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Introduction to Hemocytometry

Experiment 6 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To identify an improved Neubauer counting chamber and calculate concentration from counted cells, dilution and chamber volume.

Principle

Hemocytometry counts cells in a known volume. The improved Neubauer grid has nine large 1 mm^2 squares and a 0.1 mm depth beneath its correctly seated special coverslip. Concentration = counted cells × dilution factor ÷ counted volume. One mm^3 equals one µL.

Requirements

- Improved Neubauer chamber and its specified coverslip.
- Microscope, calibrated pipettes, instructor-prepared control suspension and approved diluent.
- Gloves and the laboratory's specimen-handling materials.

Procedure

1. Inspect the chamber

Identify the ruled areas, central counting grid and depth marking. Confirm that the chamber is the improved Neubauer type.

2. Seat the coverslip

Use the special coverslip according to the manufacturer's instructions. Incorrect seating changes chamber depth.

3. Prepare the control

Mix the instructor-provided control suspension and record the assigned dilution. Dilution factor is total mixture volume divided by original suspension volume.

4. Load and settle

Fill by capillary action without overflow, bubbles or incomplete coverage. Allow the stated settling time before counting.

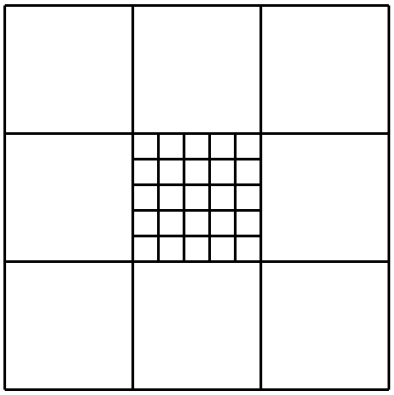
5. Count consistently

Use the assigned squares. Include cells touching top and left borders; exclude bottom and right borders, applying the same rule throughout.

6. Calculate

Record the area counted, depth and total cells. Convert the calculated concentration to the requested units.

Chamber Geometry



Improved Neubauer chamber

Grid: 3 mm × 3 mm

Each large square: 1 mm^2

Chamber depth: 0.1 mm

Central square: 25 groups

Schematic; not to scale.

Region	Area	Volume at 0.1 mm depth
One large square	1 mm^2	0.1 µL
Four corner squares	4 mm^2	0.4 µL
Five central group squares	5 × 0.04 = 0.20 mm^2	0.020 µL

Observations and Record

Quantity	Symbol / unit	Record
Cells counted	N	____
Dilution factor	D	____
Area × depth	mm^2 × mm = µL	____

Worked Example

The numbers below are illustrative; enter your own observations in the record.

- Example: 120 cells counted in 4 large squares. Volume = 4 × 1 mm^2 × 0.1 mm = 0.4 µL. With D = 20, concentration = 120 × 20 ÷ 0.4 = 6000 cells/µL.
- A 1:20 dilution uses 1 volume of original suspension plus 19 volumes of diluent, giving 20 total volumes.
- Counts from two chambers should be assessed for agreement under the laboratory criterion, not merely averaged after a faulty fill.

Result

For N = ____, D = ____ and counted volume = ____ µL, the calculated concentration is ____ cells/µL.

Precautions

- Do not use an ordinary slide coverslip when the chamber requires a special coverslip.
- Reject visibly faulty fills instead of attempting to compensate mathematically.
- Use controls or supplied specimens; collection and clinical testing are performed by trained staff.

Viva Questions and Answers

1. What is the chamber depth?

0.1 mm for a correctly assembled standard improved Neubauer chamber.

2. Why use a border rule?

It prevents counting a cell twice at neighbouring square boundaries.

3. Why does dilution appear in the numerator?

The diluted suspension has fewer cells per volume than the original.

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