BLOOD

PRACTICAL CLASSES IN BLOOD

BLOOD PRACTICAL CLASS – 1

Determination of red blood cell (RBC) and total white blood cell (WBC) counts, platelet or thrombocyte count, haemoglobin concentration. Haematocrit value or packed cell volume, red blood cell indices i.e., mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), granulocyte number and percentage, lymphocyte number and percentage and monocyte number and percentage. To determine osmotic fragility of red blood cells.

OBJECTIVES

To be familiar with: -

1. The principles of methods used to measure the different haematological values.
2. The normal values recorded when making these measurements.
3. The red blood cell indices i.e., the mean corpuscular volume (MCV), the mean corpuscular haemoglobin (MCH), the mean corpuscular haemoglobin concentration (MCHC), how these values can be calculated? Their normal values and their importance in diagnosis of different types of anaemias.
4. The procedures used for taking both capillary and venous blood and for every student to have some practical experience of collection of blood.
5. To know:
   a) How to measure the osmotic fragility of red blood cell.
   b) What is the clinical importance of osmotic fragility test.

INSTRUCTION TO STUDENTS: -

The Coulter MD II Series Analyzer is a delicate piece of clinical and research equipment. As this is the first time that you have attended the practical class in the department, you are asked to leave the responsibility of counting your samples to demonstrators and technical staff.

A. REAGENTS AND APPARATUS:

1. Coulter Micro Differential II Series Analyzer
2. EDTA anticoagulated blood
3. Coulter Micro-Pak (reagent I – diluent and reagent II – lytic reagent)
4. Coulter clenz concentrate (cleaning agent)
5. Coulter S-cal (calibrator kit)

B. COULTER MD II SERIES ANALYZER:

GENERAL PRINCIPLE:

The Coulter method accurately counts and measures the sizes of cells by detecting and measuring changes in electrical resistance when a particle (such as a 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 particle.

C. COULTER MICRO-PAK:

a. Diluent – Reagent I is an isotonic electrolyte solution that:
   - dilutes the whole blood samples
   - stabilizes cell membranes for accurate counting and sizing
   - conducts aperture current
   - rinses instrument components between analysis
   - prevents duplicate cell counts by using the sweep flow process

b. Lytic reagent – Reagent II is a lytic reagent that:
   - lysis red blood cells (RBCs) for WBC count and haemoglobin measurement

D. CONCENTRATION CLEANER:

Coulter clenz concentrate (cleaning agent) prevents protein buildup that occurs in and around apertures.

E. CALIBRATOR:

Coulter S-cal calibrator kit is an acceptable alternative to the whole blood reference method of calibrating.

THE CALCULATED RED BLOOD CELL INDICES

1. Mean cell volume (MCV)

This is the volume of an average red blood cell measured in cubic microns.

$$MCV = \frac{\text{Packed cell volume} \times 10}{\text{Red blood cell count}}$$

Where:- Red blood cell count is measured in millions/cubic millimeter. Could you have calculated the MCV without being given the formula?

MCV of a normal subject = $85 \pm 8\ \mu^3$ (or μm^3)

2. Mean cell haemoglobin (MCH)

This is the weight of haemoglobin in an average red blood cell measured in picograms (pg) = micro-micrograms (μμg).

$$MCH = \frac{\text{Haemoglobin concentration} \times 10}{\text{Red blood cell count}}$$

Where:- Haemoglobin concentration is measured in g/dl. Red blood cell count is measured in millions/cubic millimetre.

MCH of a normal subject = 29.5 ± 2.5 pg

Thus, if a red blood cell is small, the MCH is reduced (e.g. in iron deficiency anaemia). However, if the red blood cell is large, the MCH may be raised (e.g. in megaloblastic anaemia).

3. Mean cell haemoglobin concentration (MCHC)

This is the concentration of haemoglobin per 100 ml of red blood cells.

$$MCHC = \frac{\text{Haemoglobin concentration} \times 100}{\text{Packed cell volume}}$$

Where:- Haemoglobin concentration is measured in g/dl.

MCHC of a normal subject = 33 ± 3 g/dl

Values of MCHC below 30% indicate iron deficiency anaemia.

