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physio, practical



# BLOOD

# PSL 131 – PRACTICAL SCHEDULE

## INTRODUCTION

Each week two or three hours are made available for students to attend "practical classes". Many years ago a "practical class" was a session in which every student took part in an experiment, collected their own data and then wrote a report. In these reports, students were expected to show that they had understood the aims of the experiments.

In medical schools today "practical classes" are used in several different ways. This change has been necessary because of the greatly increased numbers of students who are studying Medicine and the development of sophisticated and expensive pieces of equipment which are currently used to obtain data. As a result some experiments: -

- (a) remain in which all can take part.
- (b) have been replaced by demonstrations where volunteers may be requested but in which other students are obliged to remain as observers. Do not hesitate, if you attend such sessions, to volunteer to be the subject of an experiment. You will not be asked to do anything that is either painful or harmful. However, perhaps surprisingly, you will find that you can understand physiology more easily if you take part in experiments.
- (c) are presented as films or videotapes.
- (d) which have never been performed in student laboratories can be introduced. In these classes, called data handling sessions, important experiments can be described and discussed. Some of these experiments may have been too long and/or unsuitable for a student laboratory.

The aims of the "practical classes" whether years ago or today remain the same. They provide valuable support for the lecture programmes you are attending. Every attempt is made to organize the "practical classes" so that they closely follow the theoretical part of your studies. In this, your first year in Physiology, you will be studying blood, nerve and muscle, the autonomic nervous system, cardiovascular, respiratory physiology, endocrinology and the functions of the gastrointestinal tract and kidney. The "practical classes" are intended to provide time so that some of the information obtained in lectures can be put in another way. Thus, for example, nerve impulses and erythrocytes are not simply learned about in lectures but can be observed and their characteristics experimentally determined.

Before you attend "practical classes" you will find it useful to read through the notes in this schedule about the particular week's work. The "practical class" will be much easier to understand and enjoyable if you do. You will know what to expect. As far as the "practical classes" themselves are concerned, make sure that you attend promptly. Usually an introductory talk, explaining what is to be attempted is given at the start of each session. Thus, if you are late you will miss this important part and find the practical difficult to follow.

As you read through this practical schedule you will find that: -

- (1) the aims (or objectives) of the practical are clearly defined. Know what these are and satisfy yourselves that they have been met.
- (2) the methods used for individual experiments have been written up for you. There should be no need to add further information unless, for example, you are particularly interested in some aspect of the equipment used.
- (3) spaces have been left for you to record the data obtained in the "practical classes" and to discuss the important findings. The "practical classes" are not examinations. Therefore, discuss what you have done in them with your colleagues. If, having done so, there are no problems to which you cannot find an answer, do not hesitate to go and ask a member of staff for advice. All the teaching staff are approachable and, if not immediately available, will arrange a time at which they could talk to you.
- (4) questions and/or problems have been set for each "practical class". Make an attempt to answer them. They are intended to make you apply the knowledge you have gained and not simply be able to regurgitate facts learned in lectures and textbooks.

You will find that answering the questions and problems is very useful. If you are successful:

- (A) you can be satisfied that you have understood the practical and should have nothing to worry about in subsequent practical examinations or in quizzes regularly given in your course.
- (B) you will be improving your capacity to apply your knowledge. Some students find this skill difficult. Nevertheless it is a vital one to develop because in your chosen career you are frequently faced with problems with answers that are not readily available in books.

## **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 clenx 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 clenx 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.

## F. SAMPLE PREPARATION, COUNTING:

The probe moves across and down into aspirate position. The aspiration syringe draws 12 $\mu$ l of whole blood into the probe. The Coulter MD II Series Analyzer makes the necessary dilutions with the reagents automatically for measuring the different haematological values. Some of the values are derived and computed by the computer of the machine.

Finally, all the haematological values are reported and printed.

A sample of result sheet printed by the analyzer is given. Write the different haematological values obtained. Their normal range is given for reference.

