3. Do not squeeze the finger to obtain blood, first drop of blood and it is to be wiped with sterile cotton. As first drop of blood is admixed with tissue fluids, it may interfere with the proper reporting of results. Always allow the blood to free flow.
4. The subsequent drop is collected and utilized to conduct various estimation. (For example, the blood is drawn into WBC/RBC pipette for carrying white blood cell count/red blood cell count or utilize for other practicals such as blood grouping, bleeding time and clotting time, etc.)
5. Apply firm pressure over the bleeding site by using cotton gauze and instruct the patient to hold it till the process of bleeding is stopped.

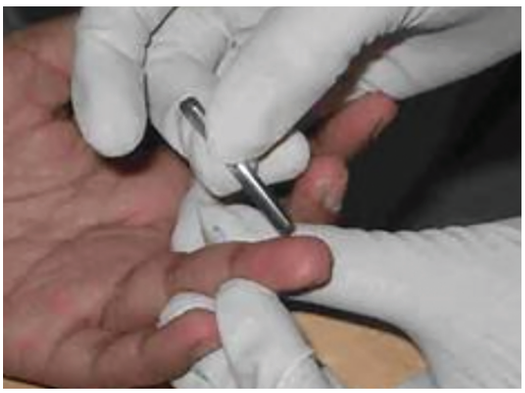

Fig. 2.1: Method of finger pricking

Precautions

1. Clean and scrub hand with soap and antiseptic solution. Dry your hand and always wear gloves for withdrawing any blood sample. Clean the fingertip area with spirit and allow it to dry.
2. The sterile disposable lancet must be used for pricking.
3. Ensure that you do not squeeze the finger in attempt to draw the blood as this dilutes the blood with tissue fluid.
4. Discard the first drop of blood as it gets admixed with tissue fluid.
5. Collect the blood from any one of the middle fingers; as palmar fascia of middle fingers is limited to hand itself while palmar fascia of thumb and little finger are in continuity with the limb.

Pricking the Ear Lobe *(Fig. 2.2)*

Method

1. Gently rub the ear lobe to make it warm. Clean the ear lobe area with methylated spirit and allow it to dry.
2. Make a single firm prick to a depth of 2–3 mm with a sterile needle of 22/23 gauze.
3. Wipe away the first few drops and collect the sample as the blood spontaneously flows thereafter.
4. Apply gentle pressure by cotton/gauze piece to ensure stoppage of bleeding.

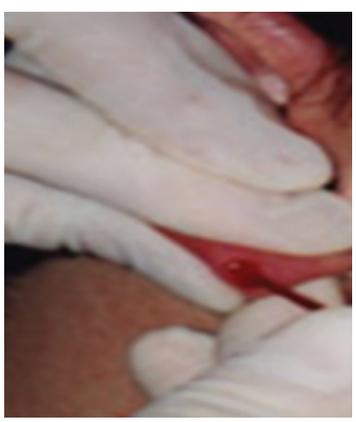

Fig. 2.2: Collection of capillary blood: Pricking of ear lobe

4. Ensure that no air bubbles are present and there is no overflow of fluid into the grooves.
5. After charging allow the cells to settle and then focus the cells under low power to ensure uniform distribution.
6. Conduct WBC count under four corner WBC squares under low power objective. Conduct RBC count in the central square of the Neubauer chamber under high power objective in the four medium sized corner square and one medium sized central square.

Precautions

1. The pipette and lancet should be clean and dry.
2. The finger should be cleaned with methylated spirit and allowed to dry.
3. The prick should be firm and bold so that after a single prick the blood flows freely.
4. The finger should never be squeezed to obtain blood.
5. The tip of the pipette should place over the drop and then sucked.
6. Ensure no air bubble is drawn into pipette.
7. Do not suck blood over 0.5 mark of the pipette.
8. Dilute the blood immediately with diluents up to 101 mark of RBC pipette or up to 11 mark of WBC pipette.
9. Ensure that tip of pipette is dipped into diluting fluid when sucking; so that air bubble do not enter in.
10. Hold the pipette gently horizontally between palms and rotate for 2–3 minutes for uniform mixing and then place it horizontally in the tray that you can charge after uniform mixing is achieved.

Rule for Cell Counting

Counting the Cells

1. **Counting cells that are on a line:** Cells that are on the line of a grid require special attention. Note that cells touching the left and lower borders are to be considered for counting while those at right and upper borders are omitted.

Precautions

1. Ensure that the Neubauer chamber, pipette, fingertip, lancet and coverslip is clean and dry.
2. The diluted blood sample after pipetting should be thoroughly mixed.
3. The air bubble should not be present in charging pipette or under coverslip of the counting chamber.
4. The chamber should be uniformly charged and ensure that there is no overflowing in the chamber
5. Note that cells touching the left and lower borders are to be considered for counting while those at right and upper borders are omitted.
6. Cell count should be started after they get equally distributed.

Sources of Error

1. Improper collection of blood sample: Blood collected less than that required for counting may give error in results.
2. Wet or contaminated pipettes: This may result in improper results.
3. Poor pipetting technique; for example, under collection below desired line with blood or diluting fluid; or over collection above desired line with blood or diluting fluid; or presence of air bubble in the pipette, etc.
4. Failure to discard 2 drops from the pipette tips before charging the haemocytometer.
5. Overcharging or undercharging the haemocytometer
6. Wet or contaminated coverslip and haemocytometers.
7. The cells in the counting chamber are unevenly distributed.
8. Error while counting cell.

