

Department of Physiology College of Medicine

# Physiology Practical For

## 1<sup>st</sup> Year Medical Students

(2018-2019)

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## List of Abbreviations

ABP	Arterial blood pressure
BP	Blood pressure
CAP	Carotid arterial pulse
CBC	Complete blood count
ECG	Electrocardiogram
ERV	expiratory reserve volume
ESR	Erythrocyte sedimentation
FEV <sub>1</sub>	Forced expiratory volume at one second of forced vital capacity
fl	Femtoliter
FVC	Forced vital capacity
FVL	Flow-volume loop
Hb	Hemoglobin
Hct	Hematocrit
IC	Inspiratory capacity
ICS	Intercostal space
ICS IRV	Intercostal space Inspiratory reserve volume
ICS IRV JVP	Intercostal space Inspiratory reserve volume Jugular venous pulse

KII	Korotkoff sound II
KIII	Korotkoff sound III
KIV	Korotkoff sound IV
KV	Korotkoff sound V
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCL	Midclavicular line
MCV	Mean corpuscular volume
$MEF_{50\%}$	Mean expiratory flow at 50% of forced vital capacity
PCV	Packed cell volume
PEFR	Peak expiratory flow rate
pg	Picogram
PIFR	Peak inspiratory flow rate
PSL	Parasternal line
RBC	Red blood cell
S <sub>1</sub>	First heart sound
$S_2$	Second heart sound
S <sub>3</sub>	Third heart sound
S <sub>4</sub>	Fourth heart sound

TLC	Total lung capacity
TV	Tidal volume
VC	Vital capacity
WBC	White blood cell

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### Introduction

Welcome to your first year in medical school. This manual has been prepared as a reference to help medical students navigate their way through 1<sup>st</sup> year physiology practical sessions. The physiology practical sessions are part of the physiology curriculum that is ingrained in the block system of the first two years of medical school. Its aim is to provide a practical aspect to some of the physiological concepts learned during mainstream lectures allowing students to have hands on experience that will strengthen their understanding of the physiological concepts. Practical sessions will also help them apply the knowledge learned in the classroom in a safe environment.

This manual is meant to be a guide for students providing the structure and topics covered in physiology practical sessions. However, students are encouraged to look for information and broaden their knowledge using other resources.

To make the best of the practical sessions, students are advised to attend the sessions on time and prepare by reading related lecture material prior to the practical sessions. During the sessions, students are encouraged to engage actively and take the opportunity to get hands on experience whenever it is feasible.

Wish you all the best!

Physiology Practical Team

## **Overview of 1<sup>st</sup> Year Medical Student Physiology Practical Sessions**

The table below provides an overview of the structure and contents of Physiology practical sessions during the first year in medical school by showing the number and title of the lab sessions provided in each block of the 1<sup>st</sup> year medical curriculum.

Block name	Block	Number of sessions	Session title
	duration		
Foundation block	≈8 weeks	3 (2-hour-sessions)	CBC & ESR
			WBC & differential
			Bleeding and clotting times and
			blood groups
Musculoskeletal	≈ 5 weeks	None	None
block			
Respiratory block	≈6 weeks	2 (2-hour-sessions)	Lung volumes & capacities
			Dynamic spirometry
Cardiovascular	≈ 5 weeks	4 (2-hour-sessions)	The electrocardiogram (ECG)
block			Jugular venous and carotid
			arterial pressure recordings
			Measurement of arterial blood
			pressure
			Heart sounds
Renal block	≈ 5 weeks	4 (2-hour-sessions)	Glomerular filtration and renal
			clearance
			Diuresis-1
			Diuresis-2
			Acid-base balance

Table 1. First v	vear medical studen	ts' physiology	practical sessions.
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# Faculty and Staff Members Involved in Physiology Practical Teaching

 Table 2. Faculty and staff members involved in teaching physiology practicals.

Male members	Contact details	Female members	Contact details
Dr. Taj	athalepota@yahoo.com	Dr. Ola Hilmi	omawlana@ksu.edu.sa
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Mr. Timhar		Mrs. Rahma	

## **Advice for Students**

- Show up to physiology practical sessions on time.
- Read related lecture material prior to the laboratory session.
- Each laboratory session starts with a pre-lab lecture that serves to explain the objectives and procedure to be done in the lab. Listen attentively to these lectures.
- Engage actively in the laboratory activity and take every opportunity to get hands on experience whenever possible.
- Answer the sample questions provided at the end of each lesson.
- Do not depend solely of this guide for information.

## Chapter 1: Foundation Block Physiology Practical

There are 3 practical physiology sessions during the foundation block which are all concerned with aspects related to blood physiology. The sessions are as follows;

- 1. Complete blood count (CBC) and red cell indices, erythrocyte sedimentation rate (ESR) and hematocrit (Hct).
- 2. White blood cell count (WBC) & differential.
- 3. Bleeding and clotting times and blood groups.

## Practical 1. Complete Blood Count (CBC), Red blood cell indices, Erythrocyte Sedimentation Rate (ESR) and Hematocrit (Hct)

#### 1.1. Objectives

At the end of session, the students should be familiar with:

- The procedures used for taking both capillary and venous blood.
- The methods used to measure the ESR and Hct.
- The normal values recorded when making these measurements.
- The method used to get CBC and assess red blood cell indices, including; mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).

#### 1.2. Equipment

- Coulter analyzer.
- EDTA tubes.
- Lancets.
- Tourniquet.
- Alcohol swabs.
- Heparinized capillary tubes.
- Plasticine.
- A centrifuge.
- Micro-hematocrit reader.

#### 1.3. Procedure

#### 1.3.1. Measurement of Hct (or packed cell volume "PCV")

Blood is drawn from capillaries in order to measure Hct using micro-hematocrit reader.



**Figure 1. Procedure of drawing blood into capillary tubes.** (A) The tip of a finger is pricked using a lancet. (B) A heparinized capillary tube is gently brought in contact with the blood drop forming on the tip of the finger.

- 1. Clean the area of the skin of a finger-tip or an ear lobe with a sterilized alcohol swab.
- 2. Prick the skin using the pen lancet, Fig-1A.
- 3. Discard the first drop of blood, because it is mixed with tissue fluid.
- 4. Allow the second drop of blood to be formed and allow it to become large enough to fill 75% of the heparinized capillary tube by the capillary action when it is brought closer to the blood, Fig-1B. Apply only gentle pressure beneath the pricked skin to help the flow of blood, because if more pronounced pressure is exerted, blood is likely to be diluted with interstitial fluid.
- 5. Seal one end of the capillary tube with plasticine.
- 6. Repeat above steps 1 5 to collect several capillary blood samples.
- 7. Put all the capillary blood samples in a centrifuge machine for 5 minutes at the speed of 3000-4000 RPM to separate plasma from cells.
- Once centrifuged, take one of the capillary blood samples to see the cells have been packed at the bottom of the tube and the light-weight clear plasma visible above the cells.

9. Hct or PCV can then be determined as a percentage of the total volume using micro-hematocrit reader.

#### **1.3.2.** Measuring the erythrocyte sedimentation rate (ESR)

To measure ESR, the following equipment will be needed;

- Westergren's sedimentation apparatus, Fig-2.
- EDTA tubes.
- Disposable sterile syringes and needles.



Figure 2. Westergren's tubes and apparatus.

#### 1.3.2.1. Procedure

- 1. Using a sterile syringe, draw 1.6 ml of blood from a suitable vein.
- 2. Transfer the blood to a test tube containing EDTA to prevent clotting.
- 3. Fill the Westergren's tube with blood up to the zero mark.
- 4. Place the tube upright in the stand and leave like this for one hour.

5. Note down the depth of the column of clear plasma at the top of red blood cells in the tube after one hour. This will be E.S.R. reading.

Normally the value of E.S.R. ranges from 0mm to 7 mm and it is slightly higher in females than males due to less number of red blood cells.

#### 1.3.3. Counting peripheral blood cells using the Coulter analyzer

Blood is drawn from a superficial vein in the antecubital fossa using a needle attached to a syringe.



- 1. Clean the area of the skin to be pricked. Usually the blood is drawn from median cubital vein in front of the elbow joint to collect venous sample, Fig-3.
- 2. Apply the tourniquet above the elbow joint to impede the flow of venous blood towards the heart for a while.
- 3. Use a disposable syringe to draw the blood from the vein.
- 4. Immediately transfer the collected blood from the syringe to EDTA anticoagulated tube to prevent blood from clotting.

- Activate the Coulter analyzer machine and a probe will move across and down into aspirate position. The aspiration syringe draws 12 µl of whole blood into the probe.
- 6. The Coulter Analyzer makes the necessary dilutions with the reagents automatically and accurately counts and measures the sizes of cells by detecting and measuring changes in electrical resistance when a particle (such as cell) in the conductive liquid passes through a small aperture. As each cell goes through the aperture, it impedes the current and causes a measurable pulse. The number of pulses signals the number of particles. The height of each pulse is proportional to the volume of that cell.
- 7. Finally all the hematological values are reported and printed.

#### 1.3.4. Calculation of red blood cell indices

#### 1.3.4.1. Mean corpuscular volume (MCV)

This is the average volume of a red blood cell in an individual measured in *femtoliters* (fl). MCV can be calculated from the Hct and total red blood cell (RBC) count using the following formula:

$$MCV = Packed Cell Volume x \frac{10}{RBC Count}$$

Normally, MCV ranges between 78-98 fl. A low MCV denotes smaller than normal RBCs which are then called *microcytes*. Whereas, a high MCV denotes a larger than normal RBCs which are known as *macrocytes*.