RESULTS RBC count =

WBC count =

Haemoglobin concentration =

PCV =

I. Calculate the following red blood cell indices and compare your results with the results provided:

i. MCV

ii. MCH

iii. MCHC

I. What is the clinical importance of knowing the red blood cell indices?

QUESTIONS AND PROBLEMS:

1. Anaemia is usually classified as being due to:-
   (a) blood loss
   (b) impaired red blood cell formation
   (c) increased red blood cell destruction

Briefly discuss how these different types of anaemia may be produced.

2. An examination of the blood of 2 adult males (A and B) provided the following data:-

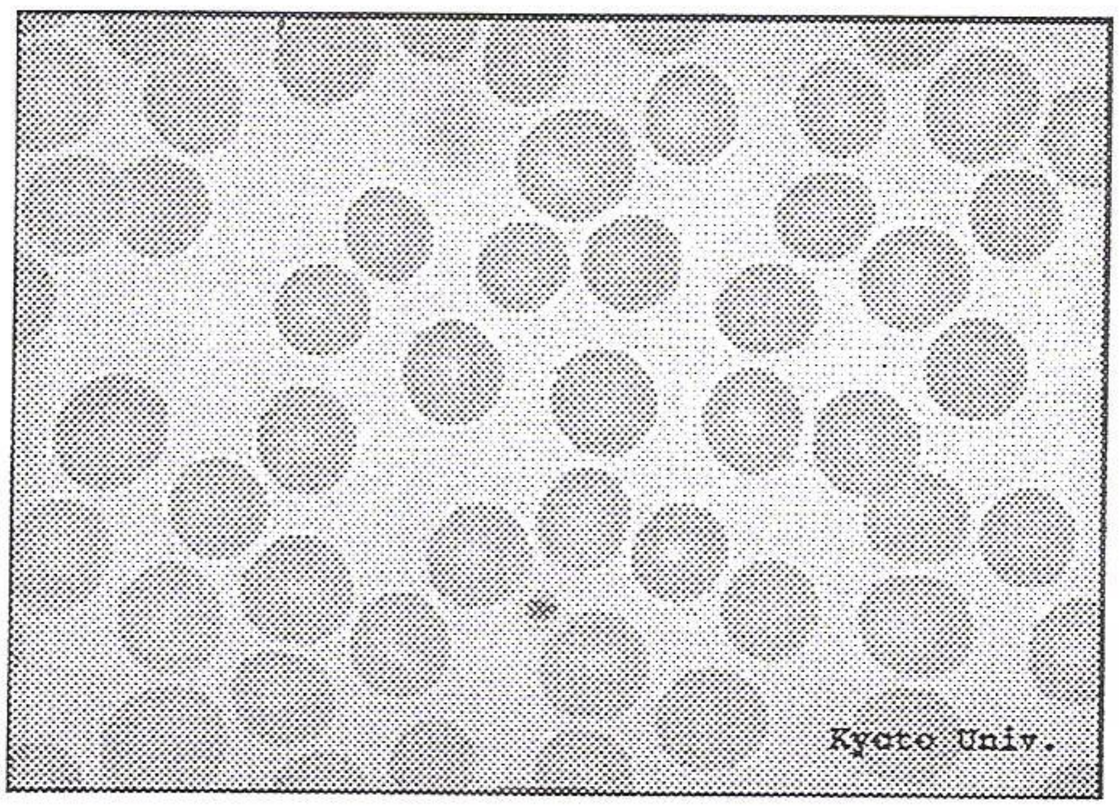
	Subject A	Subject B
RBC count	3.6×10^6 / mm^3	3.0×10^6 / mm^3
Hb concentration	7.0 g / 100 ml	8.2 g / 100 ml
PCV	25%	29%

(a) Calculate MCV, MCH and MCHC for each of these subjects.

(b) What are the abnormalities encountered in these men. What are the possible causes of these abnormalities?