Values:

|     | Students'<br>Result               | Normal<br>Range |                             | Students'<br>Result | Normal<br>Range   |
|-----|-----------------------------------|-----------------|-----------------------------|---------------------|---|
| WBC | _____ x 10 <sup>3</sup> / $\mu$ L | 4.0-10.8        | x 10 <sup>3</sup> / $\mu$ L | Hct                 | _____ % 42.0-52.0 %   |
| LY% | _____ %                           | 20.5-51.1       | %                           | MCV                 | _____ fL 80.0-100.0 fL  |
| MO% | _____ %                           | 1.7-9.3         | %                           | MCH                 | _____ pg 27.0-31.0 pg   |
| GR% | _____ %                           | 42.2-75.2       | %                           | MCHC                | _____ g/dl 32.0-36.0 g/dl   |
| LYE | _____ x 10 <sup>3</sup> / $\mu$ L | 0.7-4.9         | x 10 <sup>3</sup> / $\mu$ L | RDW                 | _____ % 11.6-13.7 %   |
| MOE | _____ x 10 <sup>3</sup> / $\mu$ L | 0.1-0.9         | x 10 <sup>3</sup> / $\mu$ L | Plt                 | _____ x 10 <sup>3</sup> / $\mu$ L 130-440 x 10 <sup>3</sup> / $\mu$ L |
| GRE | _____ x 10 <sup>3</sup> / $\mu$ L | 1.5-7.2         | x 10 <sup>3</sup> / $\mu$ L | MPV                 | _____ fL 7.4-10.4 fL  |
| RBC | _____ x 10 <sup>6</sup> / $\mu$ L | 4.70-6.10       | x 10 <sup>6</sup> / $\mu$ L | Pct                 | _____ % 0.000-0.999 %   |
| HgB | _____ g/dl                        | 14.0-18.0       | g/dl                        | PDW                 | _____ 0.0-99.9  |

## 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.

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

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

$$\text{MCV of a normal subject} = 85 \pm 8 \mu^3 \text{ (or } \mu\text{m}^3\text{)}$$

### 2. Mean cell haemoglobin (MCH)

This is the weight of haemoglobin in an average red blood cell measured in picograms (pg) = micro-micrograms ( $\mu\mu\text{g}$ ).

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

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

$$\text{MCH of a normal subject} = 29.5 \pm 2.5 \text{ pg}$$

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.

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

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

$$\text{MCHC of a normal subject} = 33 \pm 3 \text{ g/dl}$$

Values of MCHC below 30% indicate iron deficiency anaemia.

## **QUESTIONS AND PROBLEMS:**

I- What you understand by the terms:

- i. polycythemia
- ii. anaemia
- iii. leucocytosis
- iv. leucopenia
- v. differential leucocyte count
- vi. thrombocytopenia
- vii. thrombocytosis

## **THE ESTIMATION OF THE HAEMATOCRIT**

The haematocrit (or packed cell volume, PCV) is the relative volume of formed elements to plasma. It is usually expressed as a percentage.

### A. Reagents and apparatus

1. Microhaematocrit centrifuge
2. Heparinized capillary tubes
3. Haematocrit reading scale

### B. Method

Capillary blood is obtained from an ear lobe or a finger tip. Only very gentle squeezing is permitted to help the flow of blood. If more pronounced pressure is exerted, blood is likely to be diluted with interstitial fluid. Allow a large drop of blood to form and then fill the heparinized capillary tube until at least  $\frac{3}{4}$  full. Seal one end of the capillary tube with a Bunsen flame or plasticine. The capillary tubes are then centrifuged for 15 minutes at 3000-4000 RPM. By this time the cells will have been "packed" at the bottom of the tube and the plasma above should be cell-free. The packed cell volume can then be determined as a percentage of the total volume using the haematocrit reader.



**RESULTS** RBC count =  $4.5 \times 10^6 / \mu\text{L}$   
 WBC count =  $7.8 \times 10^3 / \mu\text{L}$   
 Haemoglobin concentration =  $13.5 \text{ gm/dL}$   
 PCV = 45 %

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

$$\begin{aligned} \text{i. MCV} &= \frac{\text{PCV} \times 10}{\text{RBC count}} \\ &= \frac{45 \times 10}{4.5} \\ &= 100 \text{ fL} \end{aligned}$$

$$\begin{aligned} \text{ii. MCH} &= \frac{\text{Hgb} \times 10}{\text{RBC count}} \\ &= \frac{13.5 \times 10}{4.5} \\ &= 30 \text{ pg} \end{aligned}$$

$$\begin{aligned} \text{iii. MCHC} &= \frac{\text{Hgb} \times 100}{\text{PCV}} \\ &= \frac{13.5 \times 100}{45} \\ &= 30 \text{ g/dL} \end{aligned}$$

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

To determine the type of Anemia

**QUESTIONS AND PROBLEMS:**

- Anaemia is usually classified as being due to:-
  - blood loss
  - impaired red blood cell formation
  - increased red blood cell destruction