#### 1.3.4.2. Mean corpuscular hemoglobin (MCH)

This is the average weight of hemoglobin in a single red blood cell measured in picograms (pg).

$$MCH = Hemoglobin \ Concentration \ x \frac{10}{RBC \ Count}$$

Under normal conditions, the MCH ranges between 27–32 pg.

Low MCH denotes lower than normal hemoglobin weight in an RBC which is known as a hypochromic RBC. While, a high MCH denotes a higher than normal hemoglobin weight in an RBC which is called **hyperchromic** RBC.

#### **1.3.4.3.** Mean corpuscular hemoglobin concentration (MCHC)

This is the concentration of hemoglobin per 100 ml of red blood cell measured in grams/deciliters (g/dl).

$$MCHC = Hemoglobin \ Concentration \ x \frac{100}{Packed \ Cell \ Volume}$$

Normally, the MCHC ranges between 32–36 g/dl. A MCHC value below normal suggests iron deficiency anemia.

#### 1.4. Essential terminology

Find the meaning of the following medical terms;

- Polycythemia.
- Anemia.
- Leucocytosis.
- Leucopenia.
- Thrombocytosis.
- Thrombocytopenia.

#### **1.5. Practice questions**

- 1. What is the clinical importance of knowing the red blood cell indices?
- 2. Discuss briefly the etiological classification of anemia?
- 3. Peripheral blood parameters of two adult males (subject A and subject B) are shown in the table below. Using the information shown in the table answer the questions a & b.

Laboratory parameter	Subject A	Subject B
RBC count	3.6 X 10 <sup>6</sup> / mm <sup>3</sup>	2.5 X 10 <sup>6</sup> / mm <sup>3</sup>
Hb concentration	7.2 g/dl	8 g/dl
Hct	25%	25%

- a. Calculate MCV, MCH and MCHC for each of these subjects.
- b. What are the red blood cell abnormalities seen in these men. List possible causes for each of these abnormalities?
- 4. What is meant by rouleaux formation? And why does rapid rouleaux formation increase the E.S.R?
- 5. What is the clinical significance of E.S.R.?
- 6. What conditions are associated with an increased E.S.R.?

## Practical 2. White Blood Cell Count (WBC) and Differential

#### 2.1. Objectives

At the end of this session, students should be able to:

- Identify the different types of white blood cells under the microscope.
- Describe the normal values expected for each leucocyte subset.
- Understand the clinical relevance of the differential leucocyte count in disease diagnosis.

#### 2.2. Equipment

- Light microscope with an oil immersion objective.
- Mineral or cedarwood oil.
- Wright's stain.
- Microscope slides.

#### 2.3. Procedure

- 1. Venous blood is drawn into anticoagulant EDTA tubes.
- 2. After gentle mixing, a drop of blood is aspirated using a pipette and is placed at one end of a labelled slide. Make sure that the blood drop is as small as possible.
- 3. Then using another slide, place the second slide at a 45° angle over the first slide and slide it gently toward the blood drop.
- 4. Once the edge of the second slide touches the blood drop, allow the drop to spread along the edge of that slide then gently but swiftly pull the top slide over the bottom one to spread the blood drop over the bottom slide creating a thin film of blood, Fig-4.
- 5. Allow it to dry, and then stain the film using Wright's stain.
- 6. Set the stained blood film under the oil immersion objective in an electron microscope.

7. Identify various types of white blood cells according to their histological characteristics.



**Figure 4. Steps to doing a thin blood film.** (A) a drop of anticoagulated blood is placed near the edge of a microscope slide. The edge of a second slide is then placed at an angle of  $45^{\circ}$  over the  $1^{\text{st}}$  slide. (B) The edge of the second slide is slowly brought towards the blood drop. Once it touched the blood drop, the blood drop is allowed to spread along the edge of the  $2^{\text{nd}}$  slide, then the  $2^{\text{nd}}$  slide is pulled gently but swiftly over the forst one to spread the blood over the  $1^{\text{st}}$  slide creating a thin blood film (C).



Figure 5. Schematic representation of the types of white blood cells that can be seen in a peripheral blood film with a representative image of each WBC subset.

#### 2.4. Practice questions

1. Fill the table below by writing the histologic features of each WBC subset, its normal value in the blood and the disease conditions in which they may be elevated.

Cell type	Neutrophil	Eosinophil	Basophil	Monocyte	Lymphocyte
Histologic					
features					
Normal					
value in					
blood					
biood					
O a su all'i l'a su a					
Conditions					
causing an					
increase in					
its level					

#### 2. What stains are used in the preparation of blood films?

#### Practical 3. Blood groups, Bleeding & Clotting Times

#### 3.1. Objectives

At the end of the session, the students should be able to:

- Understand and practice the method used in determining blood groups.
- Be familiar with the ABO and Rh systems of blood grouping and explain their importance in blood transfusion.
- Discuss the normal ranges of bleeding time and clotting time and determine their own values experimentally.
- Recognize the importance of bleeding time and clotting time in hemostasis.

#### 3.2. Determination of blood groups

#### 3.2.1. Equipment

- High titer anti-A, anti-B and anti-D sera.
- A microscope.
- Tooth picks.
- Microscope slides.
- Alcohol swabs.
- Lancet.

#### 3.2.2. Procedure

- 1. Take 3 microscope slides and label them clearly as "A", "B" and "D".
- 2. Sterilize the fingertip with an alcohol swab.
- 3. Prick the finger using a lancet and place one drop of blood in each of the 3 microscope slides.
- 4. Quickly add a drop of anti-A, anti-B and anti-D sera to slides labeled as "A", "B" and "D" respectively.

- 5. Stir the mixture on each slide with the help of different pieces of tooth picks for a minute or two.
- 6. Examine the mixtures carefully for the signs of red blood cell agglutination. When red blood cells clump together (agglutination), they have a speckled or peppered appearance. If there is a doubt, examine the slides using the low power of a microscope. An example is shown in Fig-6.



Figure 6. Blood group determination. (A)  $O^+$  blood sample, (B)  $B^+$  blood sample and (C)  $A^+$  blood sample.

#### **3.2.3. Practice questions**

1. What are the agglutinogens and agglutinins found in people with different blood groups in ABO system?

Blood group	Agglutinogen	Agglutinin
A		
В		
AB		
0		

2. For each blood group in the table, write to which blood group/s it can donate blood to and receive blood from in the setting of a blood transfusion.

Blood Group	Can give blood to	Can receive blood from
AB <sup>+</sup>		
AB <sup>-</sup>		
A <sup>+</sup>		
Α.		
B⁺		
В.		
0+		
0.		

- 3. Apart from classical ABO and Rh groups systems, are there other blood group systems?
- 4. What is the distribution of the ABO and Rh blood groups in Saudi Arabia?

Blood group	Percent in population
0	
0+	
A+	
B+	
AB+	
0-	
A-	
В-	
AB-	

- 5. Does the distribution of blood groups in Saudi Arabia differ from that found in rest of the world?
- 6. What is hemolytic disease of the newborn?
- 7. Under what circumstances does Rh incompatibility develop and how?

8. How is Rh incompatibility treated? And how can it be prevented?

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#### 3.3. Determination of clotting time

#### 3.3.1. Equipment

- Capillary tubes.
- Petri-dish.
- Alcohol swabs.
- Lancets.
- Plasticine.
- Water bath set at 37°C.
- A watch.

#### 3.3.2. Procedure

- 1. Prick a finger of the subject observing the usual precautions and note the time at which the prick is made.
- 2. Wipe away the first drop of blood.
- 3. Then while the blood is still freely flowing, place one end of the capillary tube on it and let the tube fill with it by the capillary action.
- 4. Close both ends of this filled capillary tube with the plasticine.
- 5. Place this capillary tube in the water bath.
- 6. Repeat all the above steps with many capillary tubes.
- Two minutes after making the prick, break a capillary tube and separate the two halves slowly and look for a thread like clot between the two broken halves of the tube.
- 8. Repeat step 7 at 30 seconds interval with the remaining tubes until you see a thread-like clot between the broken halves of one of the capillary tubes.
- 9. Note the time. The time from pricking the finger to the appearance of the clot is the clotting time.

#### 3.3.3. Practice questions

- 1. What is the normal range for clotting time?
- 2. What is/are the clinical condition/s in which the clotting time is greater than normal?
- 3. Can you name some substances that are used as anti-coagulants.

- 4. What is the clinical significance of clotting time?
- 5. What is the source of heparin in the body?

#### 3.4. Determination of bleeding time

#### 3.4.1. Equipment

- Blotting paper.
- Stop watch.
- Alcohol swabs.
- Lancets.

#### 3.4.2. Procedure

- 1. Prick a finger of the subject observing the usual precautions and note the time at which the prick is made. (The pricked skin should not be touched until the experiment is over.)
- 2. Apply a piece of filter paper (blotting paper) to the emerging drop of blood from the pricked skin every 30 seconds until the bleeding stops.
- 3. Note the time when the bleeding stops. The time from pricking the finger to the stop of bleeding is the bleeding time.

#### 3.4.3. Practice questions

- 1. What is the normal range of bleeding time?
- 2. Which blood cell when affected will lead to prolongation of the bleeding time?
- 3. Name one condition in which bleeding time is prolonged (increased)?

## Chapter 2: Respiratory Block Physiology Practical

There are 2 practical physiology sessions during the respiratory block which include;

- 1. Static spirometry (lung volumes & capacities).
- 2. Dynamic spirometry.

