Department of Physiology
College of Medicine

Physiology Practical

For

1st Year Medical Students

(2018-2019)

Contents

List of Abbreviations

CBC	Complete blood count
ESR	Erythrocyte sedimentation
fl	Femtoliter
Hb	Hemoglobin
Hct	Hematocrit
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
PCV	Packed cell volume
pg	Picogram
RBC	Red blood cell
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 1st 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 1st 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 1st year medical curriculum.

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

Faculty and Staff Members Involved in Physiology Practical Teaching

Male members	Contact details	Female members	Contact details
Dr. Taj	Ahalepota@ksu.edu.sa	Dr. Ola Hilmi	omawlana@ksu.edu.sa
Dr. Mustafa	mkmemon@gmail.com	Dr. Reem Al-Twairgi	raltaweraqi@ksu.edu.sa
Dr. Yahya		Mrs. Sulafa Al-Thubaiti	Snaak-2009@hotmail.com
Mr. Jarouni		Mrs. Shurooq Al-Saidi	snaalsuod@gmail.com
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.

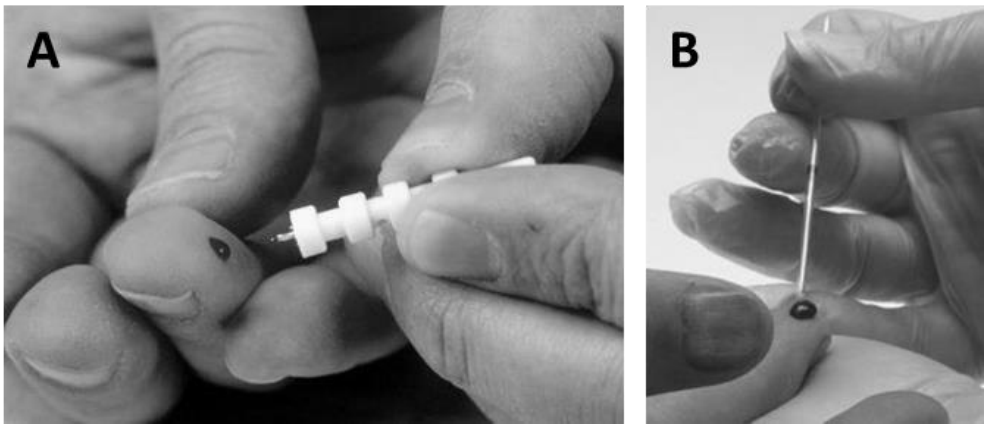

Figure 1. Procedure of drawing blood into capillary tubes. (A) The tip of a finger is pricked using a lancet. (B) A heparinized capillary tube is gently brought in contact with the blood drop forming on the tip of the finger.

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.
8. 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.

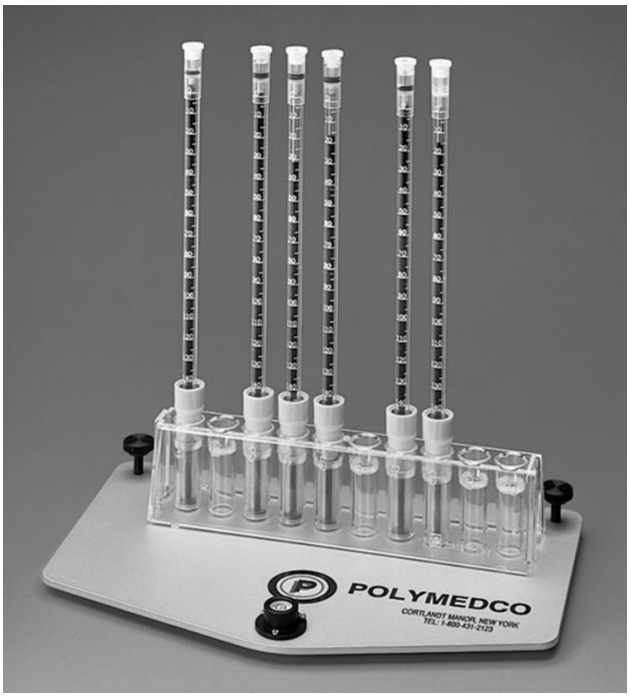

Figure 2. Westergren's tubes and apparatus.

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.