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

(a) BLOOD LOSS  $\left\{ \begin{array}{l} \text{Acute (haemorrhage)} \\ \text{Chronic (Iron-Deficiency)} \end{array} \right.$

(b) IMPAIRED RBC FORMATION  $\rightarrow$  Aplastic Anaemia  $\rightarrow$  failure of bone marrow to produce RBCs due to its destruction by radiations, drugs, chemicals etc

(c) INCREASED RBC DESTRUCTION  $\left\{ \begin{array}{l} \text{SICKLE CELL ANEMIA} \\ \text{HEREDITARY SPHEROCYTOSIS} \end{array} \right.$

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

|                  | Subject A                       | Subject B                       |
|------------------|---------------------------------|---------------------------------|
| RBC count        | $3.6 \times 10^6 / \text{mm}^3$ | $3.0 \times 10^6 / \text{mm}^3$ |
| Hb concentration | 7.0 g / 100 ml                  | 8.2 g / 100 ml                  |
| PCV              | 25%                             | 29%                             |

- Calculate MCV, MCH and MCHC for each of these subjects.

SUBJECT A

$$\text{MCV} = \frac{25 \times 10}{3.6} = 69.4 \text{ fL}$$

$$\text{MCH} = \frac{7 \times 10}{3.6} = 19.4 \text{ pg}$$

$$\text{MCHC} = \frac{7 \times 100}{25} = 28 \text{ g/dL}$$

SUBJECT B

$$\text{MCV} = \frac{29 \times 10}{3} = 96.7 \text{ fL}$$

$$\text{MCH} = \frac{8.2 \times 10}{3} = 27.33 \text{ pg}$$

$$\text{MCHC} = \frac{8.2 \times 100}{29} = 28.2 \text{ g/dL}$$

Microcytic Hypochromic Anemia

Macrocytic Normochromic Anemia

- What are the abnormalities encountered in these men. What are the possible causes of these abnormalities?

Subject A  $\rightarrow$  IRON DEFICIENCY ANEMIA

Subject B  $\rightarrow$  MEGALOBLASTIC ANEMIA

## EXPERIMENT – THE OSMOTIC FRAGILITY OF RED BLOOD CELLS

### A. Reagents and apparatus

1. Test tubes
2. Distilled water
3. Pipettes
4. NaCl in distilled water at the following concentrations – 0.55, 0.50, 0.45, 0.40, 0.35, 0.30 and 0.25g/dl.

### B. Procedure

- (a) Arrange 8 dry test tubes in a rack (see figure).
- (b) Place 5ml of each of the above solutions into the tubes in serial order.
- (c) To each test tube add 2-3 drops of freshly collected blood in EDTA tube.
- (d) Gently invert each tube to ensure mixing (do not shake the tubes).
- (e) Allow the tubes to stand for 30 minutes. Do not disturb them.
- (f) Note (a) the depth of colour of the supernatant fluids, and (b) the volumes of erythrocytes at the bottoms of the tubes. Normally haemolysis starts in a saline concentration of 0.45g/dl (i.e. 0.45%) and is complete in 0.35%-0.30% saline. If the fragility is increased haemolysis starts and is completed in saline of higher strengths.
- (g) Centrifuge the tubes at 1500 rpm for 5 minutes. Then examine the supernatants and sediments. Determine the concentrations at which haemolysis began and was completed. Compare these findings with those recorded in the first experiment.

