Aim: Enumeration of Total Red Blood Corpuscles (RBC) Count.

References

1. Cheesbrough, M. (2006). *District Laboratory Practice in Tropical Countries*. Cambridge University Press.
2. Henry, J. B. (2001). *Clinical Diagnosis and Management by Laboratory Methods*. W.B. Saunders Company.
3. Articles from Google Scholar/PubMed on manual RBC counting techniques.

Introduction:

Red Blood Corpuscles (RBCs), also known as erythrocytes, are specialized cells in the blood responsible for transporting oxygen from the lungs to tissues and carbon dioxide from tissues back to the lungs for exhalation. They are the most abundant cell type in the blood and are essential for maintaining life.

Objective

To determine the total number of red blood cells (RBCs) per cubic millimeter of blood using a hemocytometer.

Principle

Red blood cells (RBCs), or erythrocytes, are responsible for oxygen transport in the body. Their enumeration is crucial for diagnosing anemia, polycythemia, and other hematological conditions. The hemocytometer is employed to count RBCs manually within a defined grid under the microscope.

Materials Required

1. **Reagents**

Hayem's or Gower's solution (RBC diluting fluid)

2. **Apparatus**

Hemocytometer (Neubauer chamber), Microscope, Capillary pipette or micropipette, Cover slip, Sterile lancet, Cotton and antiseptic, Test tubes

Procedure

1. Sample Preparation

- Prick the fingertip using a sterile lancet or collect venous blood in an anticoagulant-treated tube.
- Use a capillary pipette to draw blood to a marked volume (e.g., 0.02 mL).

2. Dilution of Blood Sample

- Add the blood sample to the RBC diluting fluid (e.g., 0.38 mL of Hayem's solution) in a test tube.
- Mix gently to ensure proper dilution and prevent cell clumping.

3. Charging the Hemocytometer

- Clean the hemocytometer and cover slip thoroughly.
- Place the cover slip over the Neubauer chamber.
- Fill the chamber with the diluted blood sample using a micropipette, avoiding overfilling or underfilling.

4. Microscopic Counting

- Place the hemocytometer under the microscope.
- Focus on the central grid (5x5 small squares within the large center square).
- Count RBCs in the 5 smaller squares, as per the counting pattern under high power (40x objective).

Calculation

The total RBC count is calculated using the formula:

$$\text{RBC Count (cells/mm}^3\text{)} = \frac{\text{Number of cells counted} \times \text{Dilution factor}}{\text{Volume of one square (mm}^3\text{)}}$$

- **Number of cells counted:** Sum of RBCs in 5 smaller squares.
- **Dilution factor:** As prepared (e.g., 200 if 0.02 mL blood + 0.38 mL diluting fluid).
- **Volume of one small square:** 0.004 mm^3 for each small square in the Neubauer chamber.

Sample Data Table

Square No.	RBC Count (cells)
1	108
2	112
3	115
4	110
5	113
Total	558

Example Calculation

$$\text{RBC Count} = \frac{558 \times 200}{0.02} = 5,580,000 \,\text{cells/mm}^3$$

Interpretation

- **Normal RBC count:**

Males: 4.5–6.0 million cells/mm³

Females: 4.0–5.5 million cells/mm³

Newborns: 4.1–6.1 million cells/mm³

- Abnormal values indicate conditions such as anemia (low count) or polycythemia (high count).

Precautions

1. Ensure the hemocytometer and pipettes are clean to avoid errors.
2. Mix the blood and diluting fluid thoroughly but gently.
3. Avoid overcharging or undercharging the hemocytometer.
4. Count systematically to ensure accuracy.

Result: The RBC count of the given blood sample is **5.58 million cells/mm³**, which falls within the normal range.