PHYSIOLOGY LAB REQUIREMENTS AND INFORMATION

- Follow the class policies listed in course syllabus and schedule of class of College of Applied Medical Sciences
- Please do not eat or drink in the classroom.
- Do not wear open- toe shoes to lab.
- Read your lab manuals before coming to the lab.
- Clean after yourself after each lab.
- Work as a group. However, **FINISH YOUR WRITE UP INDIVIDUALLY**. Otherwise you will get zero.
- Lab workbook will be collected at the end of each lab.
- Each bad lab practice such as not to clean after yourself will be deducted.

CONTENT

S. NO.	NAME
1	Lab Safety
2	Study of the Compound Microscope
3	Blood sample collection
4	Determination of the ABO and Rh blood groups
5	Analysis of Human Blood Pressure
6	Examination of Previously Recorded and Printed ECG Curves
7	Analysis of Human Blood: Estimation of Hemoglobin by Sahli's Hemocytometer
8	Investigating the Human Respiration: Measurement of Respiratory Function (Spirometry)
9	Analysis of Human Blood: Determination of Bleeding time
10	Analysis of Human Blood: Determination of Clotting time
11	Examination of previously Recorded and Printed EEG Curves
12	Investigating Colour Vision for Color Blindness
13	Determination of Visual Acuity with Snellen's Charts
14	Analysis of Human Blood: Estimation of White Blood Count (CBC)
15	Determining Blood Glucose Level

Compound Microscope

EXPERIMENT 1 **DATE:** .....

AIM: To study the parts of a compound microscope and to view the slides, under microscope.

APPARATUS REQUIRED

Compound microscope, cedar wood oil, stained slides, and light source.

ACTIVITY 1: Identifying the Parts of a Microscope

1. Obtain a microscope and bring it to the laboratory bench. (Use the proper transport technique!)

Compare your microscope with the figure on the following page and identify the following microscope parts:

- **Base:** Supports the microscope.
- **Substage light:** Located in the base, the light passes directly upward through the microscope.
- **Stage:** The platform the slide rests on while being viewed. The stage has a hole in it to permit light to pass through both it and the specimen. The mechanical stage permits precise movement of the specimen.
- **Condenser:** Concentrates the light on the specimen. The condenser has a height-adjustment knob that raises and lowers the condenser to vary light delivery. Generally, the best position for the condenser is close to the inferior surface of the stage.
- **Iris diaphragm dial:** Dial attached to the condenser that regulates the amount of light passing through the condenser. The iris diaphragm permits the best possible contrast when viewing the specimen.
- **Coarse adjustment knob:** Used to focus on the specimen when on 4x or 10x.
- **Fine adjustment knob:** Used for precise focusing once coarse focusing has been completed. Use only this knob when on 40x or 100x.
- **Head or body tube:** Supports the objective lens system, and the ocular lenses.
- **Arm:** Vertical portion of the microscope connecting the base and the head.
- **Ocular (or eyepiece):** There are two lenses at the superior end of the head, through which observations are made. An ocular lens has a magnification of 10x. If your microscope has a pointer, it is attached to the right ocular and can be positioned by rotating the ocular lens

- **Nose piece:** Has four objective lenses and permits sequential positioning of these lenses over the light beam passing through the hole in the stage. Use the nose piece to change the objective lenses.

- **Objective lenses:** Adjustable lens system that permits the use of a scanning lens, a low-power lens, a high-power lens, or an oil immersion lens. The objective lenses have different magnifying and resolving powers.

2. Look at the objective lenses carefully. The shortest lens is the scanning lens, and has magnification of 4x. The low power lens is 10x. The high-power objective lens is 40x. The oil immersion objective lens is usually the longest of the objective lenses and has a magnifying power of 100x. Record the magnification of each objective lens of your microscope in the first row of the summary chart.

3. Rotate the lowest power objective lens until it clicks into position, and turn the coarse adjustment knob about 180 degrees. Notice how far the stage (or objective lens) travels during this adjustment. Move the fine adjustment knob 180 degrees, noting again the distance that the stage (or objective lens) moves.

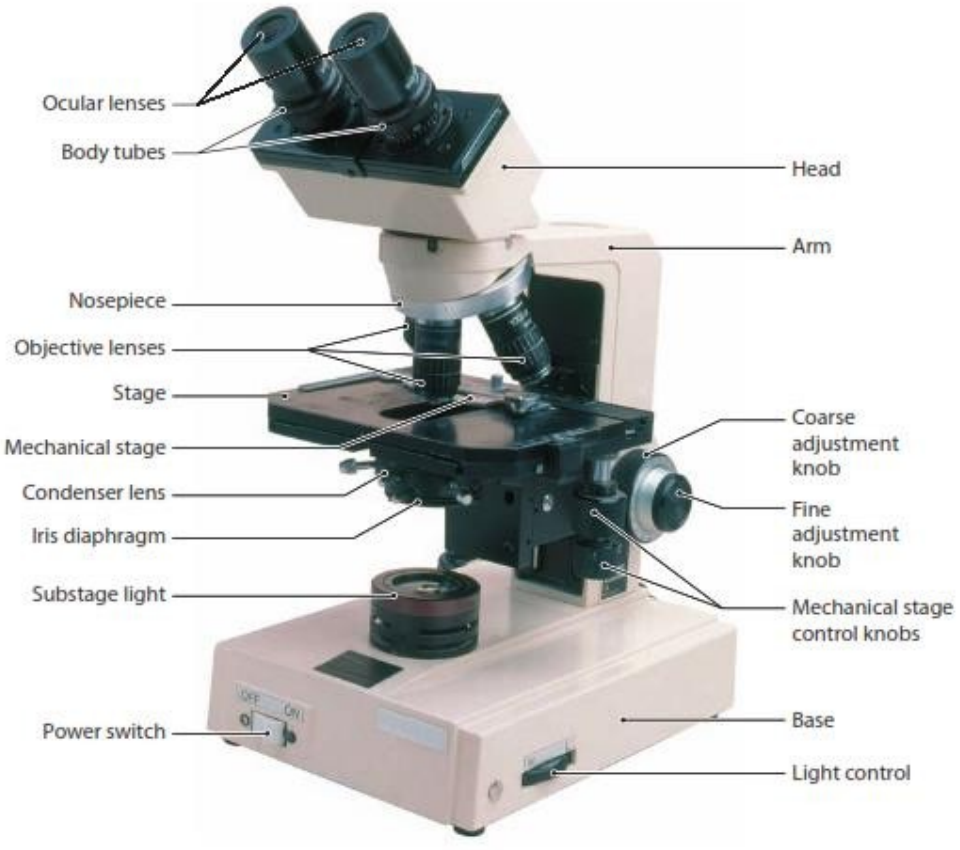


Figure 1.1: Compound microscope

PRECAUTIONS WHILE USING THE COMPOUND MICROSCOPE

The microscope must always be handled properly. You must observe the following rules for its transport, cleaning, use, and storage:

- Transport in an upright position with one hand on the arm and the other supporting the base. Set it down carefully at your work station. Do not drag it across the table.
- Use only special lens paper to clean the lenses. Clean all lenses before and after use. Slides should also be cleaned.
- Always begin the focusing process with the 4x or 10x objective lens in position, changing to the higher-power lenses as necessary.
- The coarse adjustment knob may be used with the 4x or 10x lens, but use only the fine adjustment with 40x or 100x.
- Adjust lighting appropriately. Turn off the light when not in use.
- Always use a cover slip with temporary (wet mount) preparations.
- When you put the microscope away, remove the slide from the stage, and rotate the lowest-power objective lens into position. Wrap the cord around the clips on the back, not around the base.
- Never remove or loosen any parts from the microscope.
- Inform your instructor of any mechanical problems.

Blood Sample Collection

EXPERIMENT 2 **DATE: .....**

AIM

To study the different methods of blood collection.

APPARATUS REQUIRED

Sterile lancet, disposable syringe and needle, anticoagulant container, cotton, spirit.

PROCEDURE

Collection of Capillary Blood by Finger Prick Method

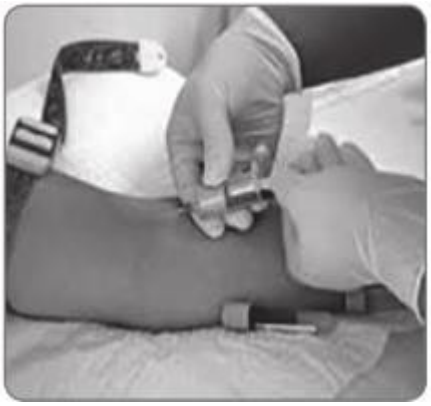
1. Clean the tip of the ring or middle finger with a cotton swab dipped in spirit.
2. Allow the fingertip to dry as spirit can cause the hemolysis of blood and pain.
3. Using a sterile lancet prick the finger deep enough to ensure a free flow of blood (Fig. 2.1).
4. Wipe out the first few drops and collect the sample when the blood is flowing spontaneously. Do not squeeze the finger as tissue fluid can mix with blood and may cause error.

Figure 2.1: Pricking the finger tip

Collection of Venous Blood

Venous blood is usually collected from antecubital vein. Clean the antecubital fossa with spirit and allow it to dry. After applying a tourniquet around the upper arm, introduce a needle under the skin and then puncture the vein from the side. During this procedure, open and close the fist repeatedly so that the vein gets engorged. When the blood flows into the syringe, release the

tourniquet and draw the required amount of blood. Withdraw the needle and apply a cotton swab over the site of puncture and apply pressure until the bleeding stops (Fig. 2.2). To prepare serum, blood can be emptied into a container without anticoagulant. To prepare plasma blood has to be emptied into a container with anticoagulant.



Collection of venous blood

Blood Grouping or Blood typing.

EXPERIMENT 3 **DATE:** .....

INTRODUCTION

It is essential to know the blood group of a person if he encounters anyone of the following circumstances:

1. Blood transfusion
2. Paternity dispute
3. Medicolegal problem
4. Organ transplantation.

The current system of blood grouping was discovered by Landsteiner in 1900 and is known as the Landsteiner's ABO system.

PRINCIPLE

The RBCs contain a series of antigens known as agglutinogens on their cell membrane while the plasma contains antibodies known as agglutinins. To find out a person's blood group the RBCs are made to react with sera containing known agglutinins. The slide is then observed for the presence or absence of agglutination and hemolysis of RBC. This can be done with the naked eyes but is ideally done by viewing the slide under the microscope.

APPARATUS AND REAGENTS REQUIRED

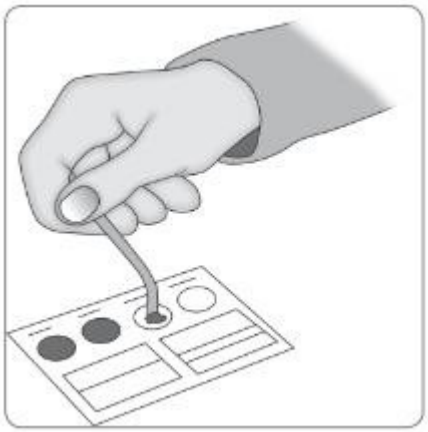
1. Sterile lancet
2. Cotton swab
3. Alcohol
4. Glass dropper
5. Toothpicks
6. Microscope
7. Porcelain tile
8. 3.8% sodium citrate in normal saline
9. Anti-A serum
10. Anti-B serum
11. Anti-D serum.

Note: Anti-A serum is tinted blue and Anti-B serum is tinted yellow (Fig. 10.1).

Figure 10.1: Antiserum A, B and D

PROCEDURE

With a glass marking pencil, the porcelain tile is divided into 3 portions. Under aseptic conditions, the subject whose blood group is to be determined is asked to stick out his finger, which is pricked, and 3–4 drops of blood is then obtained. Following this the blood is diluted with 1 mL of 3.8% sodium citrate in normal saline taken in a test tube. A drop of anti-A, anti-B and anti-D sera is placed on each of the 3 portions on the tile. Now, 1 drop of the diluted blood is put on each of the 3 portions containing the sera. This is mixed with 3 separate toothpicks. After 10–15 minutes each portion is examined for clumping and agglutination first with the naked eyes and then under the microscope (Figure 10.2).



