MARRI LAXMAN REDDY INSTITUTE OF PHARMACY

(Approved by AICTE & PCI, New Delhi and Affiliated to JNTUH)

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HUMAN ANATOMY AND PHYSIOLOGY – II

LAB MANUAL

B. PHARMACY I-II

About MLRIP

To be an educational Institute of par excellence and produce competent pharmacy professionals to serve the community through research and the ever-increasing needs of Industry.

1. Imparting quality education and innovative research for various career opportunities.
2. Creating conducive academic environment to produce competent pharmacy professionals.
3. Indoctrination of students adorned with high human values and make them aware of their responsibility as health care professionals.

Program Educational Objectives

PEO 1: To produce graduates with sound theoretical knowledge and technical skills required for their career opportunities in various domains.

PEO 2: To incite the students towards research and to address the challenges with their innovative contributions for the benefit of the mankind.

PEO 3: To instill the essence of professionalism, ethical commitment to become a health care professional with sound integrity and adherence to the core human values in the service of the society.

1. **Pharmacy Knowledge:** Possess knowledge and comprehension of the core and basic knowledge associated with the profession of pharmacy, including biomedical sciences; pharmaceutical sciences; behavioral, social, and administrative pharmacy sciences; and manufacturing practices.
2. **Planning Abilities:** Demonstrate effective planning abilities including time management, resource management, delegation skills and organizational skills. Develop and implement plans and organize work to meet deadlines.
3. **Problem analysis:** Utilize the principles of scientific enquiry, thinking analytically, clearly and critically, while solving problems and making decisions during daily practice. Find, analyze, evaluate and apply information systematically and shall make defensible decisions.
4. **Modern tool usage:** Learn, select, and apply appropriate methods and procedures, resources, and modern pharmacy-related computing tools with an understanding of the limitations.
5. **Leadership skills:** Understand and consider the human reaction to change, motivation issues, leadership and team-building when planning changes required for fulfillment of practice, professional and societal responsibilities. Assume participatory roles as responsible citizens or leadership roles when appropriate to facilitate improvement in health and well-being.
6. **Professional Identity:** Understand, analyze and communicate the value of their professional roles in society (e.g. health care professionals, promoters of health, educators, managers, employers, employees).
7. **Pharmaceutical Ethics:** Honour personal values and apply ethical principles in professional and social contexts. Demonstrate behavior that recognizes cultural and personal variability in values, communication and lifestyles. Use ethical frameworks; apply ethical principles while making decisions and take responsibility for the outcomes associated with the decisions.
8. **Communication:** Communicate effectively with the pharmacy community and with society at large, such as, being able to comprehend and write effective reports, make effective presentations and documentation, and give and receive clear instructions.
9. **The Pharmacist and society:** Apply reasoning informed by the contextual knowledge to assess societal, health, safety and legal issues and the consequent responsibilities relevant to the professional pharmacy practice.
10. **Environment and sustainability:** Understand the impact of the professional pharmacy solutions in societal and environmental contexts, and demonstrate the knowledge of, and need for sustainable development.
11. **Life-long learning:** Recognize the need for and have the preparation and ability to engage in independent and life-long learning in the broadest context of technological change. Self-assess and use feedback effectively from others to identify learning needs and to satisfy these needs on an ongoing basis.

DETERMINATION OF BLEEDINGTIME

AIM: To determine the bleeding time.

REQURIMENTS: Sterlised needle, filter paper, cotton, spirit, stopwatch.

PRINCIPLE:-The time required for complete stopping of blood flow from the punctured blood vessels by formation of fibrin network. On the surface of punctured skin is called the bleeding time. Normal**l**y it is 1-3minutes for a normal human blood.

The bleeding time test is a useful tool to test for platelet plug formation and capillary integrity. The bleeding time is dependent upon the efficiency of tissue fluid in accelerating the coagulation process on capillary function and the number of blood platelets present their ability to form a platelet plug.

=>Four methods used in determination of bleeding time:

1) Duke's method.

2) Ivy method.

3) Mielke method.

4) Simplate/Surgicut method.

PROCEDURE:

**Duke's method-

->Get a deep finger-prick under aseptic condition, get free flowing blood. Start the stopwatch

And note the time.

->Absorb/remove the blood drops every 30sec by touching the puncture site with filter paper

Along its edges, without pressing or squeezing the wound. Number the bloodspots.

->Note the time when bleeding stop i.e, when there is no trace of blood spot on the filter paper.

->Encircle this part and number this as wel this is the end point. Count the number of blood

Spots and express your result minute&sec.

->Normal bleeding time is1-5minutes.

PRECAUTIONS:

->Needle should be sterilized.

->Time should be noted properly.

->Discard first two drops of blood

CLINICALSIGNIFICANCE:

1)It plays a significant role

*To study the haemorrhagic disorders.

*To study the coagulation effect.

*To know platelet count

Bleeding time is prolonged in few disorders like vascular rlesions,platelet defect,severe liver diseases,uremia,and anti-coagulant drug administration.

OBSERVATION:The bleeding time of my own blood is observed to be 2minutes or 120seconds.

REPORT:

Patient name :

Date :

Method used :

Bleeding time :

Normal value :2to6minutes.

DETERMINATION OF CLOTTING TIME

Aim: To determine the clotting time of my own blood sample by capillary tube method (Wright's Capillary glass tube method)

Apparatus: Sterile needle, alcohol swab, cotton swab, capillary tube, stop clock.

Principle: It's a time interval in between onset of bleeding to forming a clot. The blood clot formed stops bleeding. The platelets play an important role to initiate clotting of the blood. There are 13 clotting factors that take part in process of clotting. It takes place in following steps.

1. Activation of prothrombin
2. Conversion of prothrombin into thrombin
3. Conversion of fibrinogen to fibrin (clot).

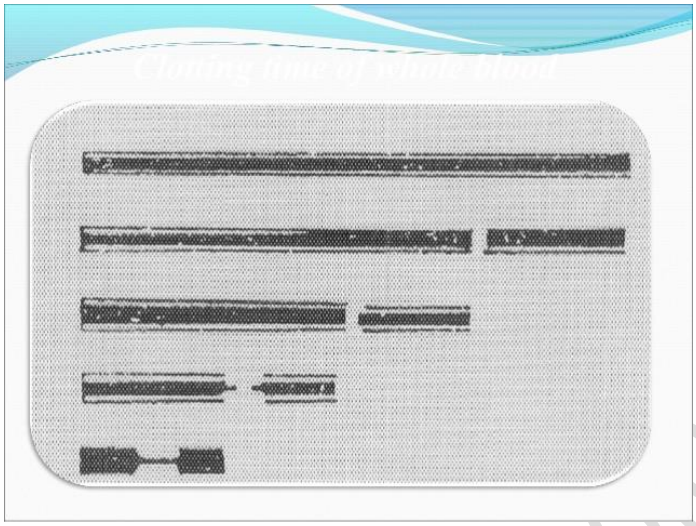
Methods used :-

- Capillary blood clotting/ Wright's capillary glass tube method.
- Drop method

Procedure:-

i. Sterilize the finger tip and give a deep prick for the free flow of blood absorb the first 2 drops of blood on a separate filter paper and allow a large drop.
ii. Now dip one end of the capillary tube in the blood, the blood rises into tube by capillary option.
iii. Note the time when the blood starts to end the time, this is zero time.
iv. Hold the capillary tube between the palms of your hands to keep the blood near body temperature
v. Gently break 1cm bits of glass tube from one end at intervals of 30 secs and look for the formation of fibrin threads between the broken ends. Note the time

Normal Clotting Time : 3-6 mins.



Clinical Significance:

i. The clotting of blood with this method involves both intrinsic and extrinsic systems of clotting. There is injury to blood (coming in contact glass, intrinsic pathway) injury to the tissues (extrinsic pathway) The clotting time is prolonged in haemophilia and other clotting disorders because thrombin normally can't be generated.

Precautions:

a. The skin sight chosen for clotting time should be scrubbed well with alcohol to increase blood flow.

b .The skin should be dry and puncture should be 3-4 mm deep to give free flowing blood. Do not squeeze.

Observation: The clotting time of my own blood sample was observed to be _____

Report:

Patient name:

Date:

Method used:

Clotting time:

Normal value:

STUDY OF EQUIPMENTS USED IN HAEMATOLOGICAL EXPERIMENTS

Aim:-To study the appliances used in haematological experiments.

Requriments:-i)Hemocytometer (RBC pipette, WBC pipette, Neubauer's chamber, coverslip.

Ii)Haemoglobinometer or haemometer.

Principle:-

Haemometer:- Hemocytometry is the procedure of counting the number of cells in a sample of blood; the red cells, the white cells, and the platelets being counted separately.
→Since the number of blood cells is very high, it is difficult to count them even under the microscope. This difficulty is partly overcome by diluting the blood to a known degree with suitable diluting fluids and then counting them.
→The sample of blood is diluted in a special pipette and is then placed in a capillary space of known capacity (volume) between a specially ruled glass slide (counting chamber) and a coverslip. The cells spread out in a single layer which makes their counting easy. Knowing the dilution employed, the number of cells in undiluted blood can then easily be calculated.

Units for reporting:-

The result of cell counting is usually expressed as "so many cells per cubic millimeter (c mm; mm3; µl) of blood".