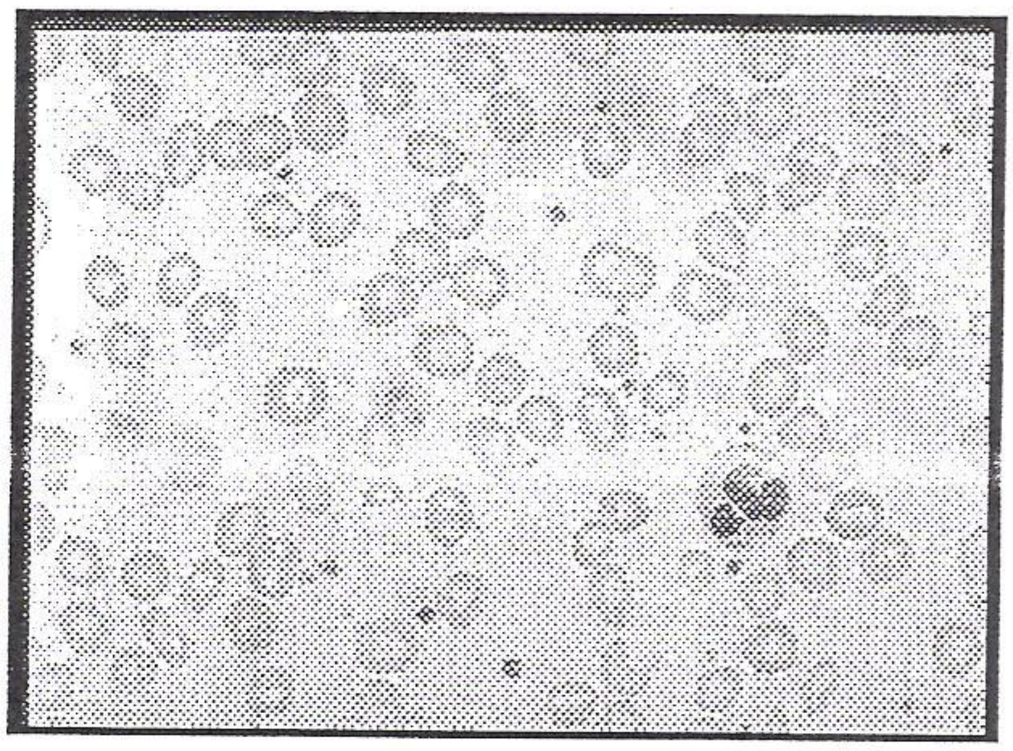
Photomicrographs of blood films

A healthy adult



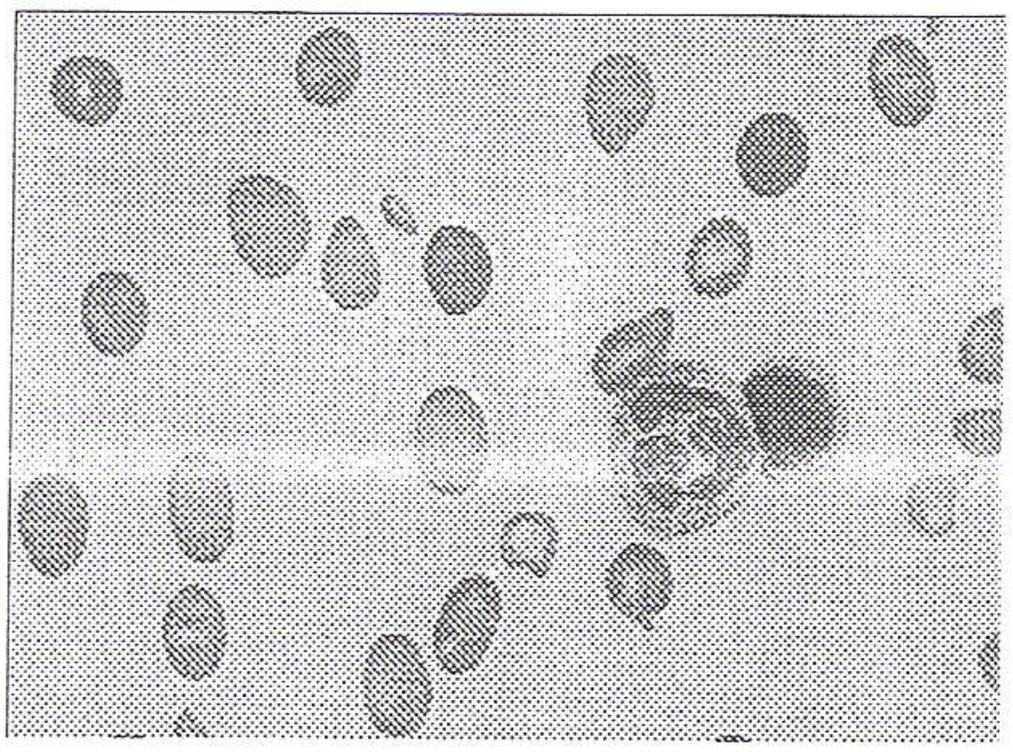
Iron deficiency anaemia

Shows a marked hypochromasia, Microcytosis and anisocytosis, and a few poikilocytes and cell fragments



Pernicious anaemia

Shows macrocytes, poikilocytes and cell fragments (schistocytes) and extreme anisocytosis



PRACTICAL CLASSES IN BLOOD

BLOOD PRACTICAL CLASS 2 - The differential leucocytes count.