OBSERVATION AND RESULTS

If any agglutination occurs, it is usually visible to the naked eye as dark red clumps of different sizes. The presence or absence of agglutination indicates the blood group of the subject as shown in the following table:

AGGLUTINATION OF SUBJECT'S BLOOD GROUP

With anti-A serum	With anti-B serum	Blood group
Present	Absent	A
Absent	Present	B
Present	Absent	AB
Absent	Present	O

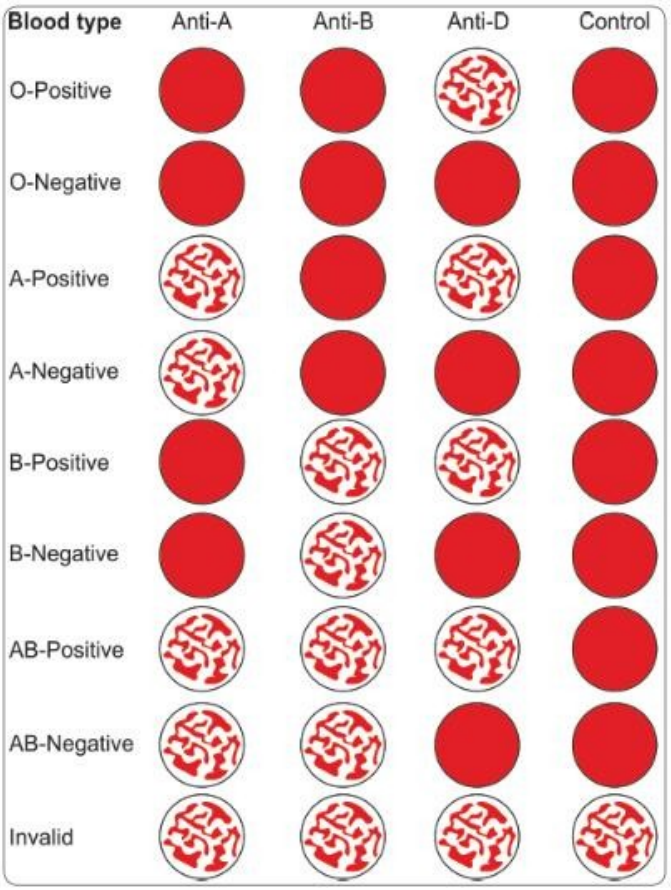


Figure 10.3: Agglutination in different blood groups

RESULT

The blood group of the given blood sample is_____

Determination of the Blood Pressure

EXPERIMENT: 4 **DATE:** .....

DEFINITION

Blood pressure (BP) is the lateral pressure exerted by the column of blood on the wall of the artery.

AIM

To determine the blood pressure of the given subject at rest and after moderate exercise.

APPARATUS

Sphygmomanometer and stethoscope.

PRINCIPLE

The pressure of blood in the artery (brachial artery) is balanced against the pressure of air in a rubber cuff surrounding the artery. The pressure of air in the cuff is then measured by means of a mercury manometer.

METHODS OF MEASUREMENTS

1. Palpatory method
2. Auscultatory method
3. Oscillatory method.

1. **Direct method:** used only for research a cannula filled with anticoagulant is inserted in artery which is then it is connected to the manometer
2. **Indirect methods:**

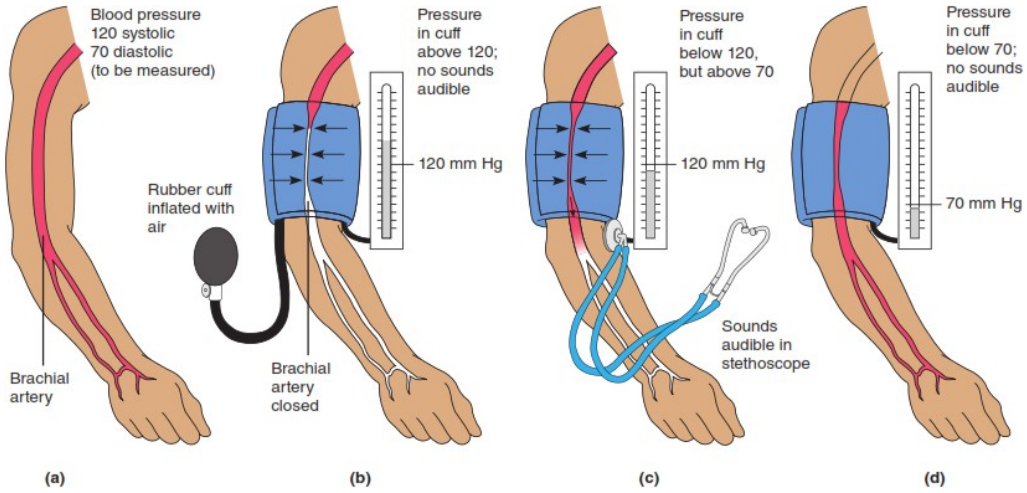
a- Palpatory method: Measures only systolic

b- Ausculatory method: It is standard method of taking a patient blood pressure by use technique developed by korotkoff in 1905.

Inflate the bag of instrument by means of a rubber squeeze bulb to pressure above the expected systolic pressure so no sound is heard with the stethoscope.

The pressure in the cuff is then lowered slowly by open release valve when the Inflation pressure falls. the small spurt of blood escapes through the cuff and Slight tapping sound heard. the

pressure at which the sound is first heard (phase 1 of Korotkoff sound) represents the systolic blood pressure. the sound become louder (phase 2) then dell sound (phase 3). muffled sound (phase 4) and finally they Disappear sound(phase5).