Haemocytometer-The hemocytometer set consists of the following:

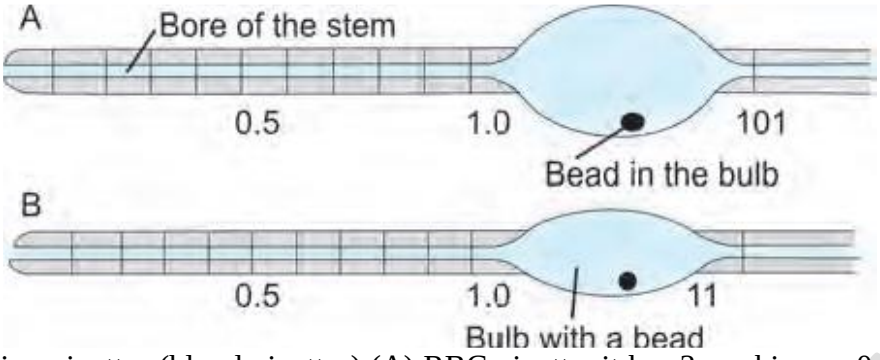
1. **The diluting pipettes** Two different glass capillary pipettes, each having a bulb, are providedfor counting RBCs and WBCs. These pipettes are sometimes called "cell pipettes" or blood pipettes.
2. **The counting chamber** It is a thick glass slide, appropriately ruled with a counting grid, i.e. squares of varying dimensions.
3. **Coverslips** Special coverslips having an optically plane and uniform surface should be preferred over ordinary coverslips.
4. **RBC and WBC** diluting fluids.
5. **Watch glasses** •Spirit swabs, •Blood lancet/needle, etc.

Study of diluting pipette:-

The two glass capillary pipettes used for diluting the blood. Each pipette has a long narrow stem (for measuring the blood), which widens into a bulb (for diluting the blood/which in turn, leads to a short stem.

Parts of a Diluting Pipette

The stem. The long narrow stem has a capillary bore and a well-grounded conical tip. It is divided into 10 equal parts (graduations) but has only two numbers etched on it—0.5 in the middle of the stem, and 1.0 (or 1) at the junction of stem and the bulb. The pipette, has a glossy white surface behind the graduations to facilitate their reading.



*The diluting pipettes (blood pipettes) (A) RBC pipette: it has 3 markings—0.5, 1.0, and 101, (B) WBC pipette: it has 3 markings—0.5, 1.0, and 11 (see text for dilutions obtained).

The bulb. The stem widens into a bulb which contains a free-rolling bead—red in the RBC pipette, and white in the WBC pipette. The bead helps in mixing the blood and the diluent and also helps in quick identification at a glance.

Rubber tube and mouthpiece. The bulb narrows again into a short stem to which a long, narrow, soft-rubber tube bearing a mouthpiece (often red in RBC pipette, and white in WBC pipette) is attached. The rubber tube should be at least 25–30 cm long to facilitate filling of the pipette by gentle suction. It also allows the pipette to be held horizontally so that one can comfortably watch the blood or diluting fluid entering the pipette.

Just beyond the bulb, the number 101 is etched on the RBC, and 11 on the WBC pipettes.

Principle:-The use of diluting pipettes:

It is important to understand that the numbers marked on the pipettes—0.5, 1.0 and 101 on the RBC pipette and 0.5, 1.0 and 11 on the WBC pipette. These figures only indicate relative volumes (parts)/or relative volumes in relation to each other. That is, half volume (from tip to mark 0.5), one volume (from the tip to mark 1.0) in both pipettes; and eleven volumes (from tip to mark 11 above the bulb in WBC pipette), and hundred one volumes (from tip to mark 101 in RBC pipette). It can be seen that the capillary bore in WBC pipette is wider than that in RBC pipette, and therefore, will hold more blood though the volume of the stem in both cases is 1.0 (one).

Differences Between the Two Pipettes

The differences between the two pipettes are given below:

	RBC pipette	WBC pipette
1.	Calibrations are 0.5 and 1.0 below the bulb and 101 above the bulb.	Calibrations are 0.5 and 1.0 below the bulb and 11 above the bulb.
2.	The capillary bore is narrow thus, it is an slow-speed pipette.	The capillary bore is wider thus, it is an fast-speed pipette.
3.	Bulb is larger and has an red bead.	Bulb is smaller and has a white bead.
4.	The volume of the bulb is 100 times of the volume contains in stem.	The volume of the bulb is 10 times of the volume contains in stem.

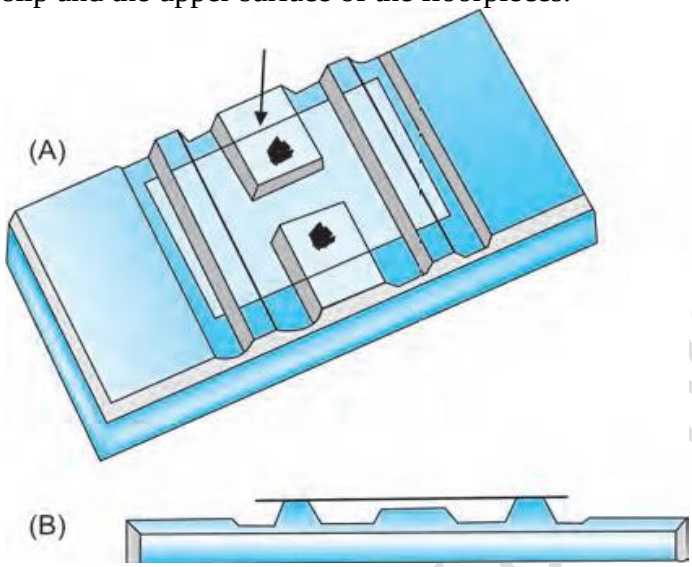
• **Note**:- Though the dilution obtained with the RBC pipette is 10 times that obtained with WBC pipette, its bulb is not 10 times bigger. The reason is the much finer bore in the red cell pipette.

Neubauer Chamber(Counting Chamber)

The counting chamber is a single, solid, heavy glass slide. Extending across its middle third are 3 parallel platforms (pillars, or flanges) separated from each other by shallow

trenches. The central platform or "floorpiece is wider, and exactly 0.1 mm lower than the two lateral pillars. The floorpiece is divided into two equal parts by a short transverse trench in its middle. Thus, there is an H-shaped trench or trough enclosing the two floorpieces.

The two lateral platforms can support a coverslip which, when in position, will span the trenches and provide a capillary space 0.1 mm deep between the under surface of the coverslip and the upper surface of the floorpieces.



*Hemocytometer, or counting chamber with improved Neubauer's ruling (A) Surface view, with the cover slip in position. (B) Side view with the coverslip in position.

Identically ruled areas, called "counting grids", consisting of squares of different sizes, are etched on each floorpiece. The two counting grids allow RBC and WBC counts to be made simultaneously if needed, or duplicate samples can be run.

The Counting Grid

The ruled area on each floor piece, the counting grid, has the following dimensions:

Each counting grid is divided into 9 large squares, each 1 mm^3, of these 9 squares, the 4 large corner squares are lightly etched, and each is divided by single lines into 16 medium-sized squares. These 4 large corner squares are employed for counting leukocytes and are, therefore, called WBC squares.

→The central densely etched large square (1 mm × 1 mm), called the RBC square, is divided into 25 medium-sized squares.

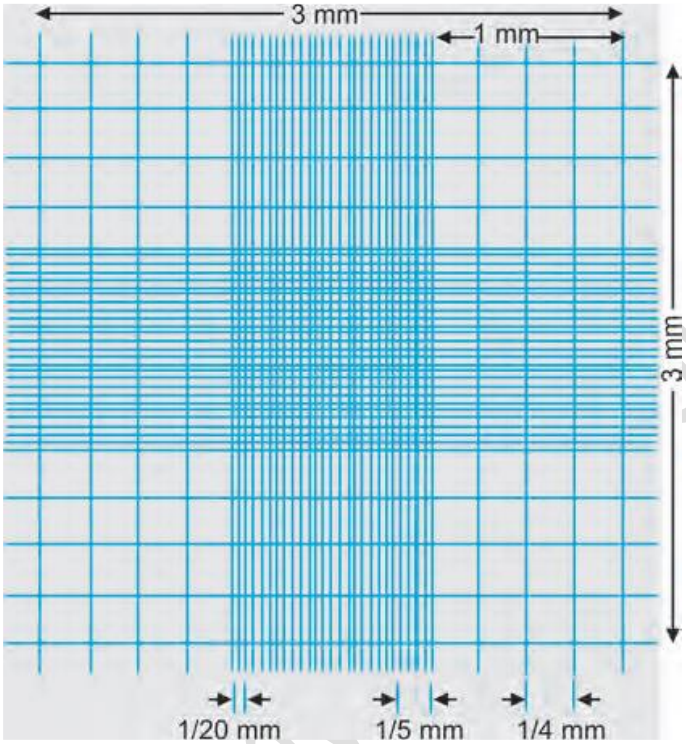
→Each of these medium squares is separated from its neighbours by very closely placed double lines extend in all directions.

→ Each of the 25 medium squares , bounded by double lines , is further divided into 16 smallest squares by single lines.

Focusing the Counting Grid

Examine the grid on each floor piece, without the coverslip, under low and high magnifications. Rack the condenser up and down, closing/adjusting the diaphragm at the same time. Find out the best combination of these two that shows the grid lines and squares clearly. When properly focused, the rulings (lines) appear as translucent darkish lines.

• With low magnification of 100 times, one large square, 1 mm × 1 mm is visible in one field, i.e. a group of 16 medium squares (for WBC counting), or a groups of 25 medium squares (for RBC counting).



Charging the Counting Chamber

The size of the drop, the angle at which the pipette should be held on the floor piece and the time needed for filling (charging) the chamber. This is called the "speed of the pipette". Obviously, it varies with the size of the capillary bore in the stem of the pipette.