OBJECTIVES

1. To be able to identify the different types of leucocytes under the microscope using theoretical knowledge of the histological characteristics of these cells.
2. To practice the procedure for differential leucocyte counting.
3. To know the normal values expected for the differential white cell count.
4. To understand the use of the differential white cell count in the diagnosis of disease processes.

A. Reagents and apparatus

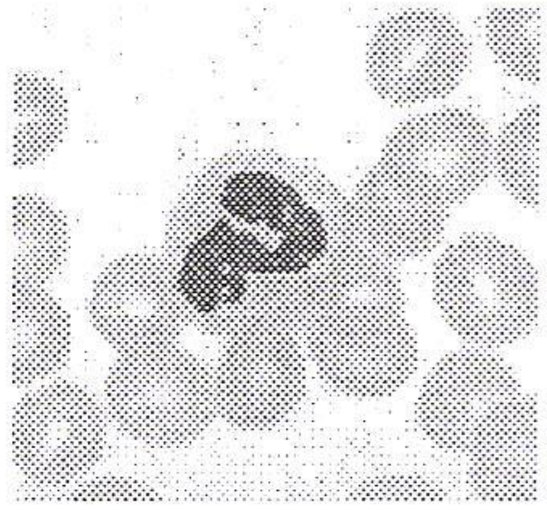
1. A microscope with an oil immersion objective
2. Mineral or cedar wood oil
3. Various dyes for staining blood films (e.g., Wright's stain)
4. Microscope slides

B. Procedure

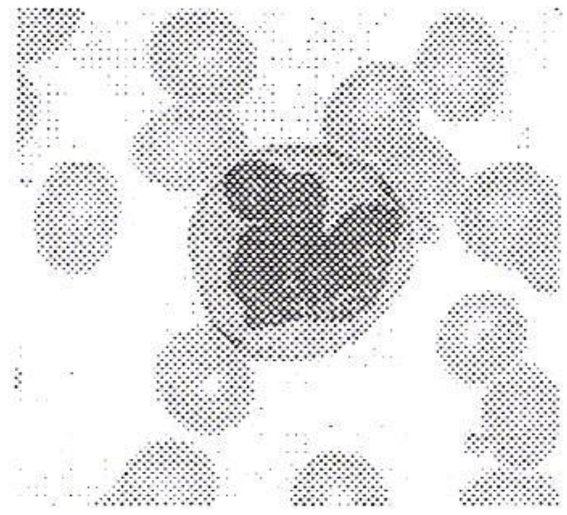
Examine the stained blood film under the oil immersion objective and identify the different leucocytes. Make coloured diagrams of these cells (on next page). Look at least 100 leucocytes and record your findings in the table below. Express your results as the percentage of the total white blood cells identified.

Type	Observed	Total	Percentage
Neutrophil			
Eosinophil			
Basophil			
Monocyte			
Lymphocyte			