PROCEDURE

1. Subject should be in relaxed setting.
2. manometer is placed at level of observer's eye.
3. all clothing should be removed from upper arm.
4. the arm of subject should be supported because failing of it causing isometric contraction which leads to false measurement.
5. Inflatable arm cuff is applied around the upper arm leaving (1-2) inches between the lower end the cubital fossa.
6. The bell of stethoscope is placed on the brachial artery.
7. cuff is inflated by a rubber squeeze bulb to pressure above expected SBP.
8. the pressure in the cuff is lowered slowly to hear soft sound by open release valve.
9. when the first sound (step 1 of korotkoff sound) is heard, the SBP is measured.
10. when the sound disappears, at this level DBP is measured in adult or listen for a muffled sound (phase 4) in child and meal exercise

Important precautions in the use of sphygmomanometer:

1. The manometer should be placed at the level of the heart.

2. The lower border of the cuff should be 2.5 cm above the cubital fossa. For children, a narrow cuff should be used.
3. Blood pressure should be preferably taken in the left arm.

CLASSIFICATION OF HYPERTENSION IN ADULTS

Category	Systolic BP	Diastolic BP
Optimal	<120	<80
Normal	120–129	80–84
High Normal	130–139	85–89
Hypertension grade I (Mild)	140–159	90–99
Hypertension grade II (Moderate)	160–179	100–109
Hypertension grade III (Severe)	≥180	≥110
Isolated systolic hypertension	≥140	<90

DISCUSSION

Blood pressure is the lateral pressure exerted by the column of blood on the wall of the vessels while flowing through it.

RESULT:

Systolic blood pressure: _____

Diastolic blood pressure:

Electrocardiography

EXPERIMENT: 5 **DATE:** .....

AIM

To record and analyze electrocardiogram (ECG) of a normal subject.

REQUIREMENTS

Electrocardiograph, ECG leads (electrodes), ECG paper, straps and ECG jelly.

Preparing the Subject

Use an alcohol swab to clean the skin of the subject at the electrode attachment sites on the inner wrists and inner ankles. Peel the electrodes off the packaging and apply to attachment sites. Clip the appropriate line to each electrode (RA = right arm, LA = left arm, RL = right leg, LL = left leg). The subject should sit in a comfortable position and not make any unnecessary movements.

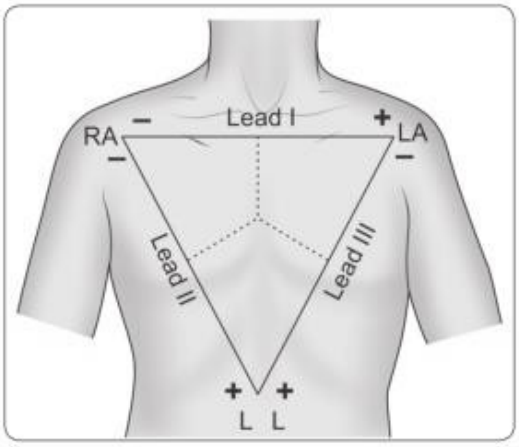


Figure: Einthoven's triangle

Baseline Readings

Run a baseline (at rest) recording for leads I-III. Be sure to record until you have a strip of stable readings long enough for each group member to have a segment for calculations.

Exercise Readings

After the baseline recording is finished, stop recording, and have the subject run in place for 2-3 minutes. As soon as the subject stops, have him/her sit down and begin recording. When you

have enough readings for each group member, stop recording. Clean up by throwing away the disposable electrodes. The subject may clean any residue off the skin with an alcohol pad. Return to your seat to perform calculations.

Understanding the Recording

P wave: atrial depolarization

QRS complex: ventricular depolarization (obscured atrial repolarization)

T wave: ventricular repolarization, may be inverted, elevated, or depressed depending on the lead sampled, or pathology

see your text for examples of abnormal ECG recordings

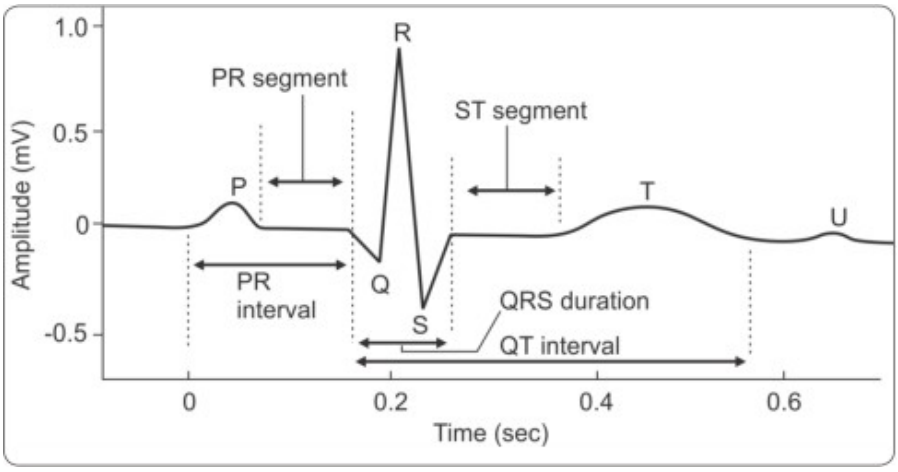


Figure: The normal electrocardiogram

PR Interval

This is the time from the initiation of SA nodal depolarization to the initiation of ventricular depolarization. It encompasses the time it takes for the action potential to pass through the AV node. A typical value, from 120-200 msec, indicates that the electrical impulses are originating from the atria and following the proper conduction pathways. The PR interval may shorten slightly during tachycardia, and lengthen during bradycardia within the stated limits. A significantly longer than normal interval may suggest a partial AV heart block caused by damage to the AV node.

QRS Duration

The normal QRS duration is 60-100 msec. A longer QRS indicates conduction problems, often caused by bundle branch blocks that cause one ventricle to contract later than the other.

QT Interval

This interval represents the time from onset of depolarization to the completion of repolarization of the ventricles. Normal values are around 300-400 msec at a heart rate of 70 beats/min. As heart rate increases, the interval becomes shorter. As heart rate decreases, the interval becomes longer. An exceptionally long QT interval may indicate slowed ventricular repolarization, possibly due to hypokalemia, or other electrolyte imbalances. Shortened QTs are seen with hypercalcemia and digitalis toxin.

