Aim: Introduction to Hemocytometry

References

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3. Cappuccino, J. G., & Sherman, N. (2013). Microbiology: A Laboratory Manual. Pearson.

Introduction

Hemocytometry is a technique used to count cells in a given volume of fluid, typically using a device called a hemocytometer. This method is widely used in biological and medical research to determine the concentration of cells in a blood sample, culture, or other cell suspension. The hemocytometer is a specialized microscope slide with a grid of known dimensions that allows for precise counting of cells.

Objectives

- To understand the principles and use of a hemocytometer.
- To learn how to prepare and load a blood or cell sample onto a hemocytometer.
- To accurately count cells and calculate their concentration in a sample.

Materials and Equipment

- Microscope

- Hemocytometer (Neubauer counting chamber)

- Cover slips

- Blood or cell suspension sample

- Diluting fluid (e.g., Turk's solution for white blood cells, isotonic saline for red blood cells)

- Pipettes and micropipettes

- Disinfectant (e.g., 70% ethanol)

- Gloves and lab coat

Procedures

Preparation of Blood Sample

1. Dilution:

If necessary, dilute the blood or cell suspension sample with an appropriate diluting fluid. For example, use Turk's solution for white blood cells or isotonic saline for red blood cells.

Mix the sample thoroughly to ensure an even distribution of cells.

Loading the Hemocytometer

1. Cleaning:

Clean the hemocytometer cover slip with 70% ethanol and dry them with lens paper.

2. Assembling:

Place the cover slip over the hemocytometer's counting grid.

3. Loading:

Using a pipette, carefully place a small drop of the diluted sample at the edge of the coverslip. Capillary action will draw the sample into the grid area.

Ensure the chamber is properly filled without overloading, which could lead to inaccurate counts.

Counting Cells

1. Microscope Adjustment:

Place the hemocytometer on the microscope stage and focus on the grid lines using low power.

2. Counting:

Switch to a higher magnification to count the cells within the grid.

Count the cells in several squares to ensure accuracy. For red blood cells, count the cells in the large center square. For white blood cells, count the cells in the four corner squares of the large grid.

Follow specific rules for counting cells that touch the boundary lines (usually, count cells touching the top and left boundaries but not the bottom and right boundaries).

Calculation of Cell Concentration

1. Formula:

$$\text{Cell concentration(cells/mL)} = \frac{\text{Number of cells counted} \times \text{Dilution factor}}{\text{Volume of the counted area}}$$

The volume of the counted area depends on the hemocytometer grid used. For example, in a Neubauer hemocytometer, the volume of the large center square is 0.1mm^3 or 0.1 µL.

2. Example Calculation:

If you counted 200 cells in 5 large squares, and the dilution factor is 10:

$$\text{Cell concentration} = \frac{200 \times 10}{0.5\ mm^3} = \frac{2000}{0.5} = 4000 \text{ cells/mm}^3 = 4 \times 10^6 \text{ cells/mL}$$

6. Observation and Documentation

- Record the number of cells counted in each grid.
- Calculate the average cell count if multiple squares were counted.
- Document the final cell concentration along with any observations or potential sources of error.

Discussion

- Discuss the importance of accurate cell counting in research and clinical settings.
- Explain the potential sources of error in hemocytometry (e.g., improper filling of the chamber, uneven cell distribution, errors in counting boundary cells).
- Relate the findings to the physiological or experimental context.

Safety and Precautions

1. Wear appropriate personal protective equipment (gloves, lab coat, safety goggles).
2. Handle blood samples with care to avoid exposure to bloodborne pathogens.
3. Disinfect the hemocytometer and work area after use.
4. Dispose of biological waste and sharps according to safety guidelines.