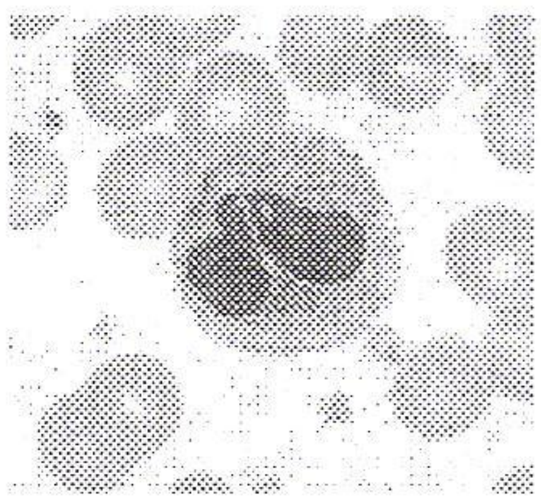
White blood cells



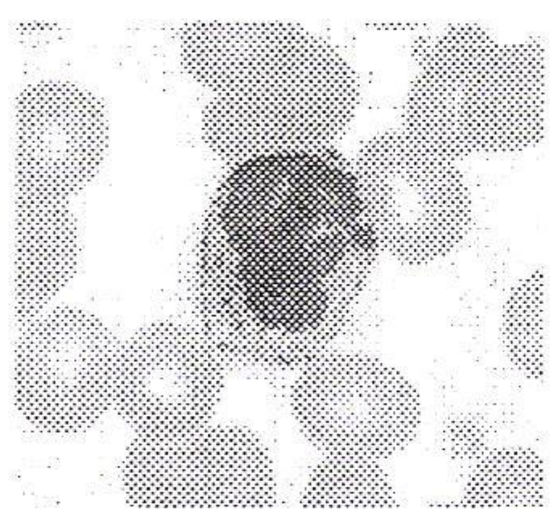
Neutrophil



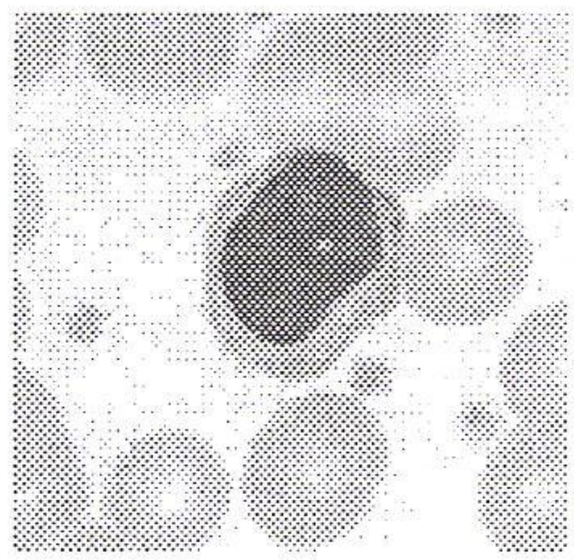
Monocyte



Eosinophil



Basophil



Lymphocyte

QUESTIONS AND PROBLEMS

1. Are the percentage of different leucocytes calculated in your experiment those you would expect in a normal subject?

2. Under what conditions are the percentage of the various types of granulocyte increased?

3. What is the significance of an increased lymphocyte count?

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

5. Show, with coloured diagrams, the characteristics of different leucocytes.

PRACTICAL CLASSES IN BLOOD

BLOOD PRACTICAL CLASS 3 - To determine blood groups, the coagulation/clotting time, the bleeding time, and the erythrocyte sedimentation rate (E.S.R.)

OBJECTIVES

1. To understand and practice the method used in determining blood groups according to the ABO and Rhesus (Rh) systems.
2. To be familiar with the ABO and Rh systems and be able to explain their importance in blood transfusion.
3. To know the normal range of values expected for the bleeding and clotting time and to have determined your own values experimentally.
4. To recognize the importance of bleeding time and clotting time in haemostasis.
5. To know (a) how to measure both the osmotic fragility of blood and the erythrocyte sedimentation rate and (b) what is the value of taking these measurements.

