

Aim: Determination of Differential count of blood

PRINCIPLE This exercise helps us to identify different kinds of leucocytes in the human blood and estimate the percentage distribution of each type. The differential blood count is based on the staining of the nucleus and cytoplasm of the white blood cells. Staining both nuclei and cytoplasm enables us to determine cells' morphology and other properties. A blood smear is stained with the Leishman stain, and different leucocytes are counted under a microscope.

MATERIALS REQUIRED

1. Glass slides and coverslip
2. Leishman Stain
3. Blood specimen
4. Distilled water
5. Cedarwood oil
6. Dropper or Coplin staining jar
7. Cotton/Filter paper
8. Oil immersion Compound Microscope

PROCEDURE

A. Preparation of blood smear

Place a drop of blood on a clean glass slide about 1-2 cm away from one end. Spread the blood drop with the help of another glass slide by placing it at an angle of 45° on the blood drop and moving sidewise. Hold the slide spreader firmly and move to the other end in a straight line. Blood smear should be 3-4cm in length. Allow the blood smear to dry.

B. Fixing and staining blood smear

1. Pour 8-10 drops of Leishman stain solution on the blood smear and leave it for 2-3 min.
2. Add distilled water over the stained smear with the help of a dropper to wash the slide and leave the slide for 8-10 min.
3. Shake off extra water and wipe the back of the slide with filter paper.
4. Set the slide in an upright position for air drying.

C. Examination of the Stained Smear under Oil Immersion

5. Cover the stained smear with the cover slip and place it under the low power of the microscope. Choose a good portion of the smear for counting and studying the distribution of patterns of the cells
6. Add a drop of the cedar wood oil to the blood film. Carefully set the oil immersion objective and focus the cell using fine adjustment.
7. Start counting the cells by the shifting slide laterally, then move forward and back upward until the total 100 cells are counted.
8. Express the count of different leucocytes as the percentage.

RESULT AND OBSERVATIONS

The WBCs can be differentiated from RBCs by the presence of a nucleus and large size. Keep in mind some key factors during the identification of WBC

1. Size of the cells
2. Colour and lobes of the nucleus.
3. Presence of pink or blue fine or coarse cytoplasmic granules
4. Nuclear/Cytoplasmic ratio.