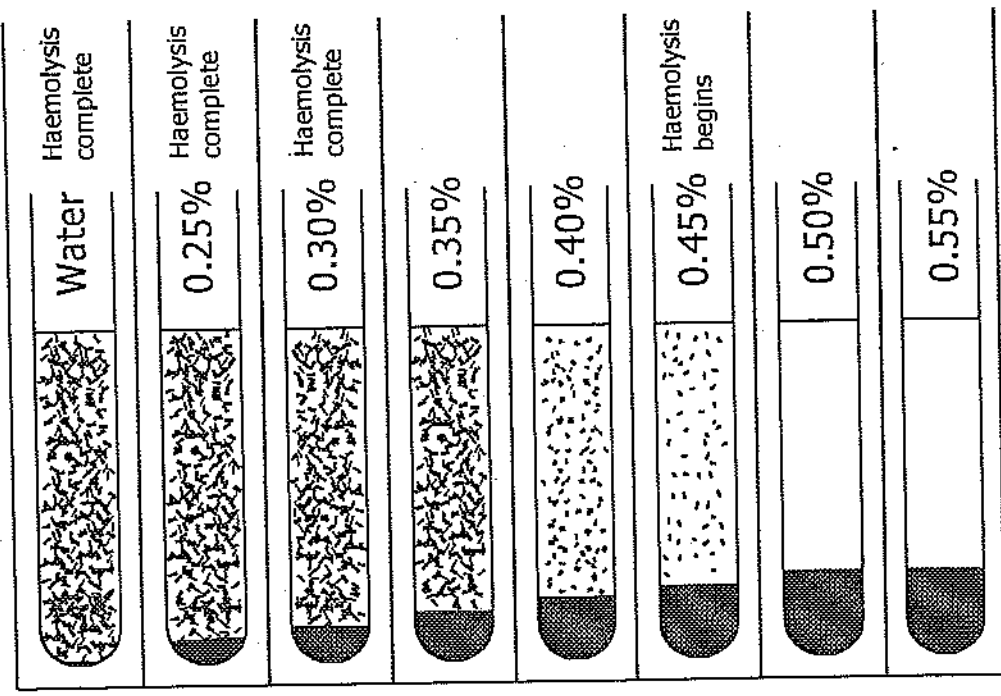
|                      | <u>1<sup>st</sup> experiment</u> | <u>2<sup>nd</sup> experiment</u> |
|----------------------|----------------------------------|----------------------------------|
| Haemolysis began     |                                  |                                  |
| Haemolysis completed |                                  |                                  |

