g. The needle should be withdrawn gently. Using a sterile cotton swab apply pressure for 2–3 minutes at the site of puncture to prevent blood loss.
h. Remove the needle from the syringe and deliver the blood into a sterile container containing anticoagulant.
i. Mix the blood with the anticoagulant by shaking the closed bottle gently over the table.
j. Rinse the needle and syringe with water and arrange for disposal.

VIVA QUESTIONS

Q 1. How should the needle, syringe and blood sample be disposed?

Ans. The needle and syringe must be disposed of in approved sharps disposal containers. Other contaminated waste must be discarded in an appropriate bio-hazard bag.

Q 2. Which toxic symptoms may occur because of sodium oxalate ingestion?

Ans. It can cause burning pain in the mouth, throat and stomach, bloody vomiting, headache, muscle cramps, convulsions, hypotension, heart failure, shock, coma, and possible death. Mean lethal dose by ingestion of oxalates is 10–15 grams (per MSDS).

Q 3. What is the role of EDTA in chelation therapy?

Ans. EDTA is used to bind metal ions in the practice of chelation therapy, e.g. for treating mercury and lead poisoning. It is also used to remove excess iron from the body.

Q 4. Which of the market products have EDTA as a component?

Ans. Shampoos, cleaners and other personal care products have EDTA salts as component and are used as a sequestering agent to improve their stability in air.

Q 5. When was sodium citrate first used as anticoagulant in blood transfusion?

Ans. Belgian doctor Albert Hustin and Argentine physician Luis Agote successfully used sodium citrate as anticoagulant in blood transfusions in 1914.

Q 6. What is the role of sodium citrate in coagulation?

Ans. The citrate ion chelates calcium ions in the blood by forming calcium citrate complexes, disrupting the blood clotting mechanism.

Q 7. Where from is heparin produced in the human body?

Ans. Yes, heparin is a naturally occurring anticoagulant produced by basophils and mast cells.

Q 8. Explain the mechanism of anticoagulant effect of heparin.

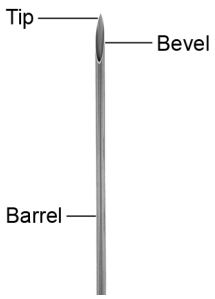
Ans. It facilitates the action of anti-thrombin III, by binding and removing clotting factors IX, X, XI and XII. Hence these clotting factors are not available for the formation of prothrombin activator and thrombin. In the absence of thrombin, fibrinogen cannot be converted to fibrin.

MCQs

Q 1. The personal protective equipment that should be worn in hematology lab is:

a. Lab coat
b. Gloves
c. Mask
d. All of the above

Q 2. Identify the object in the photo:



a. Disposable syringe
b. Plastic bottle

Chapter

5 Determination of Red Blood Cells Count and Blood Indices

LEARNING OBJECTIVES

After learning the practical the students should be able to:

1. Perform and calculate the red blood cell count in a given blood sample.
2. Discuss the physiological and pathological conditions responsible for variation in red blood cell count.
3. Classify and discuss the morphological and pathological classification of anemia.
4. Enlist the precautions to be taken for red blood cell count.

INTRODUCTION

Red blood cells are the most common type of cells in the blood, and are extremely important because they carry oxygen from the lungs to the body tissues. The RBC count ranges from 4.2 to 5 million red blood cells per micro liter of blood for women and 4.6 to 6.0 million for men. A normal red blood cell count for children is between 3.8 and 5.5 million.

Clinical Perspective

A decrease in red blood cell count is seen in anemia, acute or chronic blood loss, malnutrition, chronic inflammation or a number of nutritional deficiencies including iron, copper, vitamin B_{12} or vitamin B_6. It is biconcave in shape having a diameter of 7–8 micron and mean corpuscular volume of 90–95 cubic micron. Anemia is caused by either a decrease in production of red blood cells or hemoglobin or an increase in loss (usually due to bleeding) or destruction of red blood cells. Anemia can be a temporary problem or a chronic condition. Milder anemia can be treated with dietary changes, iron replacement (oral or IV) and vitamin supplementation.

Aims: Enumeration of the number of erythrocytes in one cubic millimeter of blood.

Materials and Chemicals: Neubauer's chamber with cover slip, red cell pipette, and microscope and diluting fluid. [The diluting fluid is the hayem fluid and it contains mercuric chloride 0.5 gm; sodium chloride 1 gm; sodium sulfate 5 gm dissolved in 200 ml of distilled water].

Procedure

1. Take a clean and dry pipette.
2. Pour the RBC diluting fluid (Hayem's fluid) in a watch glass.
3. Clean the middle or the index finger with spirit. Give a finger prick using lancet, and then suck blood up to the 0.5 mark. No air bubbles should accumulate during the process.
4. Wipe the tip of the pipette using sterile gauze, and then suck Hayem's fluid up to the 101 mark.
5. Keep the pipette initially at an acute angle while sucking in the Hayem's fluid and then gradually make it vertical as the fluid reaches the 101 mark.

Add distilled water in order to dilute it but avoid the spilling over.

4. Wait for 10 minutes. Gently blow through the blow pipe for mixing of distilled water and Leishman stain. Extra Leishman stain is now rinsed by pouring the water slowly on the slide. Dry the slide in the air.
5. Place one or two drops of cedar wood oil over the slide and place it under the oil immersion lens in contact with cedar wood oil.
6. Examine 100 (or 200) neutrophils and observe the number of lobes in each cell.