• **High-speed pipette.** Since the bore of the WBC pipette is wider, a drop will form more quickly at its tip, and it will be larger, as compared to the RBC pipette. This requires that this pipette should be held more horizontally—say, at an angle of 10–20° and for a shorter time.

• **Slow-speed pipette.** The bore of the RBC pipette being narrow, it will take a longer time for a suitable drop to form. It should, therefore, be held at a steeper angle—say, 60–70°.

Procedure:-

The whole process of cell counting involves:-

1)Keeping all the equipment readily.

2)Getting a sample of blood.

3)Pipetting i.e., filling the pipette with blood and dilute it.

4)Charging i.e., filling the counting chamber with diluted blood.

5)Counting the cells.

i)Filling the pipette:-

→Get a finger prick under aseptic conditions, wipe away first two drops of blood. Allow large drop of free flowing blood to form.

→Holding the mouthpiece of the pipette between your lips and keeping the pipette at an angle about 40^0 to the horizontal , place its tip with in the edge of the drop. Gently suck on the mouthpiece and draw blood until it is just above the mark 0.3.
→Remove the pipette from the blood drop ad clean its outer surface with cotton swab by wiping it toward the tip and keep the pipette horizontalall time, bring the blood in the stem on the exact mark 0.5.
→Holding the pipette nearly vertical , immerse its tip in the diluting fluid taken in a watch glass and suck the dilutent to the mark 11(WBC) or 101(RBC).

ii)Mixing the blood with the diluting fluid:-

Once the diluting fluid has been sucked up remove the rubber tube. Holding the short stem above the bulb between your thumb and first two fingers , and pressing the tip of the pipette against the palm of the other hand, rotate it to and fro for 3-4 minutes. So that the blood and dilutent get thoroughly mixed.
→Alternate, remove the rubber tube , close the pipette ends with thumb and forefinger of your eight hand and shake it vigorously with a finger of eight motion.
→Do not shake the pipette with an end-wise motion as this will force the cells out of the bulb into the stem.

Charging the chamber:-

→Once the blood and the dilutent have been mixed well, charge the chamber.
→Place a coverslip on the chamber so that it spans the floor pieces and the trenches around them—a process called "centering" the coverslip.
→ Roll the pipette once more between your palms to mix the contents of the bulb.
→ Keeping your finger over the top of the pipette and releasing it in a controlled manner, allow the first 2 drops to drain by gravity. This fluid contains cell-free diluting fluid in the stem which has not taken any part in the dilution of blood.
→ Hold the pipette at an appropriate angle and watching carefully, allow a drop of diluted blood to form at its tip. Then quickly place the tip of the pipette on the floor piece in gentle contact with the edge of the coverslip. As the surface of the drop touches the coverslip, the fluid will run under it by capillarity and form a uniform film. Lift the pipette as soon as the floor piece is covered with diluted blood.
→ The chamber should be charged at one go and not in parts.

Ideally-charged chamber

An ideally-charged chamber is completely filled with diluted blood. If any blood flows into the trenches, it is called **"overcharging".** If the fluid is insufficient to cover the floor piece, or if there are air bubbles, it is called **"undercharging".**

Once the chamber has been properly charged, move it to the microscope. Wait for 2–3 minutes so that the cells settle down. Counting cannot be started when the cells are moving and changing places due to currents in the fluid.

Cleaning the pipette:-

Pipette should be cleaned as soon as possible after the experiment is over. Rinse it in running water, sucking and expelling water with maximum force for few times.

Counting the cells:-

For RBC counting:-The red cells are counted in 4 corners groups and one central group medium squares, each of which has 16 smallest squares i.e., in total of 80 smallest squares.

Area of smallest squares= 1/20mm × 1/20mm =1/400 mm^2
Since the depth of chamber is 1/10 mm, then volume of the smallest square
=1/400 × 1/10=1/4000mm^2.

For WBC counting:-This count is done in the 4 corner gropus of large squares, each of which has 16 medium squares.

Area of one medium square = 1/4mm × 1/4mm=1/16 mm^2.

Volume of this square =1/16 mm × 1/10mm= 1/160mm^2.

ESTIMATION OF HAEMOGLOBIN CONTENT

Aim: - To estimate Haemoglobin content in given sample.

Apparatus and Materials: - Sahli (Sahli-Adam's) Haemoglobine meter/Haemometer, 0.1 N Hcl, distilled water, sterile needle, alcohol swab, cotton swab.

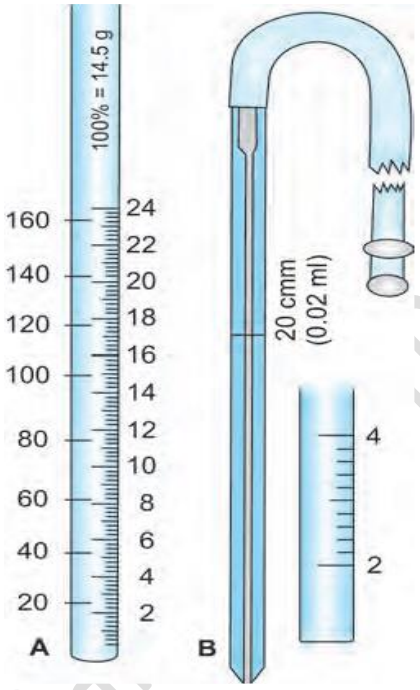
Principle: -The Haemoglobin present in a measured amount of blood is converted by dil.Hcl into acid haematin, which in dilution is golden brown in colour. The intensity of colour depends on the concentration of acid haematin which in turn depends on the concentration of Haemoglobin. The colour of solution after dilution with water is matched against golden-brown

tinted glass rods by direct vision. The readings are obtained in g %. Based on features of Haemoglobin, the various methods can be grouped into following categories.

1) Gasometric method.
2)Visual colour comparison.
3)Spectrophotometric method.
4)Electronic haematology Analysis.
5)Other methods.
These include estimation of iron content of blood and the copper sulphate specific gravity method.

Heamoglobinometry: The terms refer to measurement of concentration of haemoglobin in blood. For this purpose, an advantage is taken of the following characteristics of Haemoglobin.

1)Ability to combine with oxygen.
2)Presence of known amount of iron in each gram of Haemoglobin.
3)Ability of a solution of derivative of Haemoglobin to refract specific wavelength of light, thus giving typical absorption bands.



Sahli's method:

1)Comparator:- It is a rectangular plastic box with a slot in the middle which accommodates the calibrated Haemoglobin tube. Non-fading, standardize, golden –brown glass rods are fitted on each side of the slot for matching colour. An opaque white glass is fitted behind the slot to provide uniform illumination during direct visual colour matching.

2)Haemoglobin tube:- The square (or) round glass tube is calibrated in g Hb % (2-24 g %) in yellow colour on one side and in percentage Hb (20-40%) in red colour on other side. There is a brush to clean the tube.

3)Haemoglobin pipette:- its glass capillary pipette with only a single calibration mark-0.02ml.

4)Stirrer:- It's thin glass rod with a flattened end which is used for stirring and mixing the blood and dilution.

5)Distilled Water

Procedure:

Normal values:

For Males: 14-18g/dl

For Females: 12-16g/dl

1) Using a dropper, place 8-10 drops of Nl10 Hcl in Haemoglobin tube (or) up to the mark 20% (or) 3 g% and set it aside.

2)Get a finger prick under aseptic conditions Wipe away the first 2 drops of blood. When a large drop of free-flowing blood has formed again, draw the blood up to 20mm mark (0.02ml). Carefully wipe the blood sticking to the tip of the pipette with a cotton swab.

3)Immerse the tip of pipette to the bottom of acid solution and expel the blood gently. Avoid frothing of the mixture. Note the time.

4)Withdraw the pipette from the tube, touching in to the side of the tube, thus ensuring that no mixture is carried out of the tube. Mix the blood with acid solution with flat end of stirrer by rotating and gently moving it up and down.

5)Put the haemoglobin tube back in comparator and let it stand for 6-8mins. During this time the acid suptures the red cells, releasing their Hb into the solution. The acid acts on the Hb and converts it into acid haematin which is deep golden brown in colour.

6) Diluting and matching the colour:- dilute the acid haematin solution with distilled water till its

colour matches the colour of the standard tinted glass rods in comparator.

7)Take the Hb tube out of comparator and add distilled water drop by drop, stirring the mixture each time and comparing the colour with the standard.

8)Hold the comparator at eye level, away from the face, against bright light. Read the lower meniscus.

Clinical significance:

- Increased level of Hb, may be due to high red blood cells concentration.
  - i)Physiological: Males, new born, high altitude.

ii)Pathological: polycythemia (hypoxia due heart(or)lung disease)

- Decreased level of Hb, may be due to decreased RBC count

i)Physiological: Females during pregnancy.

ii)All cases of anaemia.

Observation: Haemoglobin convert of my own blood sample was estimated and found to be 10

gl dl.

Report:

Name:

Sex:

Date:

Normal range: 12-16gl dl

Hb content:

Method used: Visual colour compression method (Indirect method) .

DETERMINATION OF WBC COUNT OF BLOOD

Aim:- To determine the white blood cells count of my own blood sample.

Apparatus:- Turk's fluid (Glacial Acetic Acid-1.5ml, Gentian violet, needle, spirit, cotton swab, microscope.

Principle: The leukocyte count is the no. of WBC present in one cubic mm of blood. The no. Of WBC in given blood sample are many and is difficult to count cells. Sample is diluted with suitable diluents to known degree, which destroys the red cells and stains the nuclei of leucocytes. The cells are then counted in counting chamber (Neubauer's).The diluted blood sample is placed in capillary space of (0.1mm depth) between counting grid and cover slip. The cells then spread out in a single layer under capillary and form a uniform film. Number of cells are counted under low power objective and calculated are reported as WBC per mm3.

a)Physiology:- Leucocytes are two types:

1)Granular: Neuteophill, Eosiniphill, Basiophill.