### QUESTIONS AND PROBLEMS

1. What is the clinical significance of this test?

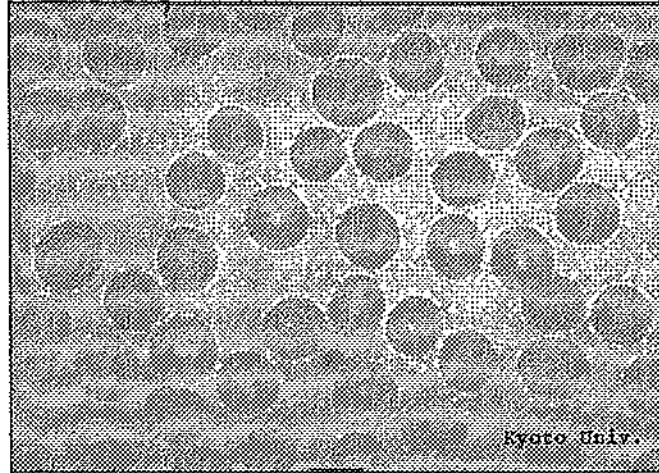
To diagnose Hereditary Spherocytosis

### Erythrocyte fragility determination



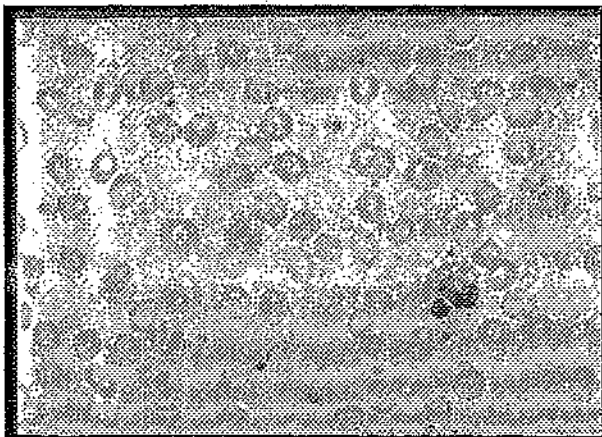
## Photomicrographs of blood films

### A healthy adult



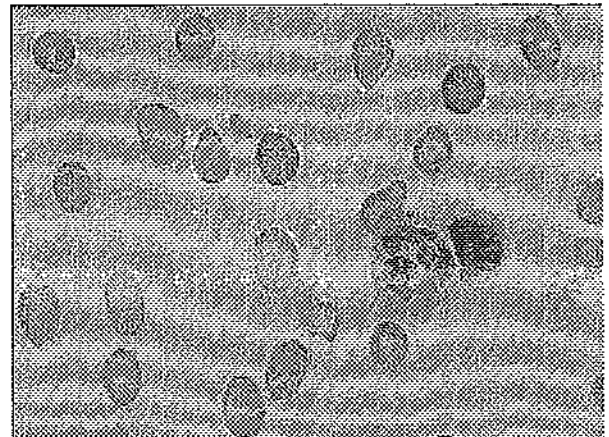
### Iron deficiency anaemia

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

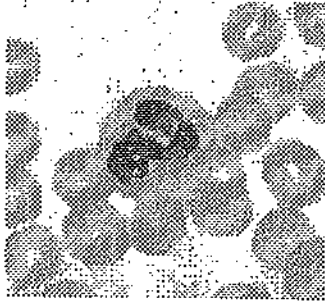
1. A microscope with an oil immersion objective
2. Mineral or cedar wood oil
3. Various dyes for staining blood films (e.g., Wright's stain)
4. Microscope slides

#### B. Procedure

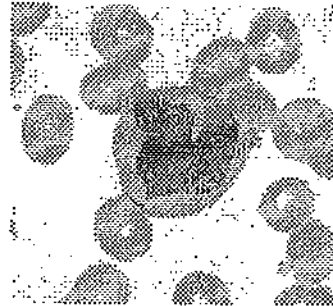
Examine the stained blood film under the oil immersion objective and identify the different leucocytes. Make coloured diagrams of these cells (on next page). Look at least 100 leucocytes and record your findings in the table below. Express your results as the percentage of the total white blood cells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

| <u>Type</u> | <u>Observed</u> | <u>Total</u> | <u>Percentage</u> |
|-------------|-----------------|--------------|-------------------|
| Neutrophil  |                 |              |                   |
| Eosinophil  |                 |              |                   |
| Basophil    |                 |              |                   |
| Monocyte    |                 |              |                   |
| Lymphocyte  |                 |              |                   |

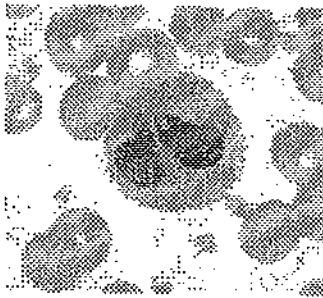
### White blood cells



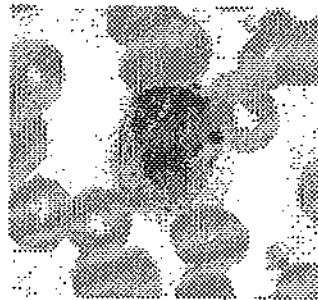
Neutrophil



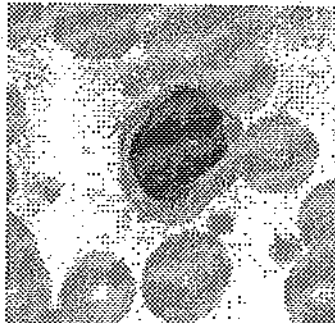
Monocyte



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?

Neutrophils → 40-70 %

Eosinophils → 1-4 %

Basophils → 0-1 %

Monocytes → 4-8 %

Lymphocytes → 20-45 %

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

NEUTROPHILS: ↑ed in Acute bacterial or fungal infections

EOSINOPHILS: ↑ed in Parasitic infections & Allergies

BASOPHILS: ↑ed in Allergies.

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

LYMPHOCYTES: ↑ed in Viral & Chronic infection & malignancies.

MONOCYTES: ↑ed in Chronic Infections.

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

① Leishman's Stain

② Wright's Stain

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.