Spirometry is one of the essential pulmonary function tests performed in clinical practice. It is concerned with the measurement of flow and volume of air entering and leaving the lungs. Two major types of spirometry measurements are usually performed: simple and dynamic. Simple spirometry is used for the determination of lung volumes and capacities, whereas dynamic spirometry measures the flow of air moving in and out of the lungs. In the following sections you will be introduced to the procedures used for measuring both simple and dynamic spirometry, their indications and result interpretation.

# Practical 1. Simple Spirometry (Lung Volumes and Capacities)

#### 1.1. Objectives

At the end of this session, students are expected to:

- Describe how a bell-type spirometer is used to measure lung volumes and capacities.
- List and define the different lung volumes and capacities.
- State the normal values of each lung volume and capacity.
- Discuss the physiological and pathological factors that may affect the different lung volumes and capacities.

#### 1.2. Equipment

- Simple spirometer (many types are available, Bell-type spirometer or watergauge spirometer), Fig-7. It would be best if students acquaint themselves with the type used in the lab.
- Nose clip.
- Disposable mouth piece.



Figure 7. Simple (volumetric) spirometer.

#### 1.3. Procedure

- 1. Insert the mouthpiece in the subject's mouth so that its edges lie between the subject's lips and gums.
- 2. Place the nose clip on the subject's nose to avoid air escaping through the nose.
- 3. Ask the subject to take normal breaths through the mouthpiece for a short while.
- 4. After recording few normal breaths, ask the subject to take a deep forceful inspiration filling their lungs to their maximum ability followed by gentle exhalation. After that, the subject can resume normal breathing.
- 5. After a few normal breaths, ask the subject to expire quickly, forcibly and as completely as possible. Once this forceful expiration is complete, the subject inhales and resumes normal breathing.
- 6. Finally, ask the subject to take a deep forceful inspiration followed immediately by a maximum, quick and forceful expiration. Once this is complete, ask the subject to breath normally for a short time.
- 7. The spirogram is recorded on a moving drum, Fig-8. An example of how the recording is done is shown in Fig-9.



**Figure 8. A spirogram recording.** The deflection of the pen upwards or downwards with each phase of respiration is dependent on machine mechanics and is subject to variability.


**Figure 9. Simple lung volumes.** The subject breaths through a mouthpiece while a nose clip is placed on the nose to avoid air escaping through it. While breathing, air moves in and out of the spirometer chamber causing displacement in the pen attached to it surface. The moving pen draws the spirometry graph on the kymograph. The degree of displacement is proportional to the volume of air moving in and out of the lungs. With proper calibration, the volume of air moving in and out of the lungs can be calculated.

#### Note to students

Depending on the mechanics of the machine used for simple spirometry measurements, the inspiratory/expiratory curves may be recorded upwards or downwards. The direction of inspiration and/or expiration will always be highlighted in any simple spirogram recording.

# **1.4. Practice questions**

- 1. Define the following terms and state/calculate their values from the data collected in the lab:
  - a. Tidal volume (TV).
  - b. Expiratory reserve volume (ERV).
  - c. Inspiratory reserve volume (IRV).
  - d. Vital capacity (VC).
  - e. Inspiratory capacity (IC).
- 2. Name a few physiological factors that may influence lung volumes and capacities and how do they exert their effects?
- Lung volumes and capacities are altered in a variety of pathological conditions. Name a few and explain how do these conditions bring about the changes are in lung volumes and capacities.

4. What is the physiological significance of the residual volume and the functional residual capacity?

5. Residual volume cannot be directly measured by spirometry. What is the technique that is used to measure it? Explain how it works.

 Using a simple ruler and the calibration provided in the graph, calculate the TV, IRV, ERV and VC from the graph below.



Parameter	Volume in liters
TV	
IRV	
ERV	
VC	

# **Practical 2. Dynamic Spirometry**

# 2.1. Objectives

At the end of this session, students should be able to:

- Perform a dynamic spirometry test on a fellow student.
- Describe the two graphs recorded by dynamic spirometry, namely: flow-volume loop (FVL) and the volume-time curve (forced expiratory curve "FEV1" curve).
- Analyze the components of each graph; FVL and FEV<sub>1</sub> and describe the characteristics of a normal FVL and FEV<sub>1</sub> graphs.
- Calculate the forced expiratory volume in the first second (FEV<sub>1</sub>) and forced vital capacity (FVC) and the FEV<sub>1</sub>/FVC ratio from the FEV<sub>1</sub> curve.
- Calculate the FVC, peak expiratory flow rate (PEFR), peak inspiratory flow rate (PIFR) and maximal expiratory flow rate at 50% of the forced vital capacity (MEF<sub>50</sub>).
- Discuss the indications of dynamic spirometry in clinical practice.
- State the normal values for FEV<sub>1</sub>, FVC and the FEV<sub>1</sub>/FVC ratio.
- State the normal values of FVC, PEFR, PIFR and MEF<sub>50</sub> in FVL.
- Describe the expected changes in FVL and FEV<sub>1</sub> curve in obstructive vs restrictive lung disease conditions.
- Describe the expected changes in FEV<sub>1</sub>, FVC and the FEV<sub>1</sub>/FVC ratio in obstructive vs restrictive lung disease conditions.
- Describe the expected changes in FVC, PEFR, PIFR and MEF<sub>50</sub> in obstructive vs restrictive lung disease conditions.

# 2.2. Equipment

- Dynamic spirometer, Fig-10.
- Nose clip.
- Disposable mouth piece.



Figure 10. Automated spirometer.

## 2.3. Procedure

- 1. Insert a new disposable mouthpiece into the flow sensor (SP-250).
- 2. Hold the sensor in an upright position.
- 3. Insert the mouthpiece in the oral cavity (mouth) and seal the lips tightly around the mouthpiece.
- 4. Place the nose clip on the subject's nose to avoid air escaping through nostrils.
- 5. While subject is standing, allow him/her to breathe normally through mouthpiece, approximately 3 normal breaths to record TV.
- 6. Then ask the subject to inhale as deep as possible and then follow it with a fast and forceful expiration. The expiration should be as fast and forceful as possible and it should continue until the subject is unable to blow out anymore.
- 7. Two types of graphs may be recorded, Fig-11.



Figure 11. Dynamic spirometry graphs. (A) Flow-volume loop, (B) FEV1 curve.

## 2.4. The Flow-volume loop (FVL)

The FVL depicts the relationship between flow and volume under maximal effort of inspiration and expiration. The shape of the loop depends on the mechanical properties of the lung and may help in the diagnosis of ventilatory dysfunction. Fig-12 shows a normal FVL. The normal expiratory portion of a well-performed flow-volume loop is characterized by a rapid increase to the peak flow rate, followed by a nearly linear decrease in flow as the subject exhales toward residual volume. While normally the inspiratory portion shows a symmetric, saddle-shaped curve (1). The parameters that each student need to be familiar with and able to extrapolate from the FVL are: the peak expiratory flow rate (PEFR), peak inspiratory flow rate (PIFR), forced vital capacity (FVC) and maximum expiratory flow at the half-way point in the forced expiratory maneuver (MEF<sub>50%</sub>)-Fig-12.

Fig-13 shows FVL in normal compared to obstructive and restrictive pulmonary disorders.



**Figure 12. A normal flow-volume loop.** The expiratory portion of the loop lies above the x-axis while the inspiratory potion of the loop lies below the x-axis. PEFR represents the maximal flow achieved during forced expiration while the PIFR represents the maximal flow achieved during inspiration. The FVC is the total expiratory volume from a maximally forced expiration maneuver. MEF<sub>50%</sub> is determined from the graph by first establishing the point at which 50% of the vital capacity has been expired (i.e. 2L in the graph above). A line perpendicular to the x-axis (volume axis) is drawn from this point towards the expiratory curve (dotted line no. 1). At the point of intersection between dotted line no. 1 and the expiratory curve, another line is drawn (dotted line no. 2) perpendicular to dotted line no. 1 towards the y-axis (flow axis). The point of intersection of the y-axis with dotted line 2 represents the MEF<sub>50%</sub>.



Figure 13. Shows a normal compared to FVLs of patients with obstructive and restrictive pulmonary disorders (reprinted from (2).

## 2.5. Volume-time graph (FEV<sub>1</sub> curve)

The volume-time graph or the  $FEV_1$  curve depicts changes in volume (x-axis) against time (y-axis). Three main parameters are measured, namely, FVC, forced expiratory volume in the 1<sup>st</sup> second (FEV<sub>1</sub>) and the ratio between these two numbers (FEV<sub>1</sub>/FVC), Fig-14. When performing the test, one must ensure that the FEV<sub>1</sub> curve has reached a plateau and that expiration is maintained for at least 6 seconds (3, 4).



Figure 14. A normal volume-time graph (FEV<sub>1</sub> curve). The FVC represents the volume of air expired at the plateau. FEV<sub>1</sub> is the volume of air expired after 1 second of forced expiratory effort. Under normal conditions, more than 70% of the FVC is expired during the first second of expiration and this is what the ratio FEV<sub>1</sub>/FVC reflects.

## 2.6. Normal values

Normal values are usually reported in 2 ways: as a volume measurement (ml or L of air), or as a percentage of the predicted normative or expected value for that patient's age, height, gender, and race from data obtained in the National Health and Nutrition Examination Survey III (NHANES III) (1).