2)Agranular: Lymphocytes, Monocytes.

The WBC play an important role in body defines mechanism against invading microbes by phagocytises; they also help in antibody formation. Normal range 4000-11000 l mm3.

Procedure:

1)Take 1 ml of Turk's in a watch glass. Place the counting chamber on microscope stage. Adjust illuminator and focus the rigtor upper group of 16 WBC squares. You will see all the squares in one field.

2)Observing all aseptic precautions get a finger prick discard the first two drops of blood and let a good sized drop to form.

3)Filling the pipette:- Dip the tip in the edge of drop, draw blood to the mark 0.5 and suck Turk's fluid to mark 1L mix them thoroughly for 3-4 mins. Draw blood up the mark 1.0 in pipette, followed by Turk's fluid to mark 1L. This gives a dilution of 1 in10.

4)Charging the chamber:-Discard first 2drops of fluid from the pipette and charge the chamber. The chamber should neither be over-charged nor under-charged.

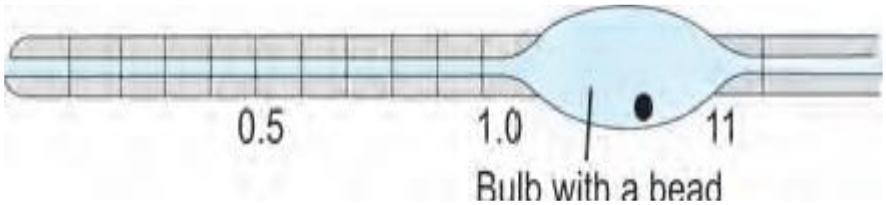
5)Allow the cells to settle for 3-4 mins, then carefully transfer the chamber to microscope use the fine adjustment again and try to identify the WBC's under low magnification; the leucocytes appear round, shiny, darkish dots with a whole around them. These dots represent the nuclei, which have been stained by gentian violet. The cytoplasm isn't stained.

Counting the cells:

- Place the haemocytometer on the microscope stage with low power objective, focus the chamber area to bring the four large (W) corners regions into view.
- Determine no. of cells in each of four specified areas. On the edges of squares count only cells and that touches the line on the left and top sides. Omit the cells touching the lines at bottom and right side.
- Count the cells in 4 graphs of 16 squares each i.e., in a total of 64 squres.
- Draw appropriate squares in your work book for entering values.
- Rinse the haemocytometer and wipe it dry.

Clinical Significance:-

1)Leucocytes:- Increased no. Of WBC than normal Ex. Pneumonia tonsillitis, diphtheria, rheumatic fever, etc.



The WBC pipette. It has 3 markings—0.5, 1.0, and 11

2)Leucoperia: - Decreased no. Of WBC than normal. Ex: Influenza, typhoid, hepatitis, cirrohor's of liver, myxedeno etc.

Precautions:-

1)When mixing blood and Turk's fluids give sufficient time for complete haemolysis of RBC.

2)Continuously rack the microscope while identifying and counting the cells, so that cells sticking to the under surface of the cover slip are not missed.

Observation: The count of WBC is performed and count was found to be.

Report:

Name :
Age :
Sex :
Date :
Normal value :
Total leucocyte count :

Calculations

The leukocytes were counted in 64 squares, the volume of one square being 1/160 mm3.
Volume of 64 squares = 1/160 × 64 = 4/10 mm3.
Thus, the total volume of diluted blood in which WBCs were counted = 4/10 mm3.
Let the count in 4/10 mm3 be = x
Then 1 mm3 of diluted blood will contain
= x × 10/4 white cells.
Since the dilution employed is 20 times (10 times in the 2nd pipette)
1 mm3 of undiluted blood
will contain = x × 10/4 × 20
= x × 200/4
= x × 50
(x × 10/4 × 10 in the 2nd pipette)

DETERMINATION OF RBC COUNT OF MY OWN BLOOD

Aim: To determine the red blood cells of my own blood sample.

Apparatus and Materials: Haemocytometer, microscope, sterile needle, cotton, sprit, RBC diluting fluid(Hayem's fluid).

Composition of RBC diluting fluid

Sodium chloride(NaCl) – 0.50g

Sodium sulphate (Na_2SO_4) - 2.50 g

Mercuric chloride (HgCl) – 0.25g

Distilled water

Characteristics of diluting fluid:

The ideal fluid for diluting the blood should be isotonic and neither cause hemolysis nor crenation of red cells. It should have a fixative to preserve the shape of RBCs and also prevent their autolysis so that they could be counted even several hours after diluting the blood if necessary. It should prevent agglutination and not get spoiled on keeping.

Physiology:

Red blood cells are biconcave discs they have no nucleus, they cannot divide. Their life span in the circulation is about 120 days. The process of development of red blood cells from stem cells takes about 7 days. Their diameter is about 7 micrometers.

Principle:

The blood is diluted 200 times in a red cell pipette and the cells are counted in the counting chamber. Knowing the dilution employed, their number in undiluted blood can easily be calculated.

Procedure:

1. Place about 2 ml of Hayem's fluid in a watch glass.
2. Examine the chamber, with the coverslip 'centred' on it, under low magnification. Adjust the illumination and focus the central 1 mm square (RBC square on the counting grid) containing 25 groups of 16 smallest squares each. All these squares will be visible in one field. Do not change the focus or the field.

• Admitting too much light is a common cause of the inability to see the grid lines and squares clearly.

3. Move the chamber to your work-table for charging it with diluted blood. (It can be charged while on the stage, but it is more convenient to charge it on the table).
4. **Filling the pipette with blood and diluting it:** Get a finger-prick. Wipe the first 2 drops of blood and fill the pipette from a fresh drop of blood up to the mark 0.5. Suck Hayem's fluid to the mark 101 and mix the contents of the bulb for 3–4 minutes as described earlier.
5. **Charging the chamber:** Observing all the precautions, fill the chamber with diluted blood.

• Since the RBC pipette is a slow-speed pipette, it will need to be kept at an angle of 70–80° while charging the chamber.

6. Move the chamber to the microscope and focus the grid once again to see the central 1 mm square with the red cells distributed all over.
7. **Counting the cells:** Switch over to high magnification (HP lens) and check the distribution of cells.
8. Move the chamber carefully and bring the left upper corner block of 16 smallest squares in the field of view.

Rules for counting:

Note that the immediate boundary of each smallest square is formed by the 4 lines forming the square (side: 1/20 mm; area: 1/400 mm2) the other lines of the tram or triple lines do not form part of the boundary of that square.

i. Cells lying within a square are to be counted with that square.

ii. Cells lying on or touching its upper horizontal and left vertical lines are to be counted with that particular square.

iii.Cells lying on or touching its lower horizontal and right vertical lines are to be omitted from that square because they will be counted with the adjacent squares.

• While counting the cells, continuously "rack", the fine adjustment up and down so that cells sticking to the underside of the coverslip are not missed.

9. We have already focused the upper left block of 16 smallest squares in the high power field. First count the cells in the upper 4 horizontal squares from left to right, then come down to the next row and count the cells in each square from right to left. Then count the cells in the 3rd row from left to right, and in the 4th row, from right to left.

10. Move the chamber carefully till you reach the right upper corner block of 16 smallest squares (there are no smallest squares above and to the right of this group), and count the cells as before. Then move on to the right lower corner and then left lower corner groups, and finally count the cells in the central block of 16 smallest squares.

Thus, the counting will have been done in 80 smallest squares, i.e., in 5 blocks of 16 squares each.

Observation: RBC count of my own blood sample is found to be

Report:

Name :
Age :
Sex :
Date :
Normal value : Males- 4.5 million to 6.5 million/mm^3
Females- 3.8 million to 5.8 million/mm^3
My own value :

Calculation of red cell count

i. Let x be the number of cells in 1/50 mm3 of diluted blood.
Cells in 1 mm3 of diluted blood = x × 50
Dilution employed was = 1 in 200
∴ Number of cells in 1 mm3 of
undiluted blood will be = x × 50 × 200
= x × 10000
Thus, adding, 4 zeros in front of x will give the RBC count per 1 mm3 of undiluted blood.

Differential Leukocyte Count (DLC)

Aim: To determine differential leucocytes count of individual

APPARATUS AND MATERIALS

1. Microscope. •2 Clean glass slides. •Sterile lancet. •Cotton and gauze swabs. •70% alcohol. •Glass dropper.
2. A drop bottle containing Leishman's stain.
3. A wash bottle of distilled water (or buffered water, if available). • Fluff-free blotting paper.

Leishman's stain It is probably one of the simplest and most precise method of staining blood for diagnostic purposes. It contains a compound dye—**eosinate of methylene-blue** dissolved in acetone-free methyl alcohol.

i. Eosin. It is an acidic dye (negatively charged) and stains basic (positive) particles—granules of eosinophils, and RBCs a pink color.

ii. Methylene-blue. It is a basic dye (positively charged) and stains acidic (negatively charged) granules in the cytoplasm, nuclei of leukocytes, especially the granules of basophils, a blue-violet color.

iii. Acetone-free and water-free absolute methyl alcohol. The methyl alcohol is a fixative and must be free from acetone and water. It serves two functions:

a. It fixes the blood smear to the glass slide. The alcohol precipitates the plasma proteins, which then act as a 'glue' which attaches (fixes) the blood cells to the slide so that they are not washed away during staining.

b. The alcohol preserves the morphology and chemical status of the cells.