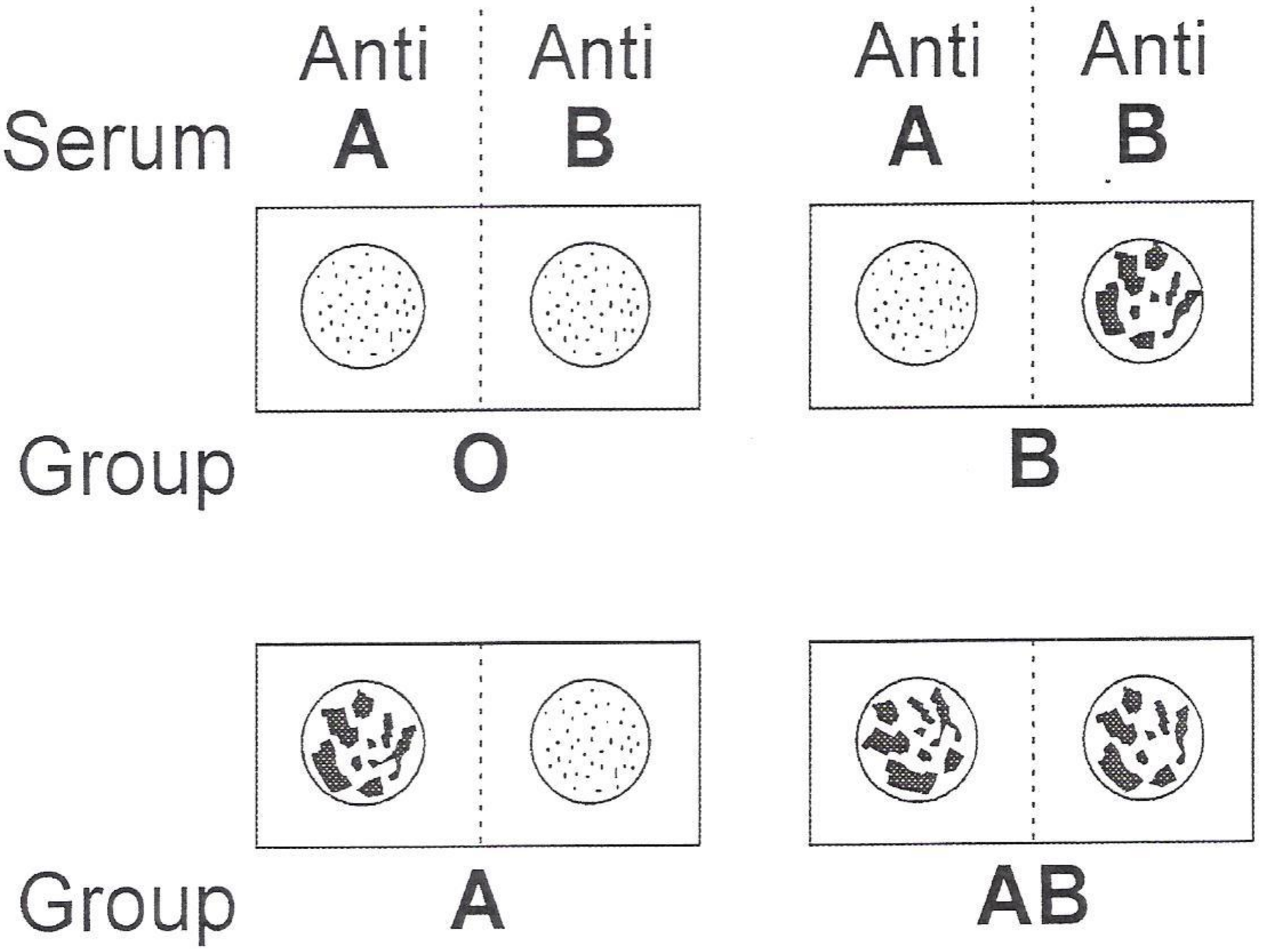
EXPERIMENT 1 – DETERMINATION OF BLOOD GROUPS

A. Reagents and apparatus

1. High titer anti-A, anti-B and anti-D sera
2. A microscope
3. A grease pencil
4. Microscope slides

B. Procedure

(a) Prick a finger and place one drop of blood in each of the compartments A, B and D. These are clearly labelled on the microscope slides provided.
(b) Quickly add a drop of anti-A, anti-B and anti-D sera to compartments A, B and D respectively.
(c) Mix the serum with the drop of blood by moving the slides gently to and fro for a minute or two. Now examine the mixtures for signs of red blood cell agglutination. When red blood cells clump together they have a speckled or peppered appearance. If you are not sure, examine the slide using the low power of a microscope.
N.B. Even the most experienced haematologist sometimes comes across a blood sample where there is doubt.



Slides showing the agglutination of blood with anti-A and anti-B sera

QUESTIONS AND PROBLEMS

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

Group	Agglutinogen	Agglutinin
A		
B		
AB		
O		

2. What is your blood group?

3. For which groups can you act as:-
   (a) a donor?

   (b) a recipient?

4. What other groups (factors) do you have to keep in mind as well as the classical ABO and Rh groups?

5. What is the distribution of the ABO and Rh blood groups in Saudi Arabia?

6. How does this distribution differ from that found in other parts of the world?

7. What is haemolytic disease of the newborn?

   i. How is it treated?

   ii. How is it prevented?