Parameter	Normal value (ATS/ERS)
FEV <sub>1</sub>	$\geq$ 70% (% predicted FEV1)
FVC	$\geq$ 70% (% predicted FVC)
FEV <sub>1</sub> /FVC ratio	≥ 70% (0.7)

 Table 3. Normal FEV1 values (% predicted).

ATS=American Thoracic Society,

ERS=European Respiratory Society

# 2.7. Diagnostic Differences between Obstructive and Restrictive Airway Diseases

Using spirometry, pulmonary disorders may be categorized into:

- Obstructive.
- Restrictive.
- Mixed.

Obstructive pulmonary disorders are characterized by expiratory airflow limitation and can be seen as a disproportionate reduction in  $FEV_1$  as compared to FVC. While restrictive pulmonary disorders are characterized by a reduction in FVC. Table-2 shows the characteristic findings in  $FEV_1$  curve in the different ventilatory defects.

|--|

	Obstructive pattern	Restrictive pattern	Mixed pattern
FEV <sub>1</sub>	$\downarrow\downarrow\downarrow\downarrow$	Normal or ↓	$\downarrow\downarrow$
FVC	Normal or ↓	$\downarrow\downarrow\downarrow\downarrow$	$\downarrow\downarrow$
FEV <sub>1</sub> /FVC (FEV <sub>1</sub> %)	< 0.7 (70%)	Normal or > 0.7 (70%)	variable

# 2.8. Practice questions

1. From the  $FEV_1$  curve produced in the lab, what is the value of the following:

_	Value	
Parameter	Litres	% predicted
FEV <sub>1</sub>		
FVC		
FEV <sub>1</sub> /FVC ratio		

- 2. What is the expected normal value for  $FEV_1$  in a normal person?
- 3. How long does it take a healthy subject to expire approximately 70% of their vital capacity?
- 4. Briefly explain what happens to FVC, FEV<sub>1</sub> and FEV<sub>1</sub> % measurements in patients with obstructive and restrictive lung diseases.

5. From the flow volume loop recorded, what is the value of the following parameters:

	Value	
Parameter	Litres	% predicted
PEFR		
PIFR		
FVC		
MEF <sub>50%</sub>		

6. Briefly describe the important characteristics of the flow-volume curve recorded in a normal healthy person.

7. Why is the force-independent part of the expiratory loop curvilinear in obstructive lung disease?

8. What is the clinical significance of MEF50 measurements?

## 2.9. Further resources

- Paraskeva et al. Spirometry. 2011. Australian Family Physician. 40 (4): 216-219.
- Johnson et al. A stepwise approach to the interpretation of pulmonary function tests. 2014. American Family Physician. 89 (5): 359-366.

# 2.10. Summary

Fig-15 summarizes the findings seen in FVL and  $FEV_1$  curve in abnormal ventilatory conditions compared to normal.



Figure 15. Typical spirograms and FVL in different ventilatory conditions (5).

# Chapter 3: Cardiovascular Block Physiology Practical

The cardiovascular system (CVS) is one of those systems where physiology is intricately related to clinical application. Understanding the procedures used for physical examination of the CVS, its symptomatology and the presentation of cardiovascular disease requires sound knowledge of its physiology. This knowledge enables better understanding of the pathophysiologic processes underlying most cardiac ailments and aids in proper interpretation of symptoms and signs of cardiovascular abnormalities. In addition, many of the crucial diagnostic tests used in the workup for cardiovascular abnormalities have their roots in physiology such as the electrocardiogram (ECG). It is thus of crucial importance that the student acquires strong understanding of the physiologic phenomena underlying cardiovascular function.

To encourage better understanding of core physiologic principles, four laboratory sessions have been incorporated into the CVS block that cover four major areas of CVS physiology, which include;

- 1. Electrocardiography (ECG).
- 2. Carotid arterial and Jugular venous pulses (CAP & JVP).
- 3. Arterial blood pressure (ABP).
- 4. Heart sounds.

# Practical 1. Electrocardiography (ECG)

The ECG is a simple and very important tool for diagnosing cardiac abnormalities. It records the electrical activity of the heart using surface electrodes placed on the precordium of the subject. The ECG records the sum of electrical activity of the heart transmitted to the surface of the body. The tracing shows waves of different shapes connected to each other by intervening intervals. By analyzing the different waves and intervals, a physician can obtain valuable information on (1) the rhythm of the heart, (2) heart's orientation, (3) size of its chambers, (4) presence of ischemia or infarction in the heart along with its location and extent, (5) electrolyte imbalance and (6) drug effects (6). It is thus of crucial importance that medical students learn how to read and interpret the ECG correctly.

This laboratory session is designed to teach students the basics behind ECG recording and to familiarize them with the normal appearance of the different ECG waves and intervals.

# 1.1. Objectives

- Define the electrocardiogram (ECG).
- Define a lead.
- List the leads used in recording the ECG.
- Compare and contrast between unipolar and bipolar leads.
- Recognize the correct position for electrode placement for each lead.
- Practice recording an ECG on a fellow student.
- Describe the normal appearance of an ECG trace.
- Identify and name the different waves on an ECG trace.
- Describe the different physiologic changes underlying each wave.
- Correlate the ECG electrical changes with the mechanical changes occurring during the cardiac cycle.
- Calculate the heart rate from the ECG trace.
- Determine the rhythm of the heart from the ECG trace.
- Calculate the cardiac axis from the ECG trace.

# 1.2. Equipment

- ECG recording machine (electrocardiograph).
- ECG graph paper.
- Disposable ECG electrodes.
- Alcohol swabs and gauze.

# 1.3. The 12-Lead ECG

A lead means a viewpoint or perspective of the heart (7). Thus, a 12-lead ECG is an ECG recoding that looks at the heart from 12 different viewpoints providing a complete picture of the heart's electrical activity. The 12-lead ECG is composed of six limb leads and six chest leads. Limb leads look at the heart in the coronal plane while chest leads look at the heart in a horizontal plane (7). Each lead looks at the heart from a different angle in its defined plane (7).

Leads are recorded by placing electrodes on the surface of the skin. Limb leads are recorded using four electrodes placed on: the left arm (LA), right arm (RA), left leg (LL) and right leg (RL). The four electrodes will record six leads (views) of the heart in the coronal plane, namely; Lead I, lead II, lead III, aVR, aVL and aVF. Leads I, II and III are bipolar leads whereas aVR, aVL and aVF are unipolar leads, Fig-16A (7). A bipolar lead records the potential difference between two active electrodes whereas a unipolar lead records the potential of one active electrode as compared to the other inactive electrodes.

Precordial (chest) leads, Fig-16B, are unipolar leads recorded using six electrodes placed in the following manner (7):

- V<sub>1</sub>: 4<sup>th</sup> intercostal space (ICS), right sternal border.
- V<sub>2</sub>: 4<sup>th</sup> ICS, left sternal border.
- V<sub>3</sub>: midway between V<sub>2</sub> and V<sub>4</sub>.
- $V_4$ : 5<sup>th</sup> ICS, mid-clavicular line (in women,  $V_4$  is placed under the breast).
- $V_5$ : 5<sup>th</sup> ICS (horizontal to  $V_4$ ), anterior axillary line.
- $V_6$ : 5<sup>th</sup> ICS (horizontal to  $V_4$ ), mid-axillary line.



Figure 16. Shows the different leads used to record the 12-lead ECG. (A) Shows the bipolar standard limb leads (leads I, II and III) and the unipolar augmented limb leads (aVR, aVL and aVF). (B) Shows the location of the unipolar precordial chest leads ( $V_1$  to  $V_6$ ).

## 1.4. Procedure

- 1. Ask a student volunteer to lie on their back on the examination bed with arms by their side.
- 2. Expose the volunteer appropriately (exposing chest and ankles).
- 3. Make sure that the skin over the precordium is dry, hairless and oil-free to ensure that electrodes stick nicely on the skin. If not, the skin can be cleaned with an alcohol swab and dried using gauze.
- The temperature of the room should neither be too hot nor too cold i.e. it should be comfortable (neutral). Sweating and muscle movements should be avoided to minimize artifacts.
- 5. Place 10 electrodes on the skin surface, table-5, and connect them correctly with the wires of the machine. When placing the electrodes avoid pressing hard on the center of the electrode to avoid gel from spilling to the edge reducing conductivity.

Electrode name	Electrode placement
RA	On the right arm
LA	In the same location as RA but on the left side (left arm)
LL	On the left leg
RL	In the same location as LL but on the right side (right leg)
V <sub>1</sub>	4 <sup>th</sup> ICS, right sternal border
V <sub>2</sub>	4 <sup>th</sup> ICS, left sternal border
V <sub>3</sub>	Midway between $V_2$ and $V_4$
V <sub>4</sub>	5 <sup>th</sup> ICS, mid-clavicular line
V <sub>5</sub>	$5^{\text{th}}$ ICS (horizontal to V <sub>4</sub> ), anterior axillary line.
V <sub>6</sub>	$5^{\text{th}}$ ICS (horizontal to V <sub>4</sub> ), mid-axillary line.

 Table 5. Electrode placement for the 12-lead ECG recording.

Adapted with modification from: <u>https://slideplayer.com/slide/10943937/</u>

6. Ask the subject to relax and start recording the ECG.

## 1.5. The ECG trace

An ECG trace similar to that shown in Fig-17 will be recorded. Analyze the trace for the following parameters:

- Calculate the heart rate of the subject.
- Comment on the rhythm of the heart.
- Name the waves and intervals and calculate their duration.
- Calculate the cardiac axis.



Figure 17. A normal ECG recording (trace).