• The alcohol must be free from acetone because acetone being a very strong lipid solvent, will, if present, cause crenation, shrinkage, or even destruction of cell membranes. This will make the identification of the cells difficult. (If acetone is present, the stain deteriorates quickly).

• The alcohol must be free from water since the latter may result in rouleaux formation and even hemolysis. The water may even wash away the blood film from the slide.

PRINCIPLE

A blood film is stained with Leishman's stain and scanned under oil immersion, from one end to the other. As each WBC is encountered, it is identified until 100 leukocytes have been examined. The percentage distribution of each type of WBC is then calculated. Knowing the

TLC and the differential count, it is easy to determine the number of each type of cell per mm3

Procedure:

Getting a blood sample from a finger-prick and making blood smears.

place a drop of blood (through the needle) on one slide and spread blood films

Air dry the slides immediately by waving them in the air.

Fixation is the process that makes the blood film and its cells adhere to the glass slide. It also preserves the shape and chemistry of blood cells as near living cells as possible. (See Q/A 8). **Staining** is the process that stains (colors) the nuclei and cytoplasm of the cells. Both these purposes are achieved by the Leishman's stain.

Pour 8–10 drops of the stain on each unfixed slide by dripping it from a drop bottle, or use a dropper. This amount of stain usually covers the entire surface and "stands up" from the edges of the slides without running off. Note the time. Allow the stain to remain undisturbed for 1–2 minutes, as advised.

add an equal number of drops of distilled water (or buffered water, if available) to the stain. If the water is carefully dripped from a drop bottle or a dropper, the entire mixture will stand up from the edges of the slides

Flush off the diluted stain in a gentle stream of distilled water for about 30 seconds and leave the slides on the rack for about a minute with the last wash of water covering them. Drain the slides and put them in an inclined position against a support, stained sides facing downwards (to prevent dust particles settling on them) to drain and dry.

[You may also try the following method on an unfixed slide which gives very good results. Cover the smear with 10 drops of stain. After 30 seconds, add 20 drops of water, and leave for 15 minutes. Pick up the slide with forceps (to avoid purple fingers), and rinse in fast-flowing tap water for 1 second only. Dry as before].

Draw 100 squares in your workbook for recording various WBCs as they are encountered and

identified one after another. Enter these cells by using the letters ‘N’ for neutrophils, ‘M’ for monocytes, ‘LL’ for large lymphocytes, ‘SL’ for small lymphocytes, ‘E’ for eosinophils, and ‘B’ for basophils. Move the slide slowly to the right (the image will move to the left) and as you encounter a leukocyte, identify it, and enter it in your workbook.

Granulocytes				
Neutrophils (40–70%)	10–14 (1.5-2X a RBC)	Blue-violet 2–6 lobes, connected by chromatin threads Seen clearly through cytoplasm	Slate-blue in color	Fine, closely-packed violet pink Not seen separately Give ground-glass appearance Do not cover nucleus
Eosinophils (1–6%)	10–15	Blue-violet 2–3 lobes, often bi-lobed, lobes connected by thick or thin chromatin band Seen clearly through cytoplasm	Eosinophilic Light pink-red Granular	Large, coarse Uniform-sized Brick-red to orange Seen separately Do not cover nucleus
Basophils (0–1%)	10–15	Blue-violet Irregular shape, may be S-shaped, rarely bilobed Not clearly seen, because overlaid with granules	Basophilic Bluish Granular	Large, very coarse Variable-sized Deep purple Seen separately Completely fill the cell, and cover the nucleus
Agranulocytes				
Monocytes (5–10%)	12–20 (1.5-3 X a RBC)	Pale blue-violet Large single May be indented horse-shoe, or kidney shaped (can appear oval or round, if seen from the side)	Abundant ‘Frosty’ Slate-blue Amount may be larger than that of nucleus	No visible granules
Small Lymphocytes (20–40%)	7–9	Deep blue-violet Single, large,	Hardly visible Thin crescent of clear, light blue	No visible granules

		round, almost fills cell. Condensed, lumpy chromatin, gives 'ink-spot' appear-ance	cytoplasm	
Large lymphocytes (5–10½)	10–15	Deep blue-violet Single, large, round or oval, almost fills cell May be central or eccentric	Large, crescent of clear, light blue cytoplasm Amount larger than in small lymphocyte	No visible granules

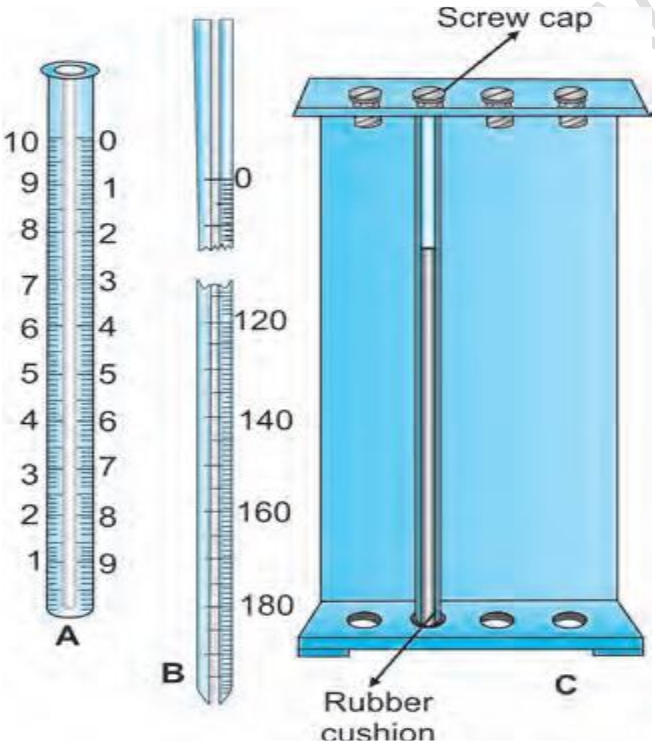
Erythrocyte sedimentation rate

AIM: to determine ESR

APPARATUS AND MATERIALS

1. Disposable syringe and needle •Sterile swabs moist with 70% alcohol •Container (discarded penicillin bottle, etc.) with double oxalate mixture, or sequestrene.
2. Pasteur pipette with a long thin nozzle.

3. Wintrobe tube and stand. a Wintrobe tube Check out its dimensions and markings once again. It is graduated 0 to 10 cm from above downwards on one side (for ESR) and 10 to 0 cm on the other side.



The Wintrobe stand can hold up to 3 (or 6) tubes at a time. It is provided with a spirit level to ensure that the tubes are held vertical throughout the test.

PRINCIPLE

In the circulating blood the red cells remain uniformly suspended in the plasma. However, when a sample of blood, to which an anticoagulant has been added, is allowed to stand in a narrow vertical tube, the red cells (specific gravity = 1.095) being heavier (denser) than the colloid plasma (specific gravity = 1.032), settle or sediment gradually towards the bottom of the tube. The rate, in mm, at which the red cells sediment, called ESR, is recorded at the end of one hour.

Sedimentation of red cells

The settling or sedimentation of red cells in a sample of anticoagulated blood occurs in 3 stages:

i. In the ***first stage,*** the RBCs pile up (like a stack of coins), and form rouleaux that become heavier during the first 10-15 minutes.

ii. During the ***second stage,*** the rouleau (pleural of rouleaux) being heavier (see below) sink to the bottom. This stage lasts for 40–45 minutes.

iii. In the **third stage,** there is packing of massed bunches of red cells at the bottom of the blood column. This stage lasts for about 10–12 minutes.

PROCEDURES

1. Draw 2.0 ml of venous blood and transfer it to a container of anticoagulant. Mix the contents gently but well by inverting the vial a few times, or by swirling it. Do not shake, as it will cause frothing.
2. Using the Pasteur pipette, fill the Wintrobe tube from below upwards as was done in Expt 1-9. Ensure that there are no air bubbles.
3. Transfer the tube to its stand and adjust the screws so that it will remain vertical. Leave the tube undisturbed in this position for one hour, at the end of which read the mm of clear plasma above the red cells.

Express your result as: .....mm 1st hour (Wintrobe).

Normal values

Males : 2–8 mm 1st hour

Females : 4–10 mm 1st hour

WESTERGREN'S METHOD

APPARATUS AND MATERIALS

1. 2 ml disposable syringe with needle •Sterile cotton/gauze swabs moist with alcohol •Container (discarded penicillin bottle).
2. Sterile solution of 3.8 percent sodium citrate as the anticoagulant.

3. Westergren pipette (tube) and stand. Figure 1-17 shows a Westergren pipette. It is 300 mm long and has a bore diameter of 2.5 mm. It is calibrated in cm and mm from 0 to 200, from above downwards in its lower two-thirds. The Westergren stand can accommodate up to 4 tubes at a time. For each pipette, there is a screw cap that slips over its top, and, at its lower end, the pipette presses into a rubber pad or cushion. When the pipette is fixed in position, there is enough pressure of the screw cap to prevent leakage of blood from its lower end. There is a spirit level to ensure vertical position of the pipette.

PROCEDURES

1. Draw 2.0 ml of venous blood and transfer it into a vial containing 0.5 ml of 3.8% sodium citrate solution. This will give a blood: citrate ratio of 4:1. Mix the contents by inverting or swirling the vial. Do not shake, as it will cause frothing.
2. Fill the Westergren's pipette with blood-citrate mixture by sucking, after placing the tip of your finger over the top of the pipette to control the flow of blood into and out of it, or with a rubber bulb. Bring the blood column to exact zero mark. (If there is a difference of 1–2 mm, it should be noted and taken into account before giving the final report at the end of one hour).
3. Keeping your finger (or the rubber bulb) over the pipette, transfer it to the Westergren stand by firmly pressing its lower end into the rubber cushion. Now slip the upper end of the pipette under the screw cap. Confirm that there is no leakage of blood and that the pipette will remain vertical.
4. Leave the pipette undisturbed for one hour at the end of which read the mm of clear plasma above the red cells.