EXPERIMENT 2 – DETERMINATION OF THE COAGULATION/CLOTTING TIME

A. Reagents and apparatus

1. A dozen capillary tubes of uniform size
2. A petri-dish
3. Alcohol swabs
4. Cotton wool
5. Plasticine
6. A water bath set at 37°C

B. Procedure

(a) Prick a finger of the subject observing the usual precautions and note the time that the prick is made.
(b) Wipe away the first drop of blood. Then while the blood is still flowing freely place one end of a capillary tube in the blood. Holding the tube horizontally let it fill by capillary action.
(c) Let an assistant close the end of the capillary tube with plasticine. Place the tube in the water bath.
(d) Repeat the process with all the tubes.
(e) Two minutes after making the puncture, break a capillary tube and separate the two halves slowly.
(f) Repeat the procedure at 30 second intervals with the remaining tubes.
(g) When the blood forms a continuous "wick-like" clot between the broken ends of the tube, the end-point has been reached. Note the time. The time from pricking the finger to the appearance of the clot is the clotting time.

Usually the clotting time measured by this method is in the range 3-6 minutes. It is important to set the temperature at that of the body and to keep it constant. As with all enzymic reactions, the rate is temperature dependent.

QUESTIONS AND PROBLEMS

1. What is your clotting time?

2. What are the clinical conditions in which the clotting time is greater than normal?

3. Name the substances which are used as anticoagulants.

4. What is the clinical significance of the clotting time?

5. What is the source of heparin in the body?

6. Produce a diagram to show the reactions that take place during the process of coagulation.

EXPERIMENT 3 – DETERMINATION OF THE BLEEDING TIME

A. Reagents and apparatus

1. Blotting paper
2. A stop-watch
3. A stylette to prick an ear lobe
4. Alcohol swabs

B. Procedure

(a) Clean the lobe of the ear or tip of the finger with an alcohol swab.
(b) When it is dry, make a single puncture with a stylette (about 3mm deep). Note the time at which the puncture is made.
N.B. The skin of the ear or finger should not be touched once the puncture has been made until the experiment is over.
(c) Apply a piece of filter paper to the blood-drop every 30 seconds until the bleeding stops.
N.B. The bleeding time estimated by this method of a normal subject is within the range of 2-5 minutes.

QUESTIONS AND PROBLEMS

1. What is the bleeding time you have estimated?

   _____ minutes

   Is your result comparable with normal values?

   If it is not, what are the most likely reasons?

The standardized template method

In clinical practice bleeding time is measured by using a much more accurate technique.

1. A sphygmomanometer cuff is applied to the subject's arm and inflated to 40mmHg.
2. The volar surface is cleaned with 70% alcohol.
3. A sterile metal template with a linear slit (11mm long) is pressed firmly against the skin.
4. A scalpel blade, with a guard, is carefully introduced so that it protrudes 1mm through the template slit. An incision, 1mm deep and 9mm long can then be made.
5. Blood is gently, but completely removed with filter paper at 15 second intervals until the bleeding stops. Normal bleeding times determined with this method are in the range 2.5-9.5 minutes.

QUESTIONS AND PROBLEMS

1. What are the advantages of the template method?

2. Write in brief about the conditions in which the bleeding time is prolonged.

EXPERIMENT 4 – THE ERYTHROCYTE SEDIMENTATION RATE – E.S.R.

A. Reagents and apparatus

1. Westergren's sedimentation apparatus
2. Anticoagulant (EDTA)
3. Disposable sterile syringes and needles

B. Procedure

(a) Using a sterile syringe remove 1.6ml of blood from a suitable vein. Transfer it to a test tube containing EDTA and then draw up blood into a Westergren tube exactly to the zero mark.
(b) Place the tube upright in the stand and leave undisturbed. The height of the column of clear plasma at the top of the tube is noted at the end of an hour and again at the end of 2 hours.

C. Results

E.S.R. after 1 hour = mm (Normal = 3-5 mm – male)

E.S.R. after 2 hours = mm (Normal = 7-15 mm – male)

N.B. In females the values are slightly higher.

QUESTIONS AND PROBLEMS

1. What is meant by rouleaux formation?

2. Why does rapid rouleaux formation increase the E.S.R.?

3. What is the clinical significance of the E.S.R. test?

4. What conditions are associated with an increased E.S.R.? Why is an increase observed?

The Westergren apparatus for the determination of the ESR