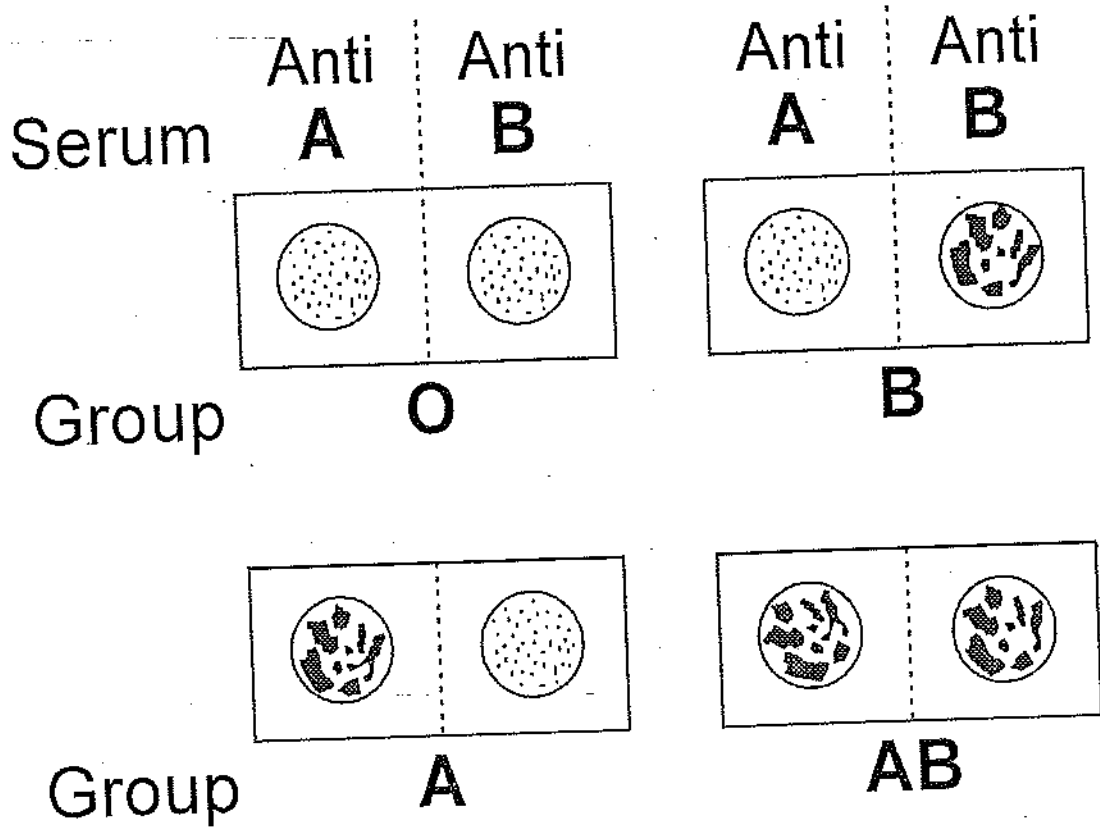
### **EXPERIMENT 1 – DETERMINATION OF BLOOD GROUPS**

#### **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?

| <u>Group</u> | <u>Agglutinogen</u> | <u>Agglutinin</u>                  |
|--------------|---------------------|------------------------------------|
| A            | A                   | Anti - B                           |
| B            | B                   | Anti - A                           |
| AB           | A & B               | No Antibody                        |
| O            | NO Antigen          | Both Anti - A, Anti - B antibodies |

2. What is your blood group?

B +ve

3. For which groups can you act as:-

(a) a donor?

B +ve, AB +ve

(b) a recipient?

B +ve, B -ve, O +ve, O -ve

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

L MN Group, Kidd-Deigo group etc.

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

O — MAXIMUM  
A } IN BETWEEN  
B }  
AB } MINIMUM

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

Same as compared to Europe, America.

B is more common in Asia than A.

7. What is haemolytic disease of the newborn?

Erythroblastosis Fetalis

- i. How is it treated?

Exchange Transfusion

- ii. How is it prevented?

By injecting Anti-D antibodies

## 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?

*hemophilia*

3. Name the substances which are used as anticoagulants.

① Heparin

② Warfarin

③ Calcium Citrate

④ Sodium Citrate

⑤ EDTA

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

- Pre-operative
- To diagnose bleeding Disorders

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

- ① Mast cells
- ② Basophils
- ③ Liver
- ④ Lungs

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?

*Thrombocytopenia*

**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?

*It's more accurate*

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)

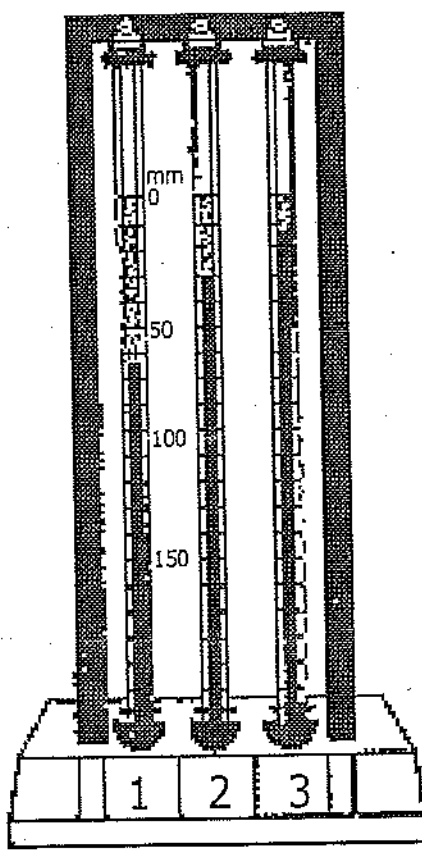
E.S.R. after 2 hours =                      mm                      (Normal = 7-15 mm – male)

