenses are fitted on the objective, these are known as objective lenses. These are fitted in the body tube, known as objective lens body. The objective lens body is fitted into the noise piece.

(d) Stage : It is a platform having a circular hole in the centre to allow the passage for light from below. It is fixed to the base by

the stand. One mirror is fixed to the stand. It is known as reflecting mirror. Below the stage is a condenser through which concentrated beam of light passes. Iris diaphragm is also attached to the condenser. The reflecting mirror reflects the light upward through the iris and diaphragm. This beam of light passes through the hole in the stage and provides light to the object kept on the slide.

There are two clips for holding the slide above the hole on the stage.

Operation (Use) : Keep a clean prepared slide in the centre of the stage. Use clips to fix the slide on the stage so that it does not move. Now move the body tube by the help of coarse adjustment knobs. Bring the slide in focus under the objective lens. Focussing should be made sharp by the use of fine adjustment knobs. When the focus is sharp then study the slide. The specimen is viewed by keeping one eye on the eye piece and the second eye should be kept open. This type of compound microscope is known as monocular compound microscope. Some compound microscopes have two body tubes. So there are 2 eye pieces and specimen can be viewed by both the eyes. Such type of compound microscope is known as binocular compound microscope. In the research work generally binocular compound microscope is used.

How to use a Compound Microscope

To use the microscope first of all rotate the nose piece until the low power objectives is in line with the body tube and clicks into position. Open the iris diaphragm. Look through the eye piece, adjust the mirror and diaphragm to set a complete field of vision. Place the slide you want to examine on the stage of the microscope and by the help of the clips fix it. Move the slide till the object comes roughly to the centre of the hole or the stage. Bring the object into focus using the coarse adjustment knob. Turn the fine adjustment knob to bring the object into sharp focus.

How much magnification the object needs will be learnt through experience. Eye lenses of 5x, 10x or 15x are available. Some way objective lenses of 4x, 10x & 40x are also available. The multiplication of magnification of eye piece and nose piece denotes the size of the object under observation.

Maintenance of Microscopes:

Microscope is costly equipment. Therefore, it should be handled carefully. Always keep the microscope in an upright position

while taking it from one place to another. As far as possible don't tilt the arm. Clean the lenses of the microscope with the lens paper or muslin cloth, never with the filter or any other kind of paper. If you are using the high power objective lens then after the observation is over, turn the nose piece and bring low power objective lens in line with the hole in the stage. Objective lens should be kept at least 1 cm above the stage. After using the microscope always keep it in the box. Take care to see that the stages of microscope, the eye piece, the objective lens are dry and clean. No chemical should stick to these. Adjustment knobs and joints should be protected from rusting by applying vaseline.

EXPERIMENT NO- 2.

Determination of Bleeding Time

Requirements: Sterile disposable needle (26G), Filter papers, Stop- watches, Cotton swab, Spirit or Antiseptic.

Procedure -

I. Select the appropriate finger, usually ring finger & clean the tip of the finger using 70% alcohol or any other suitable antiseptic.
II. Get a finger prick using sterile disposable needle to obtain free flowing blood with minimum pain.
III. Immediately, start the stop-watch & note the time.
IV. 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.
V. Number the blood spots one onwards.
VI. Note the time when bleeding stops, i.e. when there is no trace of blood spot on filter paper.
VII. Count the total number of spots & note down number at which there is no trace of blood as endpoint.
VIII. Express the result in minutes & seconds.

Normal bleeding time is 1- 5 minutes.

EXPERIMENT NO- 3.

Determination of Clotting Time

Requirements: Sterile disposable needle (26G), 10-12 cm long clean capillary tube with uniform bore diameter of 1-2 mm, Stop-watches, Cotton swab, Spirit or any other suitable antiseptic.

Procedure -

I. Select the appropriate finger, usually ring finger & clean the tip of the finger using 70% alcohol or any other suitable antiseptic.
II. Get a finger prick using sterile disposable needle (26G) to obtain free flowing blood with minimum pain.
III. Immediately, start the stop-watch & 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 tube between the palms of your hands to keep the blood near body temperature.
VII. Gently break off approximately 1cm bits of capillary tube from one end at intervals of 30 seconds & observe far formation of fibrin thread of minimum 5 mm length between the broken ends of capillary tube .This is called as rope formation. Note the time.

Normal clotting time is 3-6 minutes (by capillary method).

EXPERIMENT NO-4.

Estimation of Haemoglobin Content

Requirements: 0.1N Hydrochloric acid, distilled water, sterile disposable needle, Cotton swab, Antiseptic, Sahli's Haemoglobinometer.