## 1.6. ECG Interpretation

#### 1.6.1. The ECG paper

The ECG is recorded on graph paper. The paper runs at a standard speed of 25mm/sec. The horizontal axis denotes time while the vertical axis denotes voltage. The graph paper is divided into large and small squares. Each large square contains 5 small squares of 1 mm length. Given that the paper runs at a rate of 25 mm/sec, horizontally, one small square of 1 mm length represents 0.04 seconds, Fig-18.



**Figure 18. ECG paper calibration.** The ECG is recorded on graph paper divided into large and small squares (boxes). Each small box is 1mm in length. Five small boxes make one large box. The horizontal axis denotes time (seconds) while the vertical axis denotes voltage (mv). The paper runs at a standard speed of 25mm/sec. This means that horizontally each small box=0.04 seconds while the large box=0.2 seconds. Vertically, 10mm=1mv.

#### 1.6.2. ECG waves and intervals

ECG waves and intervals are shown in Fig-19. Each ECG wave and its physiologic significance is shown in table-6 while the intervals, their significance and normal duration are shown in table-7.



Figure 19. ECG waves and intervals.

Table 6. ECG waves.

ECG wave	Physiologic phenomenon
P wave	Atrial depolarization
QRS complex	Ventricular depolarization
T wave	Ventricular repolarization
U wave	Papillary muscle repolarization

#### Table 7. ECG intervals and their duration.

Interval	Measurement	Normal duration/characteristics
PR interval	From beginning of P wave to	0.12-0.2 seconds (3-5 small
	beginning of R wave	squares)
QRS complex	From beginning of Q wave to	Not more than 0.12 seconds (3
	the end of the S wave	small squares)
QT interval	From beginning of Q wave to	0.35-0.43 seconds
	the end of T wave	
ST interval	From end of S wave to the	0.28-0.36 seconds
	end of the T wave	
ST segment	From the end of the S wave	Normally isoelectric
	to the beginning of the T	
	wave	

#### 1.6.3. Heart rhythm determination

The heart rhythm refers to the regularity by which the heart beats. Under normal conditions, the SA node is the pacemaker of the heart and it generates impulses at regular intervals. In simple terms, heart rhythm can be determined by observing the R-R intervals on the strip recording of the ECG. If the R-R intervals have more or less the same duration, the rhythm is said to be regular, whereas if the R-R intervals are variable in length the rhythm is said to be irregular (arrhythmia).

The heart usually beats faster during inspiration and slower during expiration, showing unequal R-R intervals on the ECG strip. The R-R intervals are shorter during inspiration and wider during expiration. This pattern of arrhythmia is physiologic and is due to the different firing rate of SA node during inspiration and expiration, it is known as "sinus arrhythmia".

#### 1.6.4. Heart rate calculation

• Regular heart rhythm

If the heart rhythm is regular, the heart rate can be calculated from the ECG using one of the following formulas;

$$Heart rate = \frac{1500}{Number of small squares in one R - R interval}$$

OR

$$Heart rate = \frac{300}{Number of large squares in one R - R interval}$$

Where 1500 is the total number of small squares pulled by the machine every minute (60 sec) when the speed of ECG machine is calibrated at 25 mm/sec (60 x 25). While 300 is the total number of large squares pulled by the machine every minute (60 sec) when the speed of the ECG machine is calibrated at 25mm/sec (5 big squares/sec).

The normal range of heart rate is between 60 – 100 beats/minute. A heart rate higher than a 100 beats/minute is known as "*tachycardia*". While a heart rate less than 60 beats/minute is called "*bradycardia*".

#### • Irregular heart rhythm

The above method is not applicable when the heart rhythm is irregular. In this case, heart rate can be calculated by first, counting the number of QRS complexes in 30 large squares (which equals the number of QRS complexes in 6 seconds). Then multiply the number of QRS complexes counted in 6 seconds by 10 to get the number of QRS complexes in one minute i.e. the heart rate, Fig-20 (7).



Figure 20. Calculating the heart rate when the rhythm is irregular.

## 1.6.5. Calculation of the cardiac axis

The electrical axis is the average direction of the current flow in the heart during a cardiac cycle. The cardiac axis is expressed as an angle and is measured in degrees. The depolarization wave normally spreads through the ventricles in a direction from base of the heart to its apex. If limb leads are superimposed on each other, Fig-21, lead one will be looking at the heart from the left at an angle of 0°. This horizontal line is considered the reference or zero point. Any deviation below that line is expressed as a positive number whereas deviations above the line are expressed as negative numbers. As such, lead II is considered to be looking at the heart at an angle of +60° while lead III looks at the heart at +120°, Fig-21.



Figure 21. The axial reference system.

The normal cardiac axis lies between -30° to 90°, Fig-22. Certain pathological conditions causes the cardiac axis to deviate to the left (between -30° to -90°) which is then called *left axis deviation* (LAD) while other pathological conditions causes it to shift to the right (90° to 180°) and it is called *right axis deviation* (RAD). Beyond these values, it will be extreme or right /left axis deviation, Fig-22B.



**Figure 22. The cardiac axis.** (A) In the normal heart, the average current flow occurs primarily in the direction from base to apex with negativity at the *base* and positivity toward the *apex* of the heart at an angle between (-30° to 90°). (B) Shows the range in which the cardiac axis is considered normal (-30° to 90°), LAD (-30° to -90°), RAD (90° to 180°) and extreme axis deviation (180° to -90°).

There are several methods to determine the axis of the heart. Some are cumbersome but accurate while others are quick and easy but less accurate.

• The accurate way to determine the cardiac axis

Use two limb leads, namely leads I and III. Looking at the QRS complexes in these leads, calculate the overall size and polarity of the QRS complex in each by subtracting the depth of S wave from the height of the R wave. Construct a vector diagram and draw arrows that represent the sum of size and polarity for each lead on the diagram. The cardiac axis lies between the two arrows. Drop a perpendicular line from the tip of each arrow. The point at which the two perpendicular lines meet, constitute the tip of the cardiac axis. Draw a line from that point to zero point and this will be the cardiac axis, Fig-23.

N.B. the height of the R wave and the depth of the S wave are both measured starting from the isoelectric line.



Figure 23. Calculation of the cardiac axis. The accurate method.

• The quick and easy way to determine the cardiac axis

Is by applying the "rule of thumb" on the direction of QRS complex in leads I and III/aVF of the ECG, Fig-24.

- If the QRS complex is predominantly positive in both leads I and III, then the cardiac axis is *normal*.
- If the QRS complex is predominantly positive in lead I and predominantly negative in lead III, this means left axis deviation.
- If the QRS complex is predominantly negative in lead I and predominantly positive in lead III, this means right axis deviation.
- If the QRS complex is predominantly negative in both leads I and III, this is extreme axis deviation.



Figure 24. The quick way for cardiac axis determination. Lead III or aVF may be used.

# **1.7. Practice questions**

- 1. Looking at the ECG tracing in Fig-17, answer the following questions;
  - a. What is the rhythm like in the ECG tracing?
  - b. Calculate the heart rate from the ECG trace provided.
  - c. Calculate the cardiac axis and plot it on the hexaxial plot provided below.



- d. What is the duration of the following intervals;
  - i. PR interval=\_\_\_\_\_
  - ii. ST interval=\_\_\_\_\_
  - iii. QT interval=\_\_\_\_\_
- e. What do you think of the ST segment?

# Practical 2. Carotid Arterial and Jugular Venous Pulses

# 2.1. Objectives

- Describe the normal carotid arterial pulse (CAP) recording.
- Identify the phases of the cardiac cycle, systole & diastole, on the CAP trace.
- Explain the physiologic phenomenon behind the appearance of the dicrotic notch in the CAP recording.
- Identify systolic and diastolic pressures from the CAP recording.
- Describe the normal jugular venous pressure (JVP) recording.
- Enumerate and identify the different waves seen on the JVP trace.
- Explain the physiologic events causing each wave on the JVP trace.

# 2.2. The carotid arterial pulse (CAP)

## 2.2.1. Equipment

- Examination bed.
- Recorder (biopac machine).
- Pulse transducer.

## 2.2.2. Procedure

- 1. Ask subject to lie quietly on the examination bed with the head lifted at 45°.
- 2. Ask the subject to turn his head to the opposite side. Feel the carotid arterial pulse on the medial side of the sternocleidomastoid muscle.
- 3. Apply the transducer over the carotid artery using a soft rubber band and connect it to the recorder.

## 2.2.3. The CAP trace

When blood is forced into the aorta during ventricular systole, two things happen:

1. Blood moves forward.

2. A pressure wave is set up which travels along the wall of arteries (faster than the flow of blood). The pressure wave expands the arterial walls as it travels. The expansion of the arterial wall is palpable as the pulse.

Recording of the carotid arterial pulse produces a tracing similar to that shown in Fig-25.



Figure 25. A carotid arterial pulse (CAP) tracing.

The CAP has the following features:

1. The anacrotic limb (ana- means up):

It is the upward deflection in the carotid arterial pulse tracing. It represents increasing pressure in the carotid artery during the maximum ejection phase of ventricular systole. In healthy individuals, the arterial pressure recorded at the peak of the anacrotic limb is 120 mmHg, i.e. systolic pressure.

2. The dicrotic limb:

It is the descending phase of the carotid arterial pulse tracing. It is caused by the decrease in carotid arterial pressure during ventricular diastole. In healthy individuals, the lowest pressure recorded during this phase is about 80 mmHg due to the elastic recoil of the arterial wall.