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





الماضرة 11

اسم الآلة موري count التي تحسب عدد الكلايا في الدم  
\* تقول لازم تعرف نسبة الكلايا الطبيعية (الهيموجين والهيماتوكريت)   
فيما توريته full blood count ، الثلاث ذواتك التي منهم معادلات (تطابقها بالسنارة)  
MCH ، MCV ، MCHC ، رشي بعد اربع مروف

لا تعرف النتائج التي تحصل عليها من كذا مثلا الأنيما واعرف طرف العلاج فكيفها بعد  
\* اعرف آصفيات الأنيما (ضعسامها) (رصاصتها) أسبابها وعلاجها  
\* اعرف آ. آ. و C. آ.

|                  |                                   |
|------------------|-----------------------------------|
| clotting time    | bleeding time                     |
| نصف القلوة       | شور / سبيلو آلة                   |
| دقيقة            | (من 2 - 3 دقائق)                  |
| بال طال 7 دقائق  | بال مثال الة (دقائق)              |
| clotting factors | ثرو صبور سايتهونيا فدي التي تترده |
| هذا يقدر على ال  | بعد بعد على الصفاغ                |

bleeding time (الذي الي الصفاغ يتجمع على الكولاجين)  
Fibrin thread (التوقع التي في الصفاغ مع الفايبرين) (الذي يهايك (clotting))

مخوقات الدم (تغيرها بوجود الأنتيبيات على surface الآلة) RBC  
التي عنده أنتيجين A او B فقد يتكون anti-B ، وهذا

Rhesus (الذي تقول في ال blood ، ذياك surface) هو من الدم يقيه +  
مثلا A+ أما إذا صار فيه ريسوس (-)

نقل الدم : O- هو المتبرع العالمي universal donor  
O الدم من نفس النوع ينقل بسو له مثلك A+ (ال A+)  
A+ هو المستقبل العالمي (ترافق بالانمايات) Anti-  
(D) (مثلا A+ قد يهتج) (عنه)














ESR هو الترسب للـ RBCs (الذي له tube زبي العلم) وتكبيره عبر اناء مواد تمنع التدهش (يقول غالباً يكون لسيدي ترسب) اللهم تعبه بالدم ثم تظليه واقف لمدة ساعة. (الفرص انه الـ RBCs يترسب ما فتلل الـ ساعة ...)

بالرطل يقول يتفق انك من الحرس يقول يعني على الساعة الواحدة RBCs هفتا ترسب كم طليمتر ...

يقول مثلاً الـ T.B كرتفع فيها من كم طلي الى مثلاً 9 فتعرفنا انك كم مثلاً تروم تعرف لوالدواد وتوى ESR من جديه والطلع فيه تعرف انه الـ ESR يعني تعرف انه ESR يساعد على الـ diagnosis والـ prognosis

Rouleau form (يقول اسم الترسب) (أو كلالها العلمية اللي فيها + و -) RBCs واللع من فلالها يتم الترسب ...