Baseline Calculations

Look at the figure on the previous page and use the following equations. You do not need to memorize equations, but you do need to know how to use them.

Computing Heart Rate

Use the lead which gave the most ideal recording. Measure the distance in millimeters (the strip of paper is divided into millimeters) from the beginning of one QRS complex to the beginning of the next QRS complex (that is, measure from Q to the next Q).

$$\text{HR} = \frac{60 \text{ seconds}}{\text{RR interval (in seconds)}}$$

At a paper speed of 25 mm/second,

$$\text{HR} = \frac{25 \times 60}{\text{RR interval in no. of small squares in ECG}} = \frac{1500}{\text{RR (mm)}} = \frac{300}{\text{RR interval in no. of large squares in ECG}}$$

Heart rate. It can be determined by any of the following 2 methods:

a. By dividing 1500 by the number of small squares between 2 successive R waves.
   (1500 small squares represent 1 minute)
   For example, number of small squares between 2 R waves = 21
   Heart rate = 1500/21 = 70 per min.

b. By dividing 60 by the RR interval in seconds.

   For example, number of small squares between 2 R waves = 20

   RR interval = 20 × 0.4 = 0.80

   Heart rate = 60/0.80 = 75 per min

Computing Intervals

Compute the duration of the following intervals in msec by multiplying mm x 40

QRS interval _____

interval_____

P-R interval _____

Are the computed values for the intervals and heart rate within normal limits (see above)? As the T-P interval increases, how might this affect cardiac output?

Exercise Calculations

Which calculations do you expect to be different from baseline? Repeat the baseline calculations for the exercise readings to verify your predictions.

Diagnosing Abnormalities Using the ECG

What three types of problems can be diagnosed using an electrocardiogram? Also, define the terms tachycardia, bradycardia, arrhythmia, fibrillation, heart block, and myocardial infarction.

Analysis of Human Blood: Estimation of Hemoglobin by Sahli's Hemocytometer

EXPERIMENT: 6 **DATE:** .....

AIM

To estimate the amount of hemoglobin in 100 mL of blood by Sahli's method (acid hematin method).

PRINCIPLE

Anticoagulated blood is added to the 0.1 N HCl and kept for 5-7 minutes to form acid hematin. The color of this acid hematin should be matched with the solution, present in the calibration tube. Distilled water is added to the acid hematin until the color matches and the final reading is directly noted from the graduation in the calibration tube.

APPARATUS REQUIRED

Sahli's hemoglobinometer (Hemometer): This consists of (Figs 5.1A and B):

1. Comparator—a rectangular plastic box with a slot, which accommodates the Hb tube and non-fading standard, fixed on either side in front of an opaque white glass.
2. Hemoglobin tube—graduated in g% (2–24 g%) on one side and in percentage (20–140%) on the other.
3. Hemoglobin pipette with a 20 cu mm mark and rubber tubing with a mouthpiece.
4. A glass rod to stir.
5. N/10 hydrochloric acid.
6. Distilled water.
7. Lancet, cotton, and spirit.

PROCEDURE

N/10 HCl is taken up to the lowest mark in the graduated tube. The finger is pricked under aseptic precautions. The pipette is filled with blood up to 20 cu mm mark taking care not to allow air bubbles to enter. After wiping the blood sticking to the outside of the pipette, its tip is dipped in the acid and the blood is expelled. The pipette is rinsed 3 or 4 times with the acid solution, till all the blood is washed out. The blood and the acid are mixed well with the glass rod. The tube is allowed to stand for 8–10 min. for the formation of acid hematin. Distilled water

is added drop by drop, mixed well and the color is compared with that of the standard. Care should be taken to remove the glass rod while matching and not to view through the graduations. This is continued until the color matches. The lowest point of the meniscus is read, which directly gives the hemoglobin concentration in 100 mL of blood.

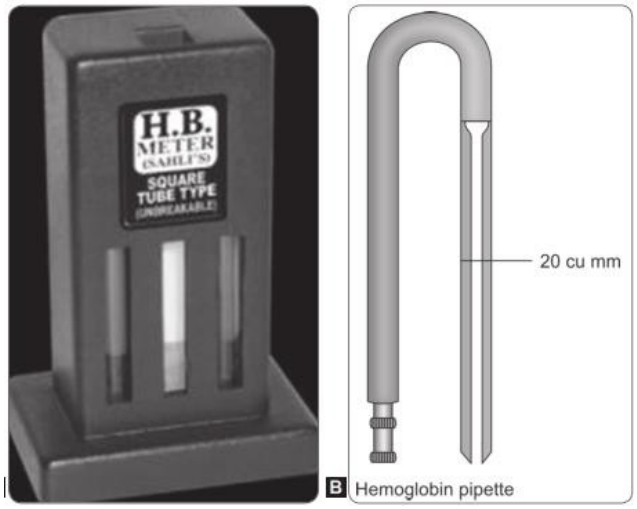


Figure 5.1: Sahli's hemoglobinometer: (A) Comparator box; (B) Hemoglobin pipette

PRECAUTIONS

i. Pipetting of blood should be done cautiously
ii. Mix the blood properly with HCl by using stirrer
iii. Match the color cautiously.

RESULT

The hemoglobin content of the given subject is…… gm/dL.

Investigation of human pulmonary function through Spirometry

EXPERIMENT: 7 **DATE:** .....

AIM: Measure and analyze the volumes and capacities of the human lung

MATERIALS REQUIRED: Computer controlled spirometer and data acquisition software, mouthpiece with bacteria filter, nose clip.