3. The dicrotic notch or incisura:

It occurs during the phase of diastole in the CAP tracing and is caused by the sudden closure of the aortic valve. It marks the beginning of ventricular diastole. It coincides with  $S_2$  when we relate it to a phonocardiogram and occurs just after the T wave when we relate it to an ECG, Fig-26.



Figure 26. Simultaneous recording of ECG, CAP and phonocardiogram.

# 2.3. The Jugular Venous Pulse (JVP)

## 2.3.1. Equipment

- Examination bed.
- Pulse transducer.
- Recorder (biopac machine).

## 2.3.2. Procedure

- 1. Position the subject at 45° on a couch or bed so that the pulsation of the internal jugular vein can be visualized.
- Ask the subject to perform a Valsalva maneuver (deep inspiration followed by a forced expiration against a closed glottis). As a result, the internal jugular vein becomes prominent.
- 3. Choose a position on the internal jugular vein away from the carotid artery.
- 4. Place the pulse transducer over the vein and keep it in position with a selfadhesive plaster and connect to the recorder.

## 2.3.3. The JVP trace

Recording of the JVP produces a tracing similar to the one shown in Fig-27. The JVP trace has three positive waves labeled as "a", "c" and "v" two negative waves or descents labeled as "x" and "y". The cause of each wave is summarized in table-8 (8).



Figure 27. The JVP waveform.

Table 8. The different waves in the JVP trace and their underlying physiologic event.

Wave name	Physiologic event		
'a' wave	Increased right atrial pressure secondary to right atrial contraction		
	occurring at the end of ventricular diastole.		
'c' wave	Increased right atrial pressure caused by the bulging of tricuspid		
	valve into the right atrium during isovolumetric ventricular		
	contraction. Or due to transmitted carotid pulsations.		
'x' descent	Decreased right atrial pressure due to downward displacement of		
	tricuspid valve secondary to contraction of papillary muscles		
	during ventricular systole.		
'v' wave	It represents the increase in right atrial pressure as it fills with		
	blood returning from the great veins against a closed tricuspid		
	valve.		
'y' descent	It represents the fall in right atrial pressure as blood flows out of		
	the right atrium and into the right ventricle upon opening of the		
	tricuspid valve.		

In Fig-28, a simultaneous recording of the JVP and ECG is shown. Correlating the events recorded on the JVP with those occurring in the ECG, one can clearly appreciate that the P wave in the ECG occurs just before the 'a' wave of JVP, thus showing atrial depolarization precedes the atrial contraction.



Figure 28. Simultaneous recording of JVP with ECG.

## 2.3.4. Clinical application of JVP

Table 9. Clinical applications of JVP.

Clinical finding	explanation	Possible causes
Prominent 'a'	Signifies increased right atrial	Right heart failure
wave	pressure	Tricuspid stenosis
		Pulmonary stenosis
		Pulmonary hypertension
Cannon 'a' wave	It occurs when the right atrial	Atrial flutter
	pressure becomes very high	Third degree heart block
	usually secondary to right atrial	(Complete heart block)
	contraction against a closed	Ventricular Tachycardia
	tricuspid valve	
Absent 'a' wave	The right atrial pressure does not	Atrial fibrillation
	increase due to failure of proper	
	contraction	
Large 'v' wave		Tricuspid regurgitation

## 2.3.5. The difference between venous and arterial pulses

- Multiphasic, venous pulse "beats" twice (in quick succession) in a cardiac cycle. The first beat represents that atrial contraction (termed 'a') and second beat represents venous filling of the right atrium against a closed tricuspid valve (termed 'v'), where as an artery only has one beat in a cardiac cycle (9).
- Non-palpable, venous pulse cannot be palpated.
- Varies with respiration, venous pulse usually decreases with deep inspiration.

# 2.4. Practice questions

1. Why is the JVP recorded from the internal jugular vein rather than the external jugular vein?

# **Practical 3. Arterial Blood Pressure (ABP)**

# 3.1. Objectives

- Define sphygmomanometer and identify its parts.
- Describe the palpatory and auscultatory methods for ABP measurement.
- Differentiate between the palpatory and auscultatory methods in measuring ABP.
- Perform ABP measurement for a fellow student using the sphygmomanometer.
- Identify Korotkov sounds and describe their use ABP determination.
- Enumerate the precautions considered before and during ABP measurement.
- Recognize the effect of exercise on the ABP.

## 3.2. Equipment

- Sphygmomanometer, Fig-29.
- Stethoscope.
- Bicycle ergometer and/or treadmill.



**Figure 29. The sphygmomanometer.** (A) shows the parts of the sphygmomanometer. (B) shows the different cuff sizes available to match patient age, size and built.

# 3.3. Procedure

## 3.3.1. Palpatory method for ABP measurement

This method only gives an estimate of the systolic blood pressure.

- 1. Ask the subject to sit comfortably on a chair with their arm resting on the bench.
- 2. Apply the cuff around the arm and over the brachial artery about 2.5cm above the antecubital crease. Make sure the arm is at heart level.
- 3. Inflate the cuff until the radial pulse disappears. By compressing the brachial artery, the pulse or pressure wave is prevented from being transmitted to the radial artery.
- 4. Deflate the cuff slowly (3-4 mmHg/second) and note the pressure at which the radial pulse returns. This will be the systolic blood pressure.

## 3.3.2. Auscultatory method for ABP measurement

This method measures both systolic and diastolic blood pressures.

- 1. Inflate the sphygmomanometer cuff until there is no radial pulsation.
- Place the diaphragm of the stethoscope over the brachial artery. The brachial artery is found in the antecubital fossa 1/3 the way from the medial epicondyle (9).
- 3. Deflate the cuff (3-4 mmHg/second). As you deflate the cuff, five different sounds are heard, known as the "Korotkoff" sounds. The first sound heard is Korotkoff I (KI) and is heard as a tapping sound and represents "systolic pressure". As the cuff continues to deflate, the sounds increase in intensity (KII), then decrease (KIII), become muffled (KIV) and finally disappears (KV). The pressure at which the sounds disappear represents "diastolic pressure", Fig-30 (9).

### 3.3.3. Further measurements

- Practice the above two methods while the subject is resting in a supine position and then in the sitting position.
- Repeat each measurement *at least three times* to establish reproducibility of the results.
• Ask the subject to perform a 10 minutes exercise on either a bicycle ergometer or a treadmill, then measure their ABP immediately after the end of the exercise and 5 minutes later.

#### 3.3.4. Precautions for measuring ABP

- The cuff should fit the patient's arm appropriately. Cuffs come in different sizes, Fig-29B. Ideally, the width of the inflatable bladder should cover 40% of the upper arm while its length should cover 80% of upper arm circumference (10).
- The cuff must be applied snuggly (not too tight and not too loose) about 2.5 cm above the antecubital fossa.
- Take care that the free margin of the cuff is not on the course of brachial artery i.e. make sure that the rubber bag within the cuff is on the medial side so that it can occlude the brachial artery when the cuff is inflated.
- It is important that the manometer is at the same level as the heart to exclude the effect of gravity while measuring the blood pressure.
- Mercury manometer should be in the vertical position.
- Check that there is adequate amount of mercury in the bulb of the instrument. This is done by checking the mercury level is at the zero position of the manometer.
- The subject must be physically and mentally relaxed and in a comfortable environment.



Figure 30. Arterial blood pressure measurement.

#### 3.4. ABP values

Ranges for normal and abnormal blood pressure (BP) measurement are shown in table-10.

|--|

Systolic BP		Diastolic BP
<120mmHg	and	<80mmHg
120-129mmHg	and	<80mmHg
130-139mmHg	or	80-89mmHg
≥ 140mmHg	or	≥ 90mmHg
> 180mmHg	and/or	> 120mmHg
> 180mmHg + target	and/or	> 120mmHg + target
organ damage		organ damage
	Systolic BP <120mmHg 20-129mmHg 30-139mmHg 140mmHg 180mmHg 180mmHg + target organ damage	Systolic BPand<120mmHg

AHA guidelines

Table-11 shows and example of BP changes with exercise.

Conditions	Blood pressure
Before exercise	120/80 mmHg
After mild exercise	140/80 mmHg
After heavy exercise	160/60 mmHg

#### Table 11. BP changes with exercise.

In the above example, we can observe that after mild exercise, systolic blood pressure increases while diastolic blood pressure remains more or less the same. Following heavy exercise, the systolic pressure increases tremendously and the diastolic pressure drops.

### 3.5. Practice questions

1. Explain how Korotkoff sounds are produced?

2. What is pulse pressure and how is it calculated?

3. What is mean arterial blood pressure? What is its significance? How is it calculated?

4. What are the effects of exercise on systolic and diastolic blood pressure? What happens to pulse pressure during exercise? Explain your answer.

## **Practical 4. Heart Sounds**

### 4.1. Objectives

- List the different heart sounds and identify the auscultatory areas recommended for auscultation of each.
- Perform heart sound auscultation on a fellow student.
- Describe the physiologic phenomenon behind the generation of each heart sound.
- Identify the timing of sounds in relation to the cardiac cycle.
- Define phonocardiography.
- Identify the major heart sounds on a phonocardiogram and correlate them to the electrical and mechanical events occurring in the heart.

## 4.2. Listening to heart sounds by auscultation

#### 4.2.1. Equipment

- Stethoscope, Fig-31.
- Examination bed.