Express your results as:………mm 1st hour (Westergren).

Normal values

Males : 3–9 mm 1st hour

Females : 5–12 mm 1st hour.

OBSERVATIONS AND RESULTS

Note that if there is no hemolysis, there is a sharp line of demarcation between the red cells and the clear, cell-free, and straw-colored plasma. The bore of the pipette if not less than 2 mm has no effect on ESR, but inclination from the vertical gives false high values. Higher values are also obtained at extremes of temperature, in anemia, and after ingesting food.

Sources of Error. These include: tilting of the tube and high temperature lead to high values while low temperature gives false low values. Hemolysed blood may obscure the sharp line separating red cells and the plasma.

PRECAUTIONS

1. Proper anticoagulant should be used for each method.
2. The blood should be collected in the fasting state.
3. The test should preferably be done within 2–3 hours of collecting the blood sample, and at a room temperature of 20–35°C.
4. The hematocrit should be checked and correction factor applied in cases of anemia (nomograms are available for this purpose).
5. Clotted or hemolysed blood must be discarded.
6. The Wintrobe tube or Westergren pipette should not be disturbed from the vertical position for the duration of the test.

Determination of Heart Rate and Pulse Rate

Aim: To determine the heart rate and pulse rate of an individual

Principle: The alternate expansion and recoil of elastic arteries after each systole of the left ventricle creates a travelling pressure wave that is called 'pulse'.

The pulse is the surge of blood that is pushed through the arteries when the heart beats. Pulse rate is the number of times your heart beats every minute or the number of times your heart beats in one minute. Pulse is lower when a person is at rest and higher when you exercise.

Heart Rate is the number of pulses over a minute and is the standard heart measurement. Heat rate can vary from person to person. The heart rate can be taken at any spot on the body at which an artery is close to the surface and pulse can be felt.

The most common place to measure the heart rate using the palpitation method is at the wrist (radial artery) and neck (carotid artery). Other places that are used sometimes are the elbow (brachial artery) and the groin (femoral artery), ankle and foot.

One should always use his/her fingers to take a pulse, not the thumb in particular when recording someone's pulse as you can sometimes feel your own pulse through your thumb.

A healthy pulse is between 60 and 100 beats per minute (bpm). Besides the pulse rate, other indicators of how a person is doing come from the regularity and strength of the pulse.

Many factors can influence heart rate including activity level, fitness level, air temperature, body position, emotions, body size and medications.

Procedure:

Manual Method – Radial pulse (wrist)

1. **Locating the pulse**

a. Have the patient hold his or her hand out with palm facing upwards

b. Place two fingers (index and middle finger) between the bone and tendon on your radial artery, which is located on the thumb, side of your wrist about half inch on the inside of the joint in line with the index finger. The pulse feels like a rhythmic thumping.

2. **Count the beats**

a. Compress the artery against a bone or a firm structure. Using a clock or a watch with seconds hand, time yourself counting the pulsating beats for one minute.

Carotid pulse (neck): Place your index and third finger on your neck to the side of your windpipe. Be careful not to press too hard. Then count the number of beats for a minute.

Clinical Significance:

Tachycardia – Increased heart or pulse rate over 100 bpm.

Bradycardia- Slow resting heart or pulse under 50 bpm.

Observation: Heart rate or pulse rate is observed to be …….

Report:

Name-

Age-

Sex-

Date -

Method -

Normal value-

Observed value-

Recording of Blood Pressure

Aim: To record blood pressure of individual.

Requirements: Stethoscope, sphygmomanometer

Principle:

A sufficient length of a single artery is selected in the arm (brachial artery), or in the thigh (femoral artery). The artery is first compressed by inflating a rubber bag (connected to a manometer) placed around the arm (or thigh) to stop the blood flow through the occluded section of the artery. The pressure is then slowly released and the flow of blood through the obstructed segment of the artery is studied by:

i. Feeling the pulse—the palpatory method.

ii. Observing the oscillations of the mercury column—the oscillometric method, and

iii. Listening to the sounds produced in the part of the artery just below the obstructed segment— the auscultatory method.

Apparatuses

Stethoscope (Steth = chest, scope = to inspect)

Though introduced in its present form by Laennec in 1819, it was not until 1905 that Korotkoff used it for recording the blood pressure. The sounds produced in the chest and elsewhere in the body are heard with a stethoscope. The instrument has the following 3 parts:

a. The chest-piece. The chest-piece has two end pieces—a bell and a flat diaphragm, though some have only the diaphragm.

b. The rubber tubing. In the commonly used stethoscope, a single soft-rubber pressure tube (inner diameter 3 mm) leads from the chest-piece to a metal Y-shaped connector. The plastic diaphragm causes magnification of low-pitched sounds though it distorts them a little. The bell-shaped chest-piece conducts sounds without distortion but with little magnification. Murmurs which precede, accompany, or follow the heart sounds are better heard with the bell.

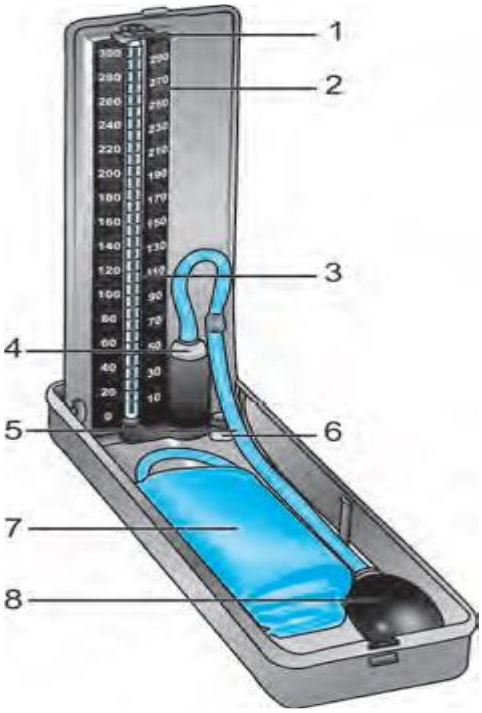
c. The ear-frame. It consists of two curved metallic tubes joined together with a flat U-shaped spring which keeps them pulled together. The upper ends of the tubes are curved so that they correspond to the curve of the external auditory meatus, i.e. they are directed forwards and downwards. Two plastic knobs threaded over the ends of the tubes fit snugly in the ear. Two rubber tubes connect the Y-shaped connector to the metal tubes.

Sphygmomanometer (Commonly called the "BP apparatus")

The sphygmomanometer is the instrument routinely used for recording arterial blood pressure in humans. The term "sphygmomanometer" is derived from three Greek roots with Latin equivalents "sphygmo" means pulse, "manos" means thin, and "metron" refers to measure. In early procedures, when physicians used to feel the pulse during measurement of BP, they described its first appearance as "thin", hence the term. Different types of BP instruments are in use, but the one in common use is the mercury sphygmomanometer. It consists of the following parts:

a. Mercury manometer. The manometer is fitted in the lid of the instrument. One arm of the manometer is the reservoir for mercury—a broad and short well that contains enough mercury to be driven up in the other limb—the graduated glass tube.

b. Graduated tube. The manometer glass tube is graduated in mm from 0 to 300, each division representing 2 mm, though actually slightly less than 2 mm. The reason for this is the greater diameter of the mercury reservoir than that of the glass tube. For example, when mercury is driven up the tube for, say, 20 mm Hg, the meniscus in the reservoir falls less so that the actual pressure on its mercury is slightly greater than 20 mm Hg



1: Spring-loaded clip; 2: Lid of the apparatus; 3: Graduated glass tube; 4 One-way valve; 5: Mercury reservoir; 6: Stop cock; 7: Armlet; 8: Air pump (rubber bulb, with leak valve.)

And, to compensate for this, the tube is calibrated with divisions that are slightly less than 2 mm apart. A stopcock between the two limbs, when closed, prevents the mercury from entering the glass tube. The one-way valve fitted at the top of the mercury well prevents spilling of mercury when the lid is closed, while allowing pressure to be transmitted from the rubber bag to the mercury reservoir. A spring-loaded clip at the top of the tube keeps it firmly pressed into a rubber washer at its lower end to prevent leakage of mercury.

c. The armlet (rubber bag; Riva Rocci cuff). The "cuff" as it is usually called, consist of an inflatable rubber bag, 24 cm × 12 cm, which is fitted with 2 rubber tubes—one connecting it to the mercury reservoir and the other to a rubber bulb (air pump). The bag is enclosed in a long strip of inelastic cloth with a long tapering free end. The cloth covering keeps the rubber bag in position around the arm when pressure is being measured. In some cuffs, 2 velcron strips are provided in appropriate locations for the same purpose. The rubber bag is 12 cm wide which is enough to form a pressure cone that reaches the underlying artery even in a thick arm. As a general rule, the width of the bag should be 20% more than the diameter of the arm, though it should be wider in an obese person. The recommended width of the bag in different age groups is as under:

Infants (below 1 year) : 2.5 cm

Below 4 years : 5 cm

Below 8 years : 8 cm

Adults : 12 cm

d. Air pump (rubber bulb). It is an oval-shaped rubber bulb of a size that conveniently fits into one's fist. It has a one-way valve at its free end, and a leak-valve with a knurled screw, at the other where the rubber tube leading to the cuff is attached. The cuff can be inflated by turning the leak valve screw clockwise, and alternately compressing and releasing the bulb. Deflation of the bag is achieved by turning this screw anti-clockwise.