Results

Note the Results

Stage I N1 (Nucleus is C or U shaped and the two limbs connected by thick band of chromatin)
Percentage.....

Stage II N2 (Nucleus is bilobed and connected by thin band of chromatin)
Percentage.....

Stage III N3 (Three lobes and connected by chromatin filament)
Percentage.....

Stage IV N4 (Four Lobes and connected by thin band of chromatin)
Percentage.....

Stage V N5 (Five lobes with unclear outline)
Percentage.....

Precautions

1. Draw blood under aseptic precaution and stain appropriately as described in the method above.
2. The blood film should be thin and smooth.
3. The slides used must be clean.
4. The staining and blowing should be as gentle as possible.
5. Count the lobes and note carefully.

VIVA QUESTIONS

Q 1. Describe morphology of a neutrophil.

Ans. The neutrophils are uniform in size having a diameter between 9 and 15 micrometers. The nucleus consists of two to five lobes joined together by chromatin material. Neutrophils move along by amoeboid motion. It extends long projections called pseudopodium into which their granules flow; followed by contraction of filaments of the cytoplasm which then draws forward the nucleus and rear of the neutrophil. Thus neutrophils rapidly advance along a surface.

Q 2. How does neutrophil move to injured site?

Ans. Bone marrow holds a large number of neutrophils in reserve to be mobilized in response to infection or inflammation. The force of attraction which determines the direction of movement of neutrophil is known as chemotaxis; and is attributed to substances liberated at sites of tissue damage which acts as chemotaxins. Large numbers of neutrophils circulate outside the bone marrow (half are in the tissues and half are in the blood vessel). Nearly 50% of neutrophil of the blood pool stick to the vascular endothelium a phenomenon called 'margination'. During inflammation, these marginated neutrophils by the help of their pseudopods moves via the gap in between the endothelium of the capillary to reach the tissue and this phenomenon is called emigration or diapedesis. The emigrated neutrophil attacks the bacteria lodged in the tissue.

Q 3. Define neutrophilia.

Ans. Increase in neutrophil count above normal is called neutrophilia. Physiological neutrophilia is seen after exercise, acute mental stress, after meals, during pregnancy, etc. The pathological causes of neutrophilia included acute infections, tissue necrosis (occurs in pulmonary, renal or myocardial infarction), after surgery, burns, acute hemorrhages, etc and may be drug induced (adrenaline, glucocorticoid, etc).

Q 4. Describe the various stages of Arneth count.

Ans. The various stages of Arneth count include:

Q 2. How much is the bleeding time as per Duke's method?

Ans. The normal bleeding time as per Duke's method is 2–6 minutes.

Q 3. How much is the clotting time as per capillary glass tube method?

Ans. The normal clotting time as per capillary glass tube method is 3–8 minutes at 37°C.

Q 4. Explain the series of events involved in hemostasis.

Ans. Three major events which get involved during arrest of bleeding are: (i) Constriction of injured blood vessel due to release of 5-HT from the damaged platelets. (ii) Formation of a hemostatic plug of platelets. (iii) Seal of damaged blood vessel by the blood clot.

Q 5. Enlist two conditions each in which bleeding and clotting time is prolonged.

Ans. The two conditions in which bleeding time is prolonged are thrombocytopenic purpura and purpura hemorrhagica.

The two conditions in which clotting time is prolonged are hemophilia and vitamin K deficiency.

MCQs

Q 1. The fibrinogen is factor number:

a. I b. II
c. III d. IV

Q 2. Prothrombin activator is formed by:

a. Intrinsic pathway b. Extrinsic pathway
c. a and b both d. Thrombin

Q 3. The first important event in hemostasis following severe tissue injury is:

a. Clumping of red blood cells
b. Vascular spasm
c. Formation of a platelet hemostatic plug
d. Formation of thromboplastin

Q 4. The conversion of fibrinogen into fibrin occurs by:

a. Thrombin b. Thrombomodulin
c. Thromboplastin d. Platelets

Q 5. In clotting mechanism via intrinsic and extrinsic pathways, the key reaction is:

a. Formation of thrombin
b. Formation of fibrin
c. Conversion of factor X to its active form
d. Formation of ATP

Q 6. Intravascular clotting is prevented by circulating:

a. Heparin b. Fibrinolysin
c. Anti-thrombin C d. All of the above

Q 7. Fibrinolytic system gets activated by all of the following conditions, except:

a. Trypsin inhibitor b. Stress and strain
c. Glucocorticoids d. Violent sudden death

Q 8. Test for clotting is:

a. Capillary tube method
b. Sahli's method
c. Wintrobes method
d. Westergren method

Q 9. Hemophilia is:

a. Autosomal dominant
b. Autosomal recessive
c. X-linked recessive
d. X-linked dominant

Q 10. In purpura:

a. Platelets count may be low
b. Capillary contractility is defective
c. CT is normal but BT increases
d. All of the above

Answers: 1. a 2. c 3. b 4. a
5. c 6. d 7. a 8. a
9. c 10. d

Contents

SECTION IV
AMPHIBIAN EXPERIMENTS