A stethoscope consists of an earpiece, rubber tubing and a chest piece. The chest piece is composed of a diaphragm (the larger circle) and a bell (the smaller circle). The bell is used with light skin contact and pressure to hear low-pitched sounds while the diaphragm is used with firm skin contact to hear high-pitched sounds. Both the first ( $S_1$ ) and second ( $S_2$ ) heart sounds are high pitched while certain abnormal cardiac sounds (i.e. murmurs) are low pitched sounds. A one-way valve system prevents sounds from being transmitted by the bell when the diaphragm is being used and vice versa.



Figure 31. The stethoscope.

#### 4.2.2. Procedure

- 1. Ask the subject to lie on the examination bed in a supine position with the head of the bed elevated at 30° degrees.
- Identify the four major auscultatory areas on the precordium of the subject, Fig-32 & 33.
- 3. Place the stethoscope sequentially on the different areas and listen carefully to S<sub>1</sub> and S<sub>2</sub> at each, note your findings. You can start auscultating for heart sounds at the apex of the heart and move to the base; left 5<sup>th</sup> intercostal space (ICS) midclavicular line (MCL), moving medially to the left sternal border, then superiorly to the left 2<sup>nd</sup> ICS at sternal border then across the sternum to the right 2<sup>nd</sup> ICS at the sternal border. Otherwise, you can start the other way around beginning at the base and moving to apex, Fig-32.
- 4. While auscultating the heart, place your left index finger and middle finger on the right carotid artery to time heart sounds with the cardiac cycle.

N.B. During cardiac examination, the patient may be asked to turn to his left side (left lateral position) or sit upright and lean forward to accentuate certain murmurs when suspected by history or initial physical examination.



**Figure 32. Heart sound auscultation areas.** The picture shows the four major precordial auscultation areas, namely, the mitral area (left 5<sup>th</sup> ICS MCL), the tricuspid area (left sternal border), the aortic area (right 2<sup>nd</sup> ICS, parasternal line (PSL)) and the pulmonary area (left 2<sup>nd</sup> ICS PSL).



Figure 33. Shows the location of the different auscultatory areas in relation to the anatomic location of the corresponding cardiac valve.

#### 4.3. Heart sounds using phonocardiography

Phonocardiography is the sensitive technique, by which heart sounds are recorded using a transducer placed on specific areas of auscultation. If the phonocardiogram (PCG) is recorded simultaneously with the ECG, the sound that coincides with the QRS complex is  $S_1$  while  $S_2$  coincides with the T wave. As such,  $S_1$  marks the beginning of systole while  $S_2$  marks the beginning of diastole. The period between the two sounds represents systole whereas the period between  $S_2$  and the next  $S_1$  signifies diastole, Fig-34.





#### 4.4. Heart sounds

Table 12.Heart sounds and their description.

Heart sound	Abbreviation	Description
1 <sup>st</sup> heart sound	S <sub>1</sub>	Normal.
		• Heard as "lub".
		Caused by closure of AV valves.
		Best heard at mitral and tricuspid areas.
		<ul> <li>Occurs at the beginning of systole.</li> </ul>
		• Coincides with the QRS complex on the ECG.
2 <sup>nd</sup> heart sound	<b>S</b> <sub>2</sub>	Normal.
		• Heard as "dub".
		Caused by closure of semilunar valves.
		Best heard at aortic and pulmonary areas.
		Occurs at the beginning of diastole.
		<ul> <li>Coincides with the T-wave on the ECG.</li> </ul>
3 <sup>rd</sup> heart sounds	S <sub>3</sub>	• May be heard normally in children, thin adults,
		and pregnant women or after exercise.
		Caused by the rapid gush of blood into the
		ventricles during the phase of rapid ventricular
		filling.
		Occurs during early diastole.
4 <sup>th</sup> heart sounds	S <sub>4</sub>	Rarely heard in normal adults.
		Caused by the oscillation of blood and cardiac
		chambers secondary to atrial contraction.
		<ul> <li>Occurs just before S<sub>1</sub> during late diastole.</li> </ul>

## 4.5. Physiologic splitting of S<sub>2</sub>

If either the first or second heart sound is heard as two distinct components, they are said to be split.  $S_2$  splitting is a normal physiological phenomenon that may occur during deep inspiration.

As a person takes a deep breath, the chest wall expands and the intra-thoracic pressure falls. This fall in intra-thoracic pressure increases the venous return into the right atrium. This inspiration-induced increase in venous return delays closure of the pulmonary valve causing the audible splitting of  $S_2$  (6).

We can hear a clear "dub, dub" when we auscultate the heart at either aortic or pulmonary areas during deep inspiration. This is what we call physiological splitting of the  $S_2$ . This splitting can be appreciated by the human ear only if the two components of the sound are separated by more than 0.2 seconds.

### 4.6. Practice questions

- 1. Below is a picture of the chest. On the picture below shadow and label the precordial areas for heart sound auscultation, and answer the following questions;
  - a. S<sub>1</sub> is heard best in which area/s?
  - b. S<sub>2</sub> is heard best in which area/s?



- The tracing below, shows simultaneous recording of the ECG and the phonocardiogram (PCG). Using the knowledge acquired in the lab identify the following on the PCG tracing;
  - a. The first and second heart sounds.
  - b. Systole and diastole.



# **Chapter 4: Renal Block Physiology Practical**

The kidney is involved in the regulation of key physiologic parameters such as, ABP, electrolytes, water and hydrogen ions. Based on that, it is not surprising that the consequences of disruption of renal function are echoed in almost every other organ system of the body, such as: the heart, muscles, nervous system, lungs, blood..etc. It is thus deemed necessary that students gain a solid understanding of the role played by the kidney in regulating each of the above parameters and the mechanisms underlying such regulation. In this regard, renal physiology practical consist of 4 sessions that aim at covering key aspects in renal physiology, namely:

- 1. Glomerular filtration and renal clearance.
- 2. Diuresis-I.
- 3. Diuresis-II.
- 4. Basics of acid-base.

The glomerular filtration and renal clearance as well as the basics of acid-base practical sessions run as tutorial lessons highlighting the difficult concepts covered during lectures and presenting them in an easy to grasp interactive way using a CD demonstration.

The diuresis practical on the other hand aims at highlighting the role of the tubules in modifying urine output in response to various environmental changes. This is a handson lab where students will have the chance to perform a mini-experiment designed to emphasize the role of the kidney in fluid and electrolyte regulation by demonstrating the changes that occur in urine flow rate and renal excretion of certain electrolytes in response to ingestion of different fluids/diuretics. The success of this lab and maximum benefit requires full cooperation and engagement of students.

## **Practical 1. Diuresis**

#### 1.1. Objectives:

- 1. To measure the volume and determine the composition of urine excreted by 5 different groups of volunteers that have underwent different experimental interventions, as follows:
  - a. Control group.
  - b. Group drinking one liter of water.
  - c. Group drinking one liter of water and lying down.
  - d. Group drinking one liter of normal saline.
  - e. Group taking a 20mg tablet of lasix (a loop diuretic).
- 2. To explain the mechanisms underlying the different data obtained from each of the above groups.

#### 1.2. Equipment

- Calibrated cylinder, Fig-35A.
- Urine collection container, Fig-35B.
- Pipette.
- Test tubes
- Urine sample container, Fig-35C.
- Osmometer.
- pH meter.



Figure 35. Laboratory equipment. (A) Calibrated cylinders. (B) Urine collection container. (C) Urine sample container.

#### 1.3. Experimental design

- 1. Ten students are needed to participate in the experiments as volunteers.
- 2. Students will be divided into 5 groups each consisting of 2 students, as follows:
  - a. Control group.
  - b. Water only group.
  - c. Water and lie down group.
  - d. Normal saline group.
  - e. Lasix group.
- 3. **ALL** volunteers will empty their bladders two hours before the beginning of the lab session and discard this sample.
- 4. **During the two hours preceding the lab session,** volunteers will behave normally (eat and drink) but they should not go to the rest rooms.
- 5. After two hours (at the beginning of the lab session), ALL volunteers will empty their bladders and collect the urine in calibrated containers. This sample is considered the 1<sup>st</sup> urine sample and represents the control sample for each student prior to intervention.

- After collecting the 1<sup>st</sup> sample, each volunteer will ingest what they have been assigned to:
  - a. Control group  $\rightarrow$  Nothing.
  - b. Water only group  $\rightarrow$  will drink one liter of water.
  - Water and lie down group → will drink one liter of water and lie flat on an examination bed.
  - d. Normal saline group  $\rightarrow$  will drink one liter of normal saline.
  - e. Lasix group  $\rightarrow$  will take one tablet of 20mg Lasix.
- Following ingestion, urine will be collected from each volunteer every half an hour for a total of two hours. Fig-36 summarizes the experimental design.
- 8. With every urine sample collected, the following should be recorded/measured:
  - a. Urine volume.
  - b. Urine flow rate.
  - c. Urine osmolality.
  - d. Sodium and potassium urine concentration.
  - e. Urine pH.



**Figure 36. Experimetal design of diuresis laboratory session.** Volunteers will empty their bladder two hours prior to lab session and discard the sample. During the two hours after they empty their bladder, volunteers will behave normally, drink/eat but should not go to the rest room. At the beginning of the laboratory session, volunteers will empty their bladders but this time the urine will be collected in a container. Its volume will be measured and a sample will be obtained for sodium and potassium concentration measurement. Volunteers will then ingest what they are assigned to, nothing (control group), 1L water (water group), 1L water and lie down (water+lie down group), 1L 0.9% NaCl (Normal saline group) and 20mg tablet of Lasix (Lasix group). Following ingestion, urine will be collected at 30-minute-intervals and its volume will be measured and a sample will be collected for sodium and potassium concentration measurement.