PROCEDURE

1. Sterilize the mouthpiece with spirit (Fig. 11.1).
2. The subject sits comfortably in a chair, facing away from the spirometer. Turn the union of the tubes to the _spirometer‘ position and flush the breathing chamber with fresh air by raising and lowering the inverted drum
3. Adjust the pointer on the pulley to the zero mark with the drum pushed down and confirm that the pointer moves in the correct direction on pulling the drum up.
4. Raise the inverted cylinder half way up and turn the outlet to “air” which closes the inner tube and allows the subject to breathe from atmosphere. The subject applies the mouthpiece and breathes through the corrugated tube. The nose of the subject is close by a nose clip. Wait until the subject gets used to the apparatus. Instruct the subject about the procedures to be followed before each measurement. Make sure that the measurements are completed soon or disconnect the subject from the spirometer and collect air in the drum
5. Breathe at tidal volume. At the end of normal inspiration, breathe out as usual but into the mouthpiece. The volume recorded is the tidal volume.
6. Readjust the drum at the lower position with pointer at zero. Breathe again at tidal volume. At the end of normal expiration, breathe out forcibly into the mouthpiece till you can breathe out no more. This volume is the expiratory reserve volume.
7. Ask the subject to inhale as much air as possible and breathe as usual make two recordings. This volume is the inspiratory reserve volume
8. Readjust the spirometer as in step 4. After a few tidal breaths, breathe in maximally and breathe out maximally into the mouthpiece. This reading is the vital capacity (Fig. 11.2).

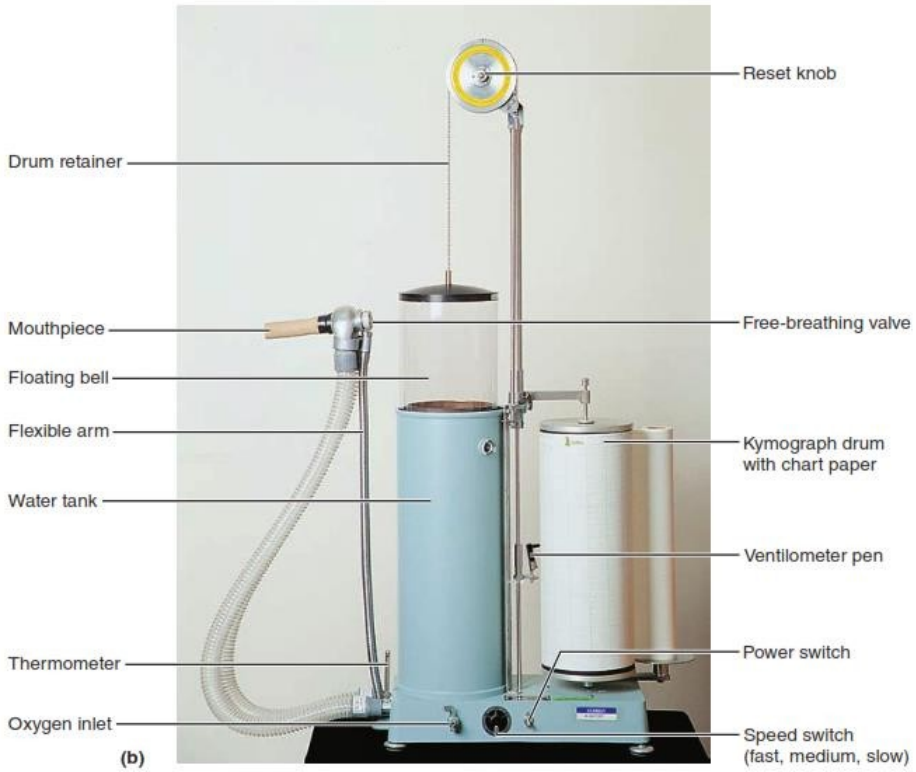


Figure 11.1: Figure Wet spirometers. The Phipps and Bird wet spirometer.

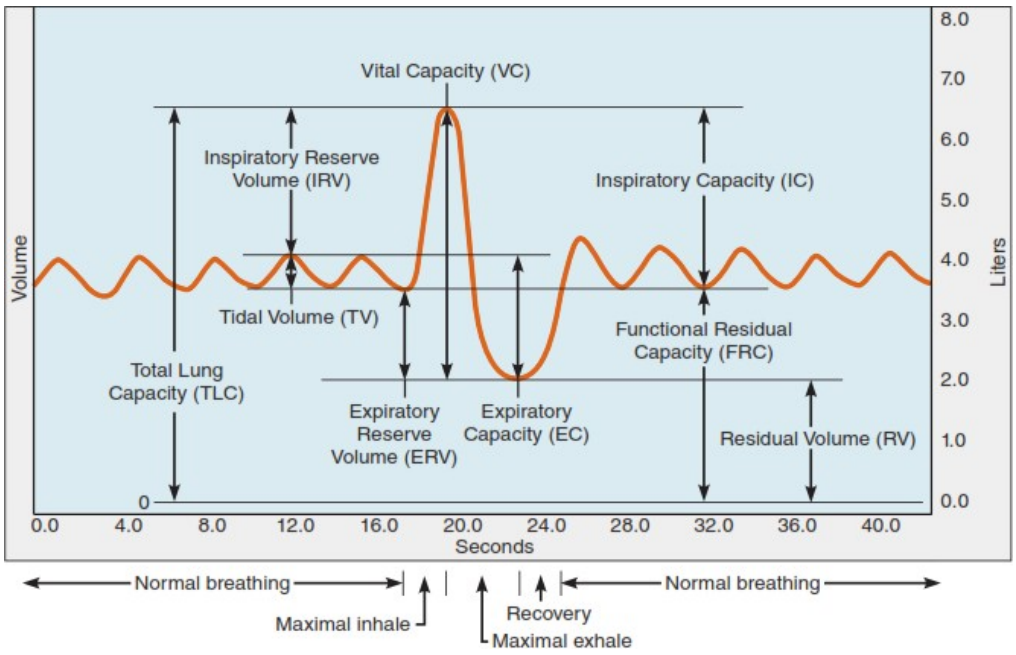


Figure 11.2: A normal spirogram

Method of prediction of lung volumes:

Lung volume depends on the build of a person. The lung volume therefore, may be expected to be related to the height and weight of a person. Formula give below are used to predict the volumes.