A. Palpatory Method (Riva Rocci 1896)

1. Make the subject sit or lie supine and allow 5 minutes for mental and physical relaxation.

2. Open the lid of the apparatus until you hear the "click". Release the lock on the mercury reservoir and check that the mercury is at the zero level. If it is above zero, subtract the difference from the final reading. If it is below zero, add the required amount of mercury to bring it to zero level.

3. Place the cuff around the upper arm, with the center of the bag lying over the brachial artery, keeping its lower edge about 3 cm above the elbow. Wrap the cloth covering around the arm so as to cover the rubber bag completely, and to prevent it bulging out from under the wrapping on inflation. The cuff should neither be too tight nor very loose.

4. Palpate the radial artery at the wrist and feel its pulsations with the tips of your fingers. Keeping your fingers on the pulse, hold the air bulb in the palm of your other hand and tighten the leak valve screw with your thumb and fingers.

5. Inflate the cuff slowly until the pulsations disappear; note the reading then raise the pressure another 30–40 mm Hg.

6. Open the leak valve and control it so that the pressure gradually falls in steps of 2–3 mm. Note the reading when the pulse just reappears. **The pressure at which the pulse is first felt is the systolic pressure**. (It corresponds to the time when, at the peak of each systole, small amounts of blood start to flow through the compressed segment of the brachial artery). Deflate the bag quickly to bring the mercury to the zero level

7. Record the pressure in the other arm. Take 3 readings in each arm, deflating the cuff for a few minutes between each determination.

Advantages of palpatory method. This method avoids the pitfall of the auscultatory method in missing the auscultatory gap

Disadvantages of palpatory method:

i. This method measures only the systolic pressure, the diastolic pressure cannot be measured.

ii. This method lacks accuracy because the systolic pressure measured by it is lower than the actual by 4–6 mm Hg. It assumes that the first escape of blood under the cuff will cause pulsations in the peripheral artery (radial in this case). However, there is no evidence that the amount of blood that escapes when the artery first opens is enough to produce a pulse wave detectable by the fingers. Thus, definite pulsation may not occur until the cuff pressure has been reduced by 6–8 mm Hg.

B. Oscillatory Method

Riva Rocci, in 1896 (i.e. before Korotkoff sounds were described) measured systolic pressure (SP) by the palpatory method while the diastolic pressure (DP) was recorded from the oscillations of the mercury column. As the cuff pressure was raised and then lowered, oscillations appeared which became maximum and then disappeared. Some workers took **the midpoint of maximum oscillations as the** DP while others considered the **lower level of these oscillations as the DP** (oscillations are best seen with an aneroid manometer). The students must have seen these oscillations in the mercury column.

In a modification of the above method, a cuff is placed on the upper arm and a lightly-inflated one on the lower arm. As the pressure in the upper cuff is raised and lowered, pulsations can be recorded from the lower cuff.

1. **Digital Blood Pressure Monitor.** It is a small, compact, battery-operated, palm-top unit with an LCD display screen and memory function. The recorder works on the 2-cuff *"oscillometric measuring"* principle described above, and automatically translates pulse wave oscillations into SP and DP. The advantage of this method is that it can be easily used by a layperson. The pressure measuring range is 0–280 mm Hg, while the HR range is 40–180/min. There are 2 input sockets, one on either side of the unit. Connect the rubber bulb to one socket and the cuff (wrapped on the upper arm) to the other. The procedure is the same as that for auscultatory method except that you do not auscultate for Korotkoff sounds. As the pressure is raised and then

lowered, the pressure and pulse readings appear on the screen and the final readings remain there until you switch off the unit.

2. Wrist Digital Blood Pressure Monitor

This innovative, compact, battery-operated BP monitor also works on the oscillometric principle. It fits over the wrist with a Velcro cuff. The controls include: Mode/set : for date and time; Start/stop, and Recall buttons.

PROCEDURE

1. Wrap the cuff on the wrist, with the palm of that arm facing up. Ensure that the wrist is at the level of the heart.

2. Press the start button. The cuff starts inflating immediately and automatically, and the readings show a gradual increase. Soon after the artery gets occluded, the readings begin to decrease in steps of 4–6 mm Hg till the final readings for SP, DP and Pulse rate appear on the screen.

C. Auscultatory Method (Korotkoff, 1905)

1. Place the cuff over the upper arm as described above, and record the BP by the palpatory method.

2. Locate the bifurcation of brachial artery (it divides into radial and ulnar branches) in the cubital space just medial to the tendon of the biceps which can be easily palpated in a semiflexed elbow as a thick, hard, elongated structure. Mark the point of arterial pulsation with a sketch pen.

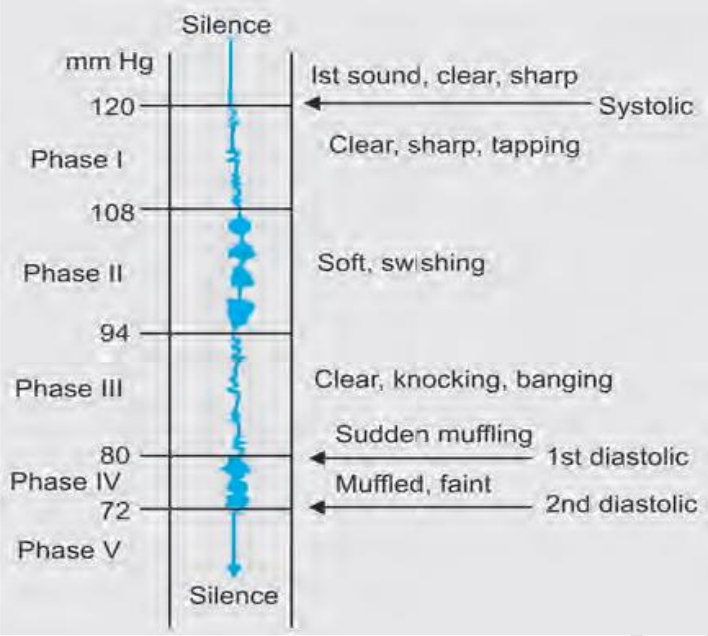
3. Place the chest-piece of the stethoscope on this point and keep it in position with your fingers and thumb of the left hand (if you are right-handed).

4. Inflate the cuff rapidly, by compressing and releasing the air pump alternately (sounds may be heard as the mercury column goes up). Raise the pressure to 40 to 50 mm Hg above the systolic level as determined by the palpatory method.

5. Lower the pressure gradually until a clear, sharp, tapping sound is heard. Continue to lower the pressure and try to note a change in the character of the sounds.

These sounds are called Korotkoff sounds and show the following phases:

Phase I This phase starts with a clear, sharp tap when a jet of blood is able to cross the previously obstructed artery. (Sometimes this phase may start with a faint tap, especially when the systolic pressure is very high). As the pressure is lowered, the sounds continue as sharp and clear taps. This phase lasts for 10–12 mm Hg fall in pressure.



Phase II The sounds become murmurish and remain so during the next 10–15 mm Hg fall in pressure when they again become clear and banging.

Phase III It starts with clear, knocking, or banging sounds that continue for the next 12 to 14 mm Hg pressure, when they suddenly become muffled.

Phase IV The transition from phase III to phase IV is usually very sudden. The sounds remain muffled, dull, faint and indistinct (as if coming from a distance) until they disappear. The muffling of sounds and their disappearance occurs nearly at the same time, there being a difference of 4–5 mm Hg (i.e. phase IV lasts for 4–5 mm Hg).

Phase V This phase begins when the Korotkoff sounds disappear completely. If you reduce the pressure slowly, you will note that total silence continues right up to the zero level.

6. Take 3 readings with the auscultatory method and repeat 3 readings on the other arm.

Phases of Korotkoff sounds, showing the changes in their character during each phase as the mercury column is gradually lowered. Systolic pressure: 1st appearance of sounds. Diastolic pressure: sudden muffling of sounds

7. Effects of posture, gravity, and muscular exercise on blood pressure are discussed in the next experiment.

PRECAUTIONS

1. The subject should be physically and mentally relaxed and free from tension and anxiety. He/ she should be assured and rested for 5 minutes or so to avoid the condition of "**white coat hypertension**" (i.e. some people have higher BP readings in the clinician's office than during their normal daytime activity). It is good practice to compare the pressures in the two arms when recording BP for the first time. If the readings are above the upper normal limits, the measurement must be repeated under basal conditions, i.e. early in the morning before the subject gets up from the bed. A diagnosis of hypertension must never be made lightly and in haste.
2. The arm, with the cuff wrapped around it, should be kept at the level of the heart to avoid the influence of gravity. The cuff tubing should lie anterolateral to the cubital fossa so that they do not rub against the chest-piece of the stethoscope.
3. The cuff should not be too tight nor too loose.
4. The cuff should not be left inflated with high pressures for any length of time, because the discomfort and reflex spasm of the artery and its branches will give false high readings.
5. Do not apply pressure on the artery with the chest-piece as this may produce partial obstruction of the artery and a fake low reading.
6. Check the pulse rate at the time of recording BP as the heart rate affects the BP.
7. The palpatory method must always be employed before the auscultatory method.
8. In suspected and known cases of hypertension, the pressure should always be raised well above 200 mm Hg; or above the level estimated by palpatory method.

Report:

Study of different Family Planning Appliances

Aim: To study different family planning appliances

Requirements: condoms, UIDs, Oral contraceptives, Diaphragm

Principle:

Family planning methods is the procedure employed to restrict the number of children by various methods that control fertility and prevent pregnancy.