#### **1.4. Measurements and calculations**

- **Urine volume** is measured by placing the urine sample in the calibrated cylinder and determining its volume. Record the volume obtained following each measurement in the table provided in the laboratory data collection sheet section.
- **Urine flow rate** is determined by dividing the volume of urine measured over time, using the following equation;

Urine flow rate 
$$(ml/min) = \frac{Urine \ volume \ (ml)}{Time \ (min)}$$

- Urine osmolality is measured using the osmometer.
- **Total urine sodium (potassium) excretion (mmol)** is calculated by multiplying urine sodium concentration by urine volume using the following equation;

 $Total urine \ sodium \ excretion \ (mmol) = \frac{Urine \ sodium \ concentration \ X \ urine \ volume}{1000}$ 

 Urine sodium (potassium) excretion rate (mmol/min) is calculated by multiplying urine sodium concentration by urine flow rate using the following equation;

Sodium excretion rate ( $\mu$ mol/min) =  $\frac{Urine \ sodium \ concentration \ X \ urine \ volume}{Time}$ 

#### 1.5. Data analysis

- 1. For each subject, record the results obtained in the tables provided in the laboratory data collection sheet section.
- 2. Draw a graph depicting the changes in each of the measured/calculated parameters over the period of the experiment. An example is provided in Fig-37.
  - a. Urine flow rate over time.
  - b. Urine osmolality over time.
  - c. Total urine sodium excretion over time.
  - d. Urine sodium excretion rate over time.
- 3. Compare between the results obtained from the different groups.
- 4. Explain the mechanisms underlying the differences seen between the groups.
- 5. Please have your report ready for the subsequent session (i.e. diuresis-II).

## **1.6.** Laboratory data collection sheet:

### 1.6.1. Group A

Name:			-
Age:			_
Gender:			_
Group:	Control	Water only	Water + lying down
	Saline	□ Lasix	

Sample No.	1	2	3	4	5	6
Time	10:00am	10:30am	11:00am	11:30am	12:00pm	12.30pm
(depending on lab	(1:00pm)	(1:30pm)	(2:00pm)	(2:30pm)	(3:00pm)	(3:30pm)
session)	100 min	20 min	20 min	20 min	20 min	
Duration	120 min	30 min	30 min	30 min	30 min	30 min
Urine volume						
(ml)						
Urine flow rate						
(ml/min)						
Urine Na <sup>+</sup>						
concentration						
(mmol/L)						
Rate of urinary Na <sup>+</sup>						
excretion						
(µmol/min)						
Total urinary						
Na⁺excretion						
(mmol)						
Urine K <sup>+</sup>						
concentration						
(mmol/L)						
Rate of urinary K <sup>+</sup>						
excretion						
(µmol/min)						
Osmolality						
(mOsmol/L)						
Urine pH						

#### 1.6.2. Group B

Name: \_\_\_\_\_

Age: \_\_\_\_\_ Gender: \_\_\_\_\_

Group: 
Control 
Water only
Group:
Gr

Comple No			2	4	E	6
	1	2	3	4	5	0
Time	10:00am	10:30am	11:00am	11:30am	12:00pm	12.30pm
(depending on lab	(1:00pm)	(1:30pm)	(2:00pm)	(2:30pm)	(3:00pm)	(3:30pm)
session)						
Duration	120 min	30 min	30 min	30 min	30 min	30 min
Urine volume						
(ml)						
Urine flow rate						
(ml/min)						
Urine Na <sup>+</sup>						
concentration						
(mmol/L)						
Rate of urinary Na <sup>+</sup>						
excretion						
(µmol/min)						
Total urinary						
Na⁺excretion						
(mmol)						
Urine K <sup>+</sup>						
concentration						
(mmol/L)						
Rate of urinary K <sup>+</sup>						
excretion						
(µmol/min)						
Osmolality						
(mOsmol/L)						
Urine pH						

#### 1.6.3. Group C

Name:\_\_\_\_\_

Age:\_\_\_\_\_

Gender:\_\_\_\_\_

Group: 
Control 
Water only
Saline Lasix

Sample No.	1	2	3	4	5	6
Time	10:00am	10:30am	11:00am	11:30am	12:00pm	12.30pm
(depending on lab	(1:00pm)	(1:30pm)	(2:00pm)	(2:30pm)	(3:00pm)	(3:30pm)
session)	100 min		20 min	20 min	20 min	20 min
Duration	120 min	30 min	30 min	30 min	30 min	30 min
Urine volume						
(ml)						
Urine flow rate						
(ml/min)						
Urine Na <sup>+</sup>						
concentration						
(mmol/L)						
Rate of urinary Na <sup>+</sup>						
excretion						
(µmol/min)						
Total urinary						
Na⁺excretion						
(mmol)						
Urine K <sup>+</sup>						
concentration						
(mmol/L)						
Rate of urinary K <sup>+</sup>						
excretion						
(µmol/min)						
Osmolality						
(mOsmol/L)						
Urine pH						

#### 1.6.4. Group D

Name:\_\_\_\_\_

Age:\_\_\_\_\_

Gender:\_\_\_\_\_

Group: 
Control 
Water only
Saline
Kater only
Group:
Group

Sample No.	1	2	3	4	5	6
Time	10:00am	10:30am	11:00am	11:30am	12:00pm	12.30pm
(depending on lab	(1:00pm)	(1:30pm)	(2:00pm)	(2:30pm)	(3:00pm)	(3:30pm)
session)	120 min	20 min				
	120 11111	30 11111	30 11111	30 11111	30 11111	30 11111
(mi)						
Urine flow rate						
(ml/min)						
Urine Na <sup>+</sup>						
concentration						
(mmol/L)						
Rate of urinary Na <sup>+</sup>						
excretion						
(µmol/min)						
Total urinary						
Na⁺excretion						
(mmol)						
Urine K <sup>+</sup>						
concentration						
(mmol/L)						
Rate of urinary $K^+$						
excretion						
(µmol/min)						
Osmolality						
(mOsmol/L)						
Urine pH						

#### 1.6.5. Group E

Name:\_\_\_\_\_

Age:\_\_\_\_\_

Gender:\_\_\_\_\_

Group:

□ Control □ Water only □ Saline □ Lasix □ Water + lying down

Sample No. 1 2 3 4 5 6 10:00am 11:00am 11:30am 12:00pm 12.30pm Time 10:30am (depending on lab (2:00pm) (1:00pm) (2:30pm) (3:00pm) (3:30pm) (1:30pm) session) Duration 120 min 30 min 30 min 30 min 30 min 30 min Urine volume (ml) Urine flow rate (ml/min) Urine Na<sup>+</sup> concentration (mmol/L) Rate of urinary Na<sup>+</sup> excretion (µmol/min) Total urinary Na<sup>+</sup>excretion (mmol) Urine K<sup>+</sup> concentration (mmol/L) Rate of urinary K<sup>+</sup> excretion (µmol/min) Osmolality (mOsmol/L) Urine pH

#### 1.7. Example

Provided below is a sample analysis of data obtained from a previously performed experiment. Students are expected to submit their data analysis in a similar format.

The data below was obtained from a subject who drank one liter of 0.9% NaCl (normal saline) and underwent the same experimental protocol shown in Fig-36.

Sample no	1	2	3	4	5	6
Time	10:00	10:30	11:00	11:30	12:00	12:30
Duration (min)	120	30	30	30	30	30
Volume (ml)	125	39	50	42	47	32
Urine flow rate (ml/min)	1.04	1.3	1.67	1.4	1.57	1.067
Urine [Na⁺] (mmol/L)	101	98	112	109	120	137
Urinary Na <sup>+</sup> excretion rate (µmol/min)	105.21	127.4	186.7	152.6	188	146.1
Total urinary sodium excretion (mmol)	12.63	3.82	5.6	4.58	5.64	4.38



Figure 37. Shows changes in urine flow rate and sodium excretion in response to ingestion of 1L of normal saline. (A) Changes in urine flow rate over the period of the experiment. (B) Changes in total sodium excretion (mmol) over the period of the experiment. (C) Changes in urinary sodium excretion rate ( $\mu$ mol/ml) over the period of the experiment. (D) Changes in total sodium excretion (mmol) over the period of the experiment. (D) Changes in total sodium excretion (mmol) over the period of the experiment. Samples were obtained at 30-minute-intervals, starting at 10:00am which represents the baseline sample before ingesting saline while the samples that follow are considered post-experimental samples.

### **1.8. Practice questions**

 The table below shows the changes in urine volume and urine sodium concentration in a subject that ingested 1L of water and underwent the same experimental protocol shown in Fig-36.

Sample no	1	2	3	4	5
Time	10:00	10:30	11:00	11:30	12:00
Duration (min)	120	30	30	30	30
Urine volume (ml)	50	10	145	217	250
Urinary sodium	211	168	29	12	14
concentration (mmol/L)					
Urine flow rate (ml/min)					
Urinary sodium excretion					
rate (µmol/min)					
Total urinary sodium					
excretion (mmol)					

Looking at the table above;

- a. Calculate the urine flow rate, total sodium excretion and urinary sodium excretion rate at each time point and record your answers on the table above.
- b. Draw a graph depicting the changes in each of the three parameters over the period of the experiment.
- c. Compare between the results obtained from this subject and those shown in Fig-37 for the subject that ingested 1L of normal saline.
- d. Explain your observations.

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