Disadvantages of Sahli's method are:

a. The standard colouration on the haemoglobinometer fades away over the year and this will give wrong result.
b. It gives results in approximation only and not accurately and cannot be fully relied upon.

Q4. Which of the methods is most accurate method for haemoglobin estimation?

Ans. Cyanmethaemoglobin method is most accurate because estimation is done with photoelectric colorimeter.

Q5. Enlist three main causes for error reporting while estimating haemoglobin level.

Ans. *The error in reporting haemoglobin levels while estimating haemoglobin concentration in blood is*:

1. Technical errors—improper mixing of blood.
2. Errors in pipetting—tissue fluid contaminating capillary blood.
3. Visual errors—taking the reading is very subjective, as it is a comparison of colours. It can vary from person to person. Hence the results may not be accurate.
4. Quality of the colour comparators can affect the reading—if the glass blocks are old or faded it can cause wrong results.

Q6. What is the adequate time required for conversion of Hb to acid haematin?

Ans. Minimum time period of ten minutes is required for complete conversion of Hb to acid haematin; otherwise it will lead to false negative result. The 95% of Hb is converted acid haematin at the end of 10 minutes, 98% at the end of 20 minutes, and the maximum colour is reached in about 1 hour.

Q7. What are the types of haemoglobin which do not get converted to acid haematin?

Ans. The types of haemoglobin which do not get converted to acid haematin are carboxyhaemoglobin, methaemoglobin and sulphaemoglobin.

Q8. What will time delay in noting the haemoglobin levels by Sahli's method lead to?

Ans. The golden brown colour of acid haematin is unstable, hence undue delay in reading the test result will lead to false result.

Q9. What are the precautions to be taken while making the reading for haemoglobin value with that of comparator?

Ans.

1. The most important precaution to be taken is that the glass rod should not be left inside the haemoglobin tube.
2. Note the reading only when the colour of the solution in the haemoglobin tube is same as that of the comparator.
3. The matching should be done against natural light.

Q10. Hb concentration of a given subject was found to be 14 gm%. Calculate its oxygen carrying capacity %.

Ans. The normal oxygen carrying capacity of blood per gram of haemoglobin is 1.34 ml; hence the oxygen carrying capacity of the subject is $14 \times 1.34 = 18.76$ ml/dl.

Q11. What are the functions of haemoglobin?

Ans. *The functions of haemoglobin are*: Haemoglobin transports oxygen from the lungs to the tissues and carbon dioxide from the tissues to the lungs. It also acts as a buffer in and helps maintaining the blood pH. Haemoglobin in tissue regulates iron metabolism and mediates antioxidant effects.

Q12. What is the name of the molecule that transports oxygen in red blood cells?

Ans. The respiratory pigment of the red blood cells is haemoglobin.

Q13. What is the molecular composition of haemoglobin? Does the functionality of haemoglobin as a protein depend upon its tertiary or upon its quaternary structure?

Ans. Haemoglobin is a molecule made of four polypeptide chains, each bound to an iron-containing molecular group called a haem group. Thus, the molecule contains four polypeptide chains and four haem groups. As a protein it is composed of association of polypeptide chains, the functionality of haemoglobin depends upon the integrity of its quaternary structure.

Q14. What is the molecular weight of haemoglobin?

Ans. Haemoglobin is a globular molecule having a molecular weight of 68,000 daltons.

CHAPTER

6

Determination of Red Blood Cell Indices

Learning Objectives

After learning the practical the students should be able to:

1. Explain the significance of determining red blood cell indices.
2. Define and discuss regarding parameters concerned with determining red blood cell indices.
3. Calculate and report the red blood cell indices.
4. Discuss the clinical significance of each red blood cell indices.

Aim of experiment: Determination of red blood cell indices.

Principle: The parameters such as haemoglobin, RBC count, haematocrit value, packed cell volume helps to evaluate certain blood indices which indicate haemoglobin concentration in red blood cell and red blood cell size that aids in accurate diagnosis of the type of anaemia which the patient is suffering from.

Snap box 1

This vital information can be obtained from blood indices:

1. Mean corpuscular volume (MCV)
2. Mean corpuscular haemoglobin (MCH)
3. Mean corpuscular haemoglobin concentration (MCHC)
4. Colour index (CI)
5. Red blood cell distribution width (RDW)

Mean corpuscular volume (MCV) determines the average size of the RBCs; Mean corpuscular haemoglobin (MCH) states the average amount of oxygen-carrying haemoglobin inside a red blood cell; and mean corpuscular haemoglobin concentration (MCHC) denotes the average concentration of haemoglobin inside a red blood cell. The variation in size of RBC can be ascertained by calculating the red cell distribution width (RDW). In pernicious anaemia the variation in RBC size (anisocytosis) along with variation in shape (poikilocytosis) increases the RDW.

Apparatus: Same as that described in chapters of estimation of haemoglobin, RBC count, haematocrit and packed cell volume.

Method

The parameters such as haemoglobin, RBC count, haematocrit value, packed cell volume are calculated by manual method or automated method and red blood cell indices are then determined.

Calculation

1. Mean corpuscular volume (MCV): It is the average volume of single red blood cells. The normal MCV averages in between 78 and 94 μm^3. MCV is increased in pernicious anaemia and megaloblastic anaemia. MCV is decreased in iron deficiency anaemia.