I. Methods Based on Physiological Principles

1. Rhythm Method ("Safe Period"; Periodic Abstinence).

Normally, only one viable ovum is released per menstrual cycle and it remains viable for about 24 hours, while the sperms, after entering the uterus survive for about 48 hours. Thus, there is a minimum period of 3 days during which intercourse must be avoided to prevent

pregnancy. For this method to be effective, the time of ovulation must be known. In most women who have regular periods, ovulation usually occurs 14 days before the onset of the next menstruation (not the 14th day from the 1st day of a cycle). For example, if the cycle starts on the first day of a month and lasts 30 days, the time of ovulation would be the 16th of that month. Pregnancy is unlikely to occur if coitus is avoided 4 days before and 4 days after the expected day of ovulation.

Note The rhythm method, though physiological, is the most unreliable method because pregnancy has been reported to occur from coitus on every day of the cycle.

2. Withdrawal method. Withdrawal of penis just before ejaculation (orgasm or climax) though practiced is not reliable, the failure rate of this method (coitus interruptus) being about 20 percent.

II. Barrier Methods

(Condom and diaphragm)

Since it is very cheap and effective, the condom (a rubber sheath worn over the penis during coitus), is the most widely used method by the males. An added advantage is the protection it gives to the male against sexually transmitted diseases (STDs) like AIDS, hepatitis, syphilis, and gonorrhea. A similar barrier, the rubber diaphragm, is fitted over the cervix by the female. In addition to these mechanical barriers, a spermicidal jelly is used by many couples at the same time.

III. Use of Spermicidal Agents

Use of creams, jellies, foams, suppositories, etc. in the female before coitus, and vaginal douches after intercourse may be combined with barriers.

IV. Interruption of the Normal Paths of Sperms or Ovum (Surgical Sterilization)

Interrupting the normal paths of sperm or ovum by vasectomy in males and tubectomy in females, appear to be the ideal methods suitable for our poor and illiterate population. However, restoration of the patency of these tubes, if required later on, has few chances of success.

V. Intrauterine Devices (IUDs) IUDs or intrauterine contraceptive devices (IUCDs) are foreign bodies (plastic or metal) that are placed in the uterus and left there. “Copper T” and “Loop D” (stainless steel) are the common devices used. They possibly make the endometrium unsuitable for implantation of fertilized egg by causing” aseptic inflammation” and/or by increasing uterine motility. IUDs have long-term use (6–10 years), can be removed when desired, and are as effective as tubectomy. (The copper in “Copper T” may also be spermicidal).

VI. Oral Contraceptives (Hormonal Methods)

It has been known for long that various doses of synthetic estrogens and progesterone given during the first half of menstrual cycle inhibit release of FSH and LH by negative feedback. This, in turn, reduces the levels of the normal ovarian estrogens and progesterone, the mid-cycle LH surge does not occur, and ovulation is not triggered. Even if ovulation does occur, changes in cervical mucus and in the endometrium prove hostile for sperms and implantation.

The pills are started early in the cycle, continued beyond the expected day of ovulation, and then stopped to allow menstruation to occur. The contraceptive "pills" are 100% effective and are used by millions worldwide. The hormonal methods include: a. The classical pill. It contains orally active progesterone-like substance—gestagen, and a small dose of estrogen. In addition to inhibiting ovulation, these pills also render the cervical mucus hostile to sperm penetration. They may also induce endometrial changes which prevent implantation of the fertilized egg. b. The sequential pill. It has a high dose of estrogen for 15 days followed by estrogen plus gestagen for 5 days. This pill inhibits ovulation by suppressing both LH and FSH. c. Luteal supplementation pill. These pills contain low doses of gestagen throughout the entire cycle. It controls fertility without inhibiting ovulation.

The hormone may be acting on the cervical mucus, or on the endometrium, or perhaps by reducing the motility of the Fallopian tubes. d. The "Morning-after Pill" (Emergency Contraception, EC). These pills have high doses of estrogens and progestin. They inhibit FSH and LH, and stop the secretion of ovarian estrogens and progesterone. The sudden fall of these hormones causes shedding of uterine endometrium, thus blocking implantation. When two pills are taken within 72 hours of unprotected coitus, and another two tablets after another 12 hours, chances of pregnancy are greatly reduced. Other Hormonal Methods Include • Subcutaneous implantation of hormone-containing capsules (they slowly release the drug into the circulation and are effective for about 5 years. • Intramuscular injection of progestin (e.g. DepoProvera) every 3 months. • Once-a-month intramuscular injection of estrogen and progesterone, skin patches containing these hormones, once a week for 3 weeks of the cycle.

Pregnancy Diagnostic Test

Aim: To perform pregnancy Diagnosis test

Requirements: Pregnancy diagnosis kit, urine sample

Principle:

Most of the laboratory tests for pregnancy are based on the detection of the presence of human chorionic gonadotropins (HCG) in the woman's urine. Some tests are so sensitive that the earliest diagnosis of pregnancy can be made within a few days of the conception, i.e. even before the next missed period.

These tests can be grouped into biological, immunological, and radiological.

I. BIOLOGICAL DIAGNOSTIC TESTS

These tests, which involve injection of urine into various animals, are time consuming and costly. They are, however, 99% accurate.

1. Aschheim-Zondek mouse test: Urine is injected subcutaneously into immature mice. Appearance of blood-filled ovulated follicles on 5th day confirms pregnancy.

2. Friedman rabbit test: Intravenous injection of urine into virgin female rabits causes ovulation in 18 hours.

3. Galli Mainini frog test: Injection of urine into the dorsal lymph sac of male frogs or toads causes shedding of sperms in about 3 hours

4. Hogben test. Adult female toads are used in this test. Injection of urine into the lymph space causes ovulation within 18 hours.

II. IMMUNOLOGICAL TESTS ****

Principle The HCG (human chorionic gonadotropins) secreted by syncytiotrophoblast cells of placenta is antigenic, and antibodies against this hormone can be produced by injecting it into rabbits. These antibodies are available commercially and are employed to detect the presence of HCG in the subject's urine or serum by precipitation, hemaggluination, or complement fixation, etc.

Procedures Collection of urine. The sensitivity level of HCG in urine is 1.5–3.5 IU/ml in slide test and 0.2–1.2 IU/ ml in test tube test. This concentration is reached by 10th day of fertilization (i.e. even before the missed period). The subject is advised to restrict water intake for 12–14 hours, and urine is collected in a clean container in the morning. The specific gravity of urine should be at least 1.015 and it should be free from protein and blood.

A. **Latex Agglutination Inhibition (LAI) Test. (Gravindex test)**

Basis of test. Small globules of latex (rubber) particles coated with pure HCG, and antiserum to HCG, are available commercially in 'kit' form.

Procedure: A sample of urine is treated with antiserum on a glass slide placed against a black background.

• If the urine contains HCG (i.e. if the woman is pregnant), then the antibodies in the antiserum are all "used up". Then if the coated latex particles are added, they do not get agglutinated. Urine (HCG present) + Antiserum + Latex particles = No agglutination.

Therefore, "No agglutination" means a positive result, i.e. the woman is pregnant.

• If the urine sample does not contain HCG, then the antibodies in the antiserum "remain free". Then if latex particles are added, antigen-antibody reaction occurs and the particles get agglutinated. Urine (HCG absent) + Antiserum + latex particles = Agglutination.

Therefore, "agglutination" means a negative result, i.e. the woman is not pregnant.

B. **Hemagglutination Inhibition (HAI) Test (Prognostican test**) In this test, sheep's RBCs coated with HCG are employed in place of latex particles. The test is done in a test tube and observations are made after 2 hours. The principle is the same as LAI

C. **One-step immunoassay test****.

Principle: This test is based on the combination of monoclonal antibody-dye conjugate with polyclonal solid phase antibodies for the qualitative detection of HCG in the urine.

Procedure: A sample of urine is applied to the TEST zone of the card or strip, and if it contains HCG, a pink-purple colored band develops. A control is provided to check the potency of the test reagents.

(A number of test kits under different proprietary names are available.)

D. **Radioimmunoassay (RIA**)

Principle:HCG radiolabeled with iodine (iodine 135) is treated with fixed amounts of antibodies and the urine/serum sample.

The method is much more sensitive and can detect as little as 0.003 IU/ml of beta subunit and 0.001 IU/ml of alpha subunit of HCG in the specimen.

E**. ELISA** (Enzyme-linked immunosorbent Assay)

Elisa has been widely used to detect a variety of antigens and antibodies.

The ***principle*** of the test is the same as that of RIA except that an enzyme is used in place of a radioactive substance.

The enzyme acts on the substrate to produce **blue color** which is **a positive test** for pregnancy.

III. ULTRASONOGRAPHY

Principle; Pulses of ultrasonic waves at high frequency are generated from a piezo-electric crystal transducer that also acts as a receiver to detect waves reflected back from various parts of the uterus.

The echoes (reflected waves) are displayed on the ultrasound screen. It is the most reliable method for detecting pregnancy. The gestational ring is evident as early as 5th week of pregnancy, cardiac pulsations by 10th week, and fetal movements by 11th week.

uses**:** in detecting fetal viability and position, site of placenta, multiple pregnancy, and fetal-maternal abnormalities, etc. For the determination of sex of the fetus.

Clinical significance:

1) Immunological tests are used routinely in all hospitals and clinics.
2) Compared to biological tests, they are less costly, simpler and easy to carry out.
3) Take very little time for reporting, and can confirm pregnancy within 10 days of conception.

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Report: Name:

Age :

Sex :Male Female

Date:

Method: N/A **One-step immunoassay**

Control: Pink-purple colored band

Observation: Pink-purple colored band was not developed

Result: Negative