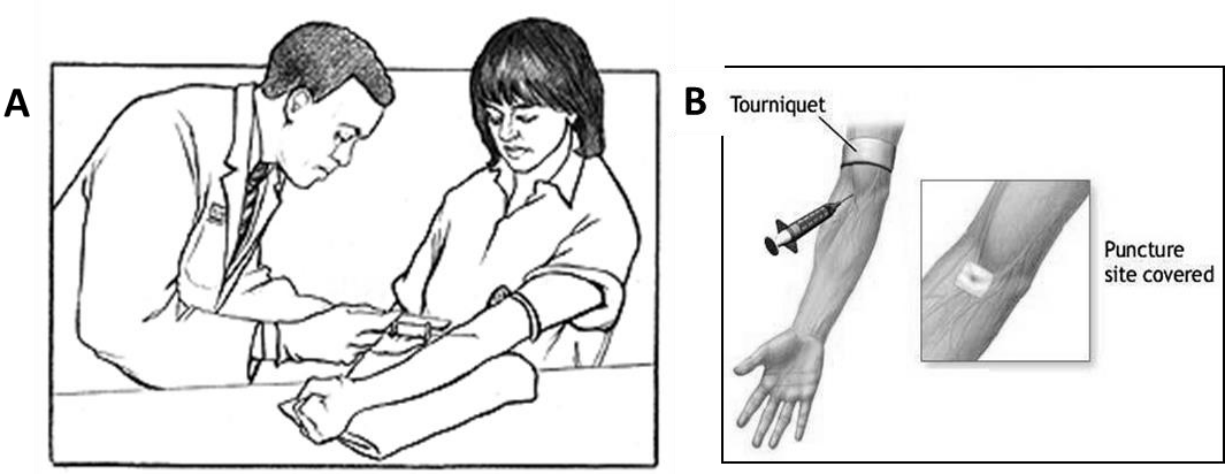

Figure 3. Drawing blood from a superficial vein in the antecubital fossa.

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 anti-coagulated tube to prevent blood from clotting.

5. 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 10^6 / mm^3	2.5 X 10^6 / mm^3
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 45º 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.

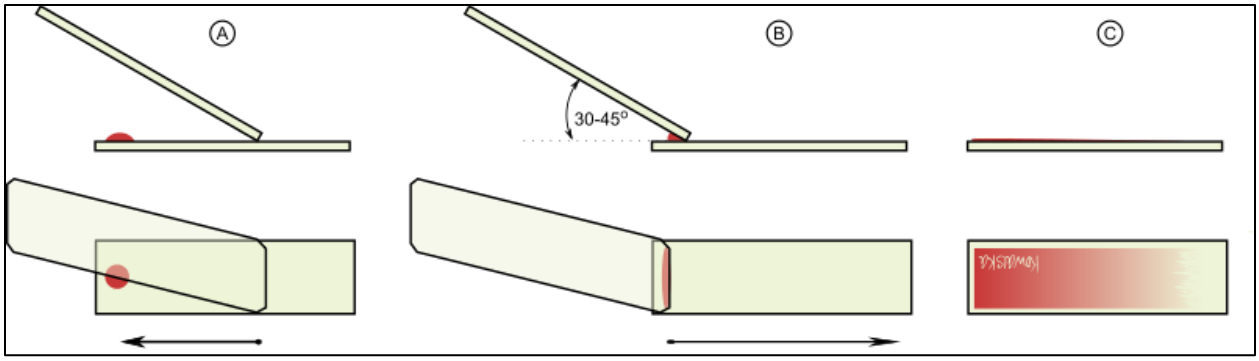

Figure 4. Steps to doing a thin blood film. (A) a drop of anticoagulated blood is placed near the edge of a microscope slide. The edge of a second slide is then placed at an angle of 45º over the 1st slide. (B) The edge of the second slide is slowly brought towards the blood drop. Once it touched the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