VC = 0.064 X [height (cm)] – 0.031 X (age) – 5.335 Male

= 0.052 X [height (cm)] – 0.018 X (age) – 4.36 Female

Total lung capacity (TLC)

TLC = 0.094 X [height (cm)] – 0.015 X (age) – 9.617 Male

= 0.097 X [height (cm)] – 0.008 X (age) – 7.49 Female

OBSERVATION

Name (male)	Predicted		TV (l)	IRV (l)	ERV (l)	VC (l)
	VC (l)	TLC (l)				
Mean						
SD ±						

Name (female)	Predicted		TV (l)	IRV (l)	ERV (l)	VC (l)
	VC (l)	TLC (l)				
Mean						
SD ±						

Determination of Bleeding Time

EXPERIMENT 8 **DATE:** .....

AIM: To determine the bleeding time of the given subject by Duke's method.

APPARATUS REQUIRED: Filter paper, stop watch, lancet, cotton, spirit.

PROCEDURE

The tip of the left ring finger is pricked with aseptic precautions (3–4 mm). The blood should flow freely without squeezing. The time of puncture is noted. With a filter paper the blood is gently blotted every 30 seconds. The successive blots become smaller. This procedure is repeated until no blot appears on the filter paper. The time is noted again. The number of blots on the paper is counted. Number of blots × 30 seconds will be the bleeding time.

DISCUSSION

Bleeding time is the time interval between the skin puncture and the cessation of bleeding- the time in minutes, which it takes for a standardized skin wound to stop bleeding.

Significance: Cessation of bleeding from a small wound as that inflicted during this procedure can be affected by vascular spasm and formation of platelet plugs. This test, therefore, measures the capillary and platelet functions in hemostasis.

Normal—1–5 minutes (by Duke's method)

RESULT

The bleeding time of the given subject is……

Determination of Clotting Time

EXPERIMENT 9 **DATE:** .....

AIM: To determine the clotting time of the given subject by Wright's method.

APPARATUS REQUIRED: Capillary glass tube 15 cm long with a bore of 0.8 mm, stop watch, lancet, cotton and spirit.

PROCEDURE

The tip of the left ring finger is pricked under aseptic precautions and the time of puncture is noted. The prick must be deep enough to allow free flow of blood without squeezing. The blood is drawn into the capillary tube by dipping one end of the tube in the blood drop. The blood fills the tube by capillary action. After 2 minutes, a small bit of the tube is broken every 30 seconds until a fine thread of fibrin appears between the broken ends. The time is again noted and the interval between the prick and the appearance of fibrin thread gives the clotting time.

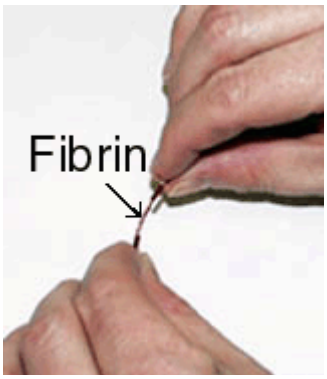


Figure . Breaking the capillary tube to view insoluble fibrin strands

DISCUSSION

Clotting time is the time interval between the skin puncture and formation of fibrin thread.

Significance: The clotting time is generally not affected by deficiency of platelets as only few platelets are required to provide enough platelet factor 3 for normal coagulation. This test assesses the intrinsic and common pathways of coagulation because the trigger for coagulation in this test is the surface activation of blood that comes into contact with the glass surface of the test tube.

Normal—2–8 minutes.

RESULT: The clotting time of the given subject is……

Estimation of Total Leukocyte Count

EXPERIMENT 10 **DATE:** .....

AIM

To enumerate the white blood cells in 1 mm

PRINCIPLE

The blood is diluted with a diluting fluid, which destroys the red blood corpuscles and stains the nuclei of the white blood cells. The leukocytes are then counted in a hemocytometer and their number in undiluted blood is calculated.

APPARATUS REQUIRED

- Anticoagulated whole blood.
- Turk's diluting fluid composed of
    - Glacial acetic acid to hemolyze RBCs.
    - Aqueous gention violet to color the nuclei of WBC.
    - Distilled water.
- WBC pipette, which is composed of a stem, mixing chamber, white bead inside the mixing chamber, aspiration tube (ruber sucking tube).
- Hemocytometer (Neubauer's counting chamber) with cover slip.
- Microscope.
- Lancet.
- Cotton.

PROCEDURE

1. **Filling the pipette and diluting the blood sample:** Clean the finger tip with spirit and prick it with a sterile lancet. The first drop of blood is discarded as it contains tissue fluid. Allow a good sized drop to form and draw blood up to 0.5 in the WBC pipette. Suck the Turk's fluid up to the mark 11. Hold the pipette horizontally and roll it between the palms to ensure thorough mixing.
2. **Charging the chamber:** Place the cover slip over the chamber. Place the chamber on the microscope stage. Roll the pipette again before charging. Discard the first two or three drops of fluid as the stem of the pipette is filled only with diluting fluid and has no blood

at all. Allow a moderate size drop of diluted blood to form at the tip of the pipette. Hold the pipette at an angle of 45º and make the drop to touch the slide and the cover slip. The fluid will run into the capillary space to fill it. The fluid should not run into the trenches. There should be no air bubbles in the chamber. After charging, allow the fluid then to settle for two to three minutes before actual counting is begun.

3. **Counting the cells:** Focus the WBC area under low power and ensure that there is no air bubble. The cells lying on the upper horizontal and left vertical lines should be counted along with those lying within the square under consideration. Count the cells in 4 large corner squares and enter in the observation table (Fig. 6.1).