Procedure –

I. Fill the Haemoglobin (Hb) tube up to the mark of 20% with 0.1N HCl.

II. Get a finger prick under aseptic conditions & fill the pipette up to the mark of 20 µL.

III. Once the pipette is filled, immediately immerse the tip of pipette to the bottom of acid solution & expel the blood gently. Rinse the pipette, 3-4 times by drawing up & blowing out the 0.1N HCl solution.

IV. Mix the blood with 0.1N HCl solution with the help of flat end of stirrer.

V. Put the tube into comparator & let it stand for 6-8 minutes (during this period formation of acid haemation takes place).

VI. Dilute this acid haemation solution with distilled water till the colour matches to that of standard tinted glass rods in the comparator.

VII. Read the lower meniscus of the coloured solution & note the reading as Hb g%.

The average level & range of Hb is as follows:

Males- 13.5 – 18.0 g%

Females- 11.5- 16 g%

EXPERIMENT NO-5.

Determination of Erythrocyte Sedimentation Rate (ESR)

Requirements- Blood sample, EDTA (or any other suitable anticoagulant), Wintrobe's tube with stand, Sterile disposable needle, Cotton swab, 70% alcohol or any other suitable marketed antiseptic, Pasteur pipette.

Procedure –

I. Take 0.2 ml of 2.5% EDTA solution in blood sample tube.
II. Draw 0.2 ml of venous blood with the help of expert technician & transfer it into the tube containing EDTA.
III. Mix the contents gently but thoroughly by inverting the tube 2-3 times. Avoid vigorous shaking as it may form foam.
IV. Fill the Wintrobe's tube with blood slowly using Pasteur pipette up to the "0" mm mark without any air gap.
V. Transfer this Wintrobe's tube to the stand & adjust the spirit level of stand to ensure that the tube is exactly vertical.
VI. Keep the tube undisturbed in position for one hour & strictly avoid tilting of the tube.
VII. Read the "mm" of clear plasma above the sediment RBCs after 10,50, & 60minutes.

Normal ESR range (first hour)
Males: 2-8 mm
Females: 4-10 mm

EXPERIMENT NO- 6.

Introduction to Haemocytometry

Process-

I. Collection of blood sample from the finger prick.
II. Filling the blood in pipette.
III. Dilution of blood with suitable diluting fluid.
IV. Filling counting chamber with diluted blood i.e. charging the chamber.
V. Counting cells & reporting result in terms of number of cells per cubic millimetre.

EXPERIMENT NO- 7.

Determination of Pulse rate

Requirements- Stop-watch

Procedure-

I. Locate the radial artery at the own wrist level.
II. Palpate the radial artery by pressing them with finger against the underlying bones (The pulse will be felt).
III. Record the pulse for 1 minute.
IV. Take three readings at the interval of 5 minutes & calculate the mean pulse rate.

Normal values of pulse rate/minute:
Neonates: 140 beats/minutes
Children: 100 beats/minutes
Adults: 60-80 beats/ minutes

EXPERIMENT NO- 8.

Determination of Heart Rate

Requirements: Stethoscope

Procedure-

I. Select the subject & make him/her to sit comfortable on the chair.
II. Place the chest piece of stethoscope against thoracic wall.
III. Record the heart beat for the period of 1 minute.
IV. Take three readings at the interval of 5 minutes & calculate the mean heart rate.

Normal values of heart rate/minute:

Foetus: 140-160 beats

Children: 140 beats

Adults: Males- 64-74 beats

Females- 72-80 beats

EXPERIMENT NO- 9.

Recording of Blood Pressure

Requirements- Stethoscope, Mercury Sphygmomanometer.

Procedure-

I. Ask the subject to sit or lie down & allow 5 minutes to relax.
II. Place the arm of the subject in a position so that it is at the level of the heart.
III. Uncover the arm up to shoulder & tie cuff around the arm, neither too tight nor too loose. Record the blood pressure first with palpatory method followed by auscultatory method.

Palpatory method-

I. Palpate the radial artery at wrist & feel the pulse.
II. Tighten the screw to leak valve & inflate the cuff slowly with air pump until the pulse disappears & note the reading.

Ausccultatory method-

I. Place the chest piece of the stethoscope on bifurcation of brachial artery at the elbow level.
II. Inflate the cuff rapidly & raise the pressure 30-40 mm Hg above the reading determined by palpatory method.
III. Release the pressure gradually using knob until a clear sharp tapping sound is heard; note this pressure as systolic pressure.
IV. Continue to release the pressure until the sound disappears; note this pressure as diastolic pressure.
V. Take three readings at an interval of 30 minutes with auscltatory method & find out the average & express the result as systolic/diastolic blood pressure.