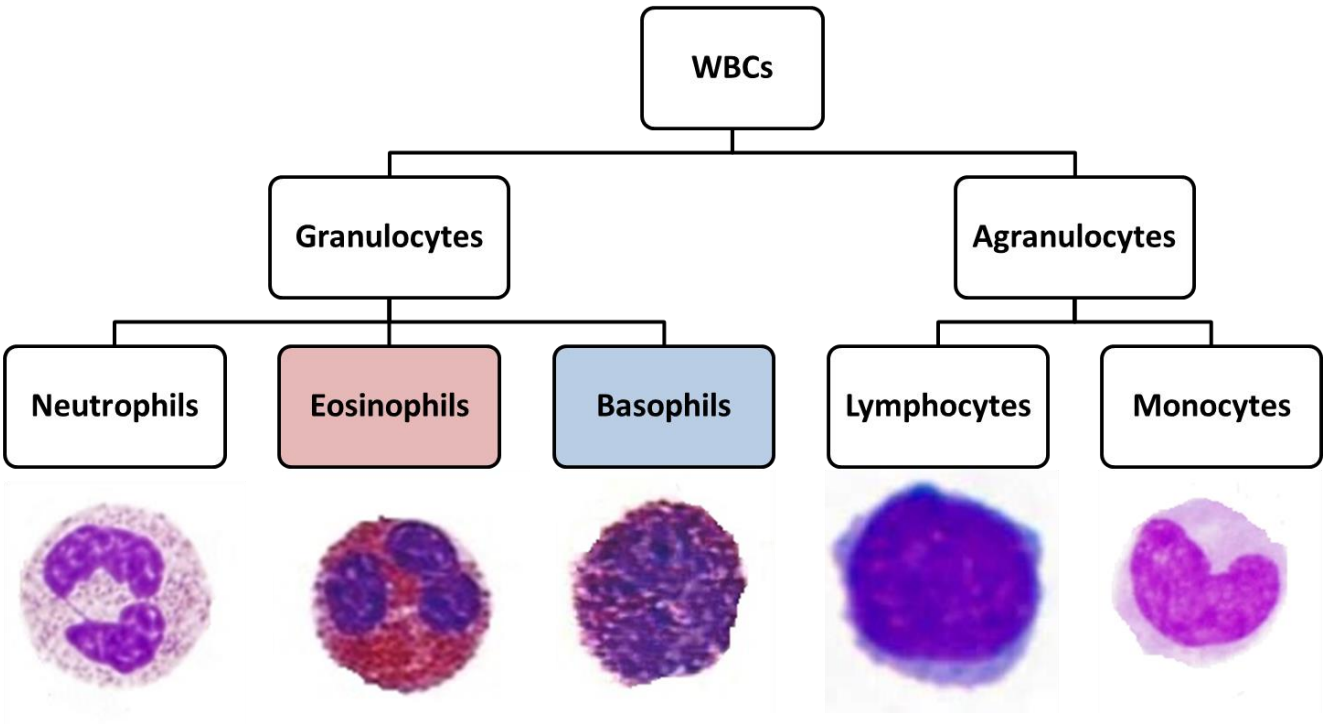

Figure 5. Schematic representation of the types of white blood cells that can be seen in a peripheral blood film with a representative image of each WBC subset.

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					
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.

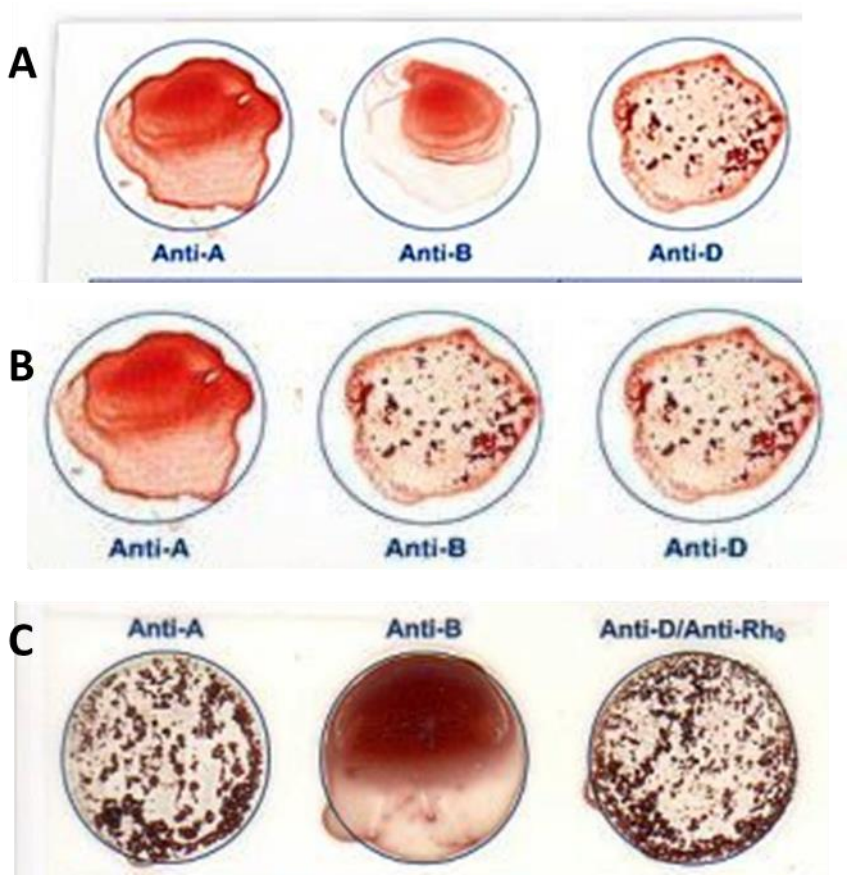

Figure 6. Blood group determination. (A) O^+ blood sample, (B) B^+ blood sample and (C) A^+ blood sample.

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		
B		
AB		
O		

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

Blood Group	Can give blood to	Can receive blood from
AB^+		
AB^-		
A^+		
A^-		
B^+		
B^-		
O^+		
O^-		

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
O+	
A+	
B+	
AB+	
O-	
A-	
B-	
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?**

_____

_____

_____

_____

_____

3.3. Determination of clotting time

3.3.1. Equipment

- Capillary tubes.
- Petri-dish.
- Alcohol swabs.
- Lancets.
- Plasticine.
- Water bath set at 37ºC.
- 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.
7. 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. **An abnormality in which blood cell may prolong the bleeding time?**

   _____

3. **Name one condition in which bleeding time is prolonged (increased)?**

   _____