| Cell type   | Occurrence in blood (per mm <sup>3</sup> ) | Cell anatomy*  | Function   |
|---|--|--|--|
| <b>Erythrocytes</b><br>(red blood cells, or RBCs)<br>                          | 4-6 million                                | Salmon-colored biconcave disks; anucleate; literally, sacs of hemoglobin; most organelles have been ejected  | Transport oxygen bound to hemoglobin molecules; also transport small amount of carbon dioxide  |
| <b>Leukocytes</b><br>(white blood cells, or WBCs)   | 4000-11,000                                |  |  |
| <b>Granulocytes</b>   |  |  |  |
| <ul style="list-style-type: none"> <li>• <b>Neutrophils</b><br/> </li> </ul>   | 3000-7000 (40-70% of WBCs)                 | Cytoplasm stains pale pink and contains fine granules, which are difficult to see; deep purple nucleus consists of three to seven lobes connected by thin strands of nucleoplasm | Active phagocytes; number increases rapidly during short-term or acute infections  |
| <ul style="list-style-type: none"> <li>• <b>Eosinophils</b><br/> </li> </ul>   | 100-400 (1-4% of WBCs)                     | Red coarse cytoplasmic granules; figure-8 or bilobed nucleus stains blue-red   | Kill parasitic worms; increase during allergy attacks; might phagocytize antigen-antibody complexes and inactivate some inflammatory chemicals   |
| <ul style="list-style-type: none"> <li>• <b>Basophils</b><br/> </li> </ul>   | 20-50 (0-1% of WBCs)                       | Cytoplasm has a few large blue-purple granules; U- or S-shaped nucleus with constrictions, stains dark blue  | Granules contain histamine (vasodilator chemical), which is discharged at sites of inflammation  |
| <b>Agranulocytes</b>  |  |  |  |
| <ul style="list-style-type: none"> <li>• <b>Lymphocytes</b><br/> </li> </ul> | 1500-3000 (20-45% of WBCs)                 | Cytoplasm pale blue and appears as thin rim around nucleus; spherical (or slightly indented) dark purple-blue nucleus  | Part of immune system; one group (B lymphocytes) produces antibodies; other group (T lymphocytes) involved in graft rejection, fighting tumors and viruses, and activating B lymphocytes |
| <ul style="list-style-type: none"> <li>• <b>Monocytes</b><br/> </li> </ul>   | 100-700 (4-8% of WBCs)                     | Abundant gray-blue cytoplasm; dark blue-purple nucleus often kidney-shaped   | Active phagocytes that become macrophages in the tissues; long-term "clean-up team"; increase in number during chronic infections, such as tuberculosis                                  |
| <b>Platelets</b><br>   | 250,000-500,000                            | Essentially irregularly shaped cell fragments; stain deep purple   | Needed for normal blood clotting; initiate clotting cascade by clinging to broken area; help to control blood loss from broken blood vessels   |





# HAEMATOLOGICAL VALUES

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|  | <u>SI units</u>              | <u>Other units</u>  |
|--|------------------------------|---|
| <u>Bleeding time</u>   |                              | 2-7 minutes   |
| <u>Clotting time</u>   |                              | 3-10 minutes  |
| <u>Erythrocyte sedimentation rate</u><br>( Westergren - figures are for patients under 60 years of age ) |                              | 0-6 mm/hour<br>( normal )<br>> 20 mm/hour<br>( abnormal ) |
| <u>Haemoglobin</u> (a) men   | 130-180 g/l.                 | 13-18 g/dl.   |
| (b) women  | 115-165 g/l.                 | 11.5-16.5 g/dl.   |
| <u>Leucocytes</u> - adults   | 4-11*10 <sup>9</sup> /l.     | 4-11*10 <sup>3</sup> /mm <sup>3</sup>                     |
| <u>Differential white cell count</u>   |                              |   |
| ✓ Neutrophil granulocytes  | 2.5-7.5*10 <sup>9</sup> /l   | 50-70 %   |
| ✓ Lymphocytes  | 1.0-3.5*10 <sup>9</sup> /l   | 20-40 %   |
| ✓ Monocytes  | 0.2-0.8*10 <sup>9</sup> /l   | 2-10%   |
| ✓ Eosinophil granulocytes  | 0.04-0.4*10 <sup>9</sup> /l  | 1-6%  |
| ✓ Basophil granulocytes  | 0.01-0.1*10 <sup>9</sup> /l  | 0-1%  |
| <u>Mean corpuscular haemoglobin</u><br>( MCH )   | 27-32 pg                     | 27-32 µg  |
| <u>Mean corpuscular haemoglobin concentration</u> ( MCHC )   | 30-35 g/dl.                  | 30-35%  |
| <u>Mean corpuscular volume</u> ( MCV )   | 78-98 fl.                    | 78-98 µ <sup>3</sup> or µm <sup>3</sup>                   |
| <u>Packed cell volume</u> ( PCV )<br>or haematocrit (a) men  | 0.40-0.54                    | 40-54%  |
| (b) women  | 0.35-0.47                    | 35-47%  |
| <u>Platelets</u>   | 150-400*10 <sup>9</sup> /l.  | 150,000-400,000/µl<br>or /mm <sup>3</sup>                 |
| <u>Red cell count</u> (a) men  | 4.5-6.5*10 <sup>12</sup> /l. | 4.5-6.5*10 <sup>6</sup> /µl.<br>or /mm <sup>3</sup>       |
| (b) women  | 3.8-5.8*10 <sup>12</sup> /l. | 3.8-5.8*10 <sup>6</sup> /µl.<br>or /mm <sup>3</sup>       |