PRECAUTIONS

1. There should be no air bubble in the column of the blood.
2. The cells should be evenly distributed.

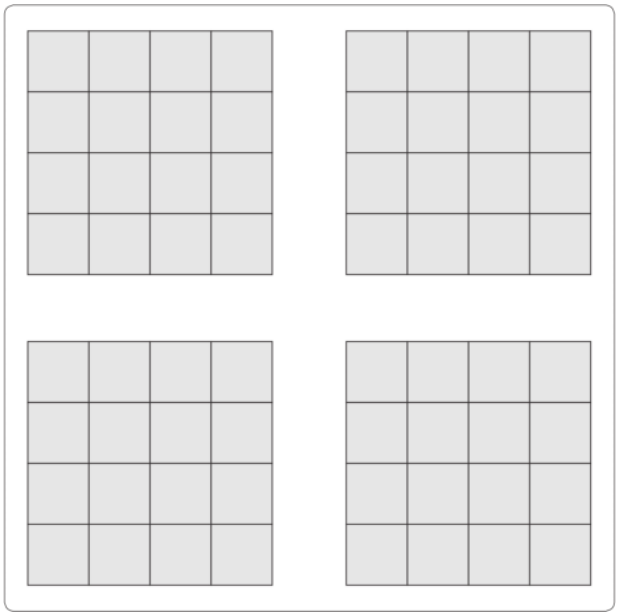


Figure 6.1: Observation table for WBC count

CALCULATION

Side of 1 WBC square	= 1 mm
Area of 1 WBC square	= 1 mm^2
Depth of chamber	= 1/10 mm
Volume of fluid present over 1 WBC square	= 1/10 mm^3
Volume of fluid present over 4 WBC square	= 4/10 mm^3

No. of cells in 4/10 cu mm of diluted blood = N

No. of cells in 1 cu mm of diluted blood = 10/4 N

Dilution factor = 1:20

No. of cells in 1 cu mm of undiluted blood = 10/4 × 20 N

= 50 N

DISCUSSION

Total leukocytes count shows variation under certain physiological and pathological conditions (Normal WBC count = 4000–11000 cell/cu mm).

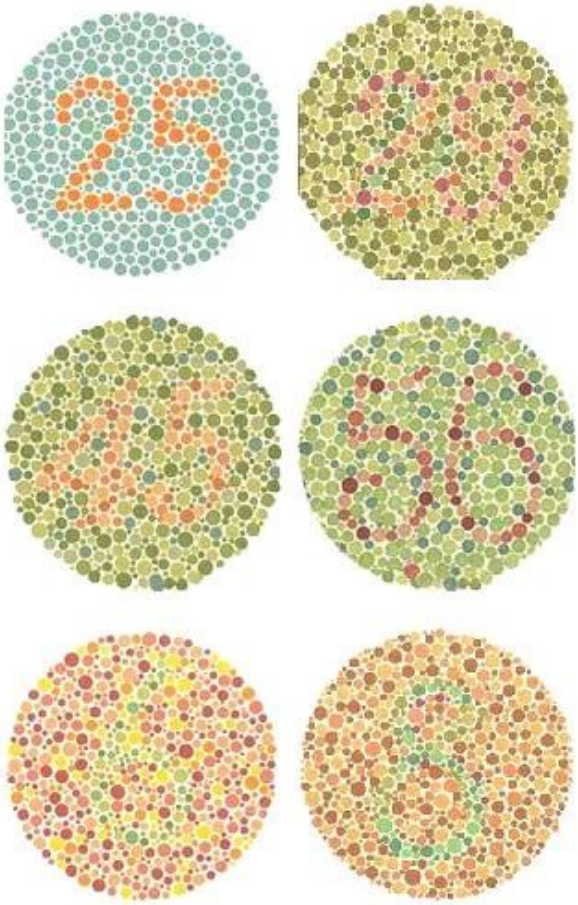
RESULT

The WBC count of the given subject is …… cells/cu mm.

Investigating Color vision for Color Blindness

EXPERIMENT 11 **DATE:** .....

The commonly used test for color blindness is the Ishihara Test. Color blindness testing may be relevant for athletes - testing the ability of athletes to distinguish team colors and the ball from the crowd and background surfaces may be appropriate for some sports.



Equipment required: The full Ishihara Test is a collection of 38 plates filled with colored dots. The dots are colored in different shades and a number is hidden inside with shades of another color.

Procedure: Subjects look at each plate of the 38 plates and have to indicate the number or shape illustrated. It is usually not necessary to conduct the full test, as a color vision deficiency is usually apparent after just a few plates.

Results: Between 10-20% of males have some degree of color blindness, and only a small percentage of females. Red-Green Color blindness is the most common

Results For Ishihara Test(above)

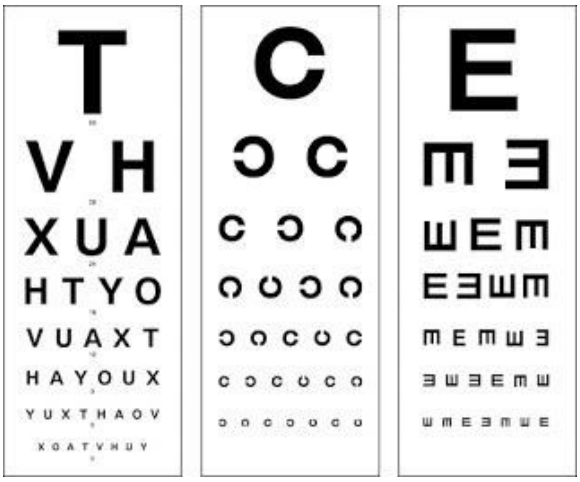
Normal Color Vision			**Red-Green Color Blind**		
	Left	Right		Left	Right
Top	25	29	Top	25	Spots
Middle	45	56	Middle	Spots	56
Bottom	6	8	Bottom	Spots	Spots

Determination of Visual Acuity with Snellen's Chart

EXPERIMENT 12 **DATE:** .....

AIM: Determination of Visual Acuity with Snellen's Chart

MATERIALS: Snellen chart,



PROCEDURE

1. Stand 20 feet (6 meter) from the Snellen chart.
2. Remove correction glasses and cover one eye with an index card.
3. Start from the top of the chart and read the letters randomly.
4. Record the number of the last line that can be read successfully.
5. Test the other eye with the same procedure.

CALCULATION:

Visual acuity (V) = d/D; where d is the distance between the chart and the subject and D is the distance from which subject should read the letter

KEY POINTS:

1. Subject should stand at a distance of 20 feet (6 meters).
2. One eye to be tested at a time. Cover the other eye with hand.
3. Ask the subject to read the Snellen's chart from top to bottom. Normally subject should be able to read till the letters in the smallest 7th line.
4. If subject unable to read even the topmost line, shift the subject towards the chart every 1 meter till able to read.

RESULT: Normal visual acuity is 6/6.