Total Red Blood Cell Count

Experiment 16 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To calculate a manual red-cell concentration and explain the chamber-area and dilution factors.

Principle

A validated isotonic RBC diluent preserves countable red cells. Five group squares in the centre of the improved Neubauer grid have total area 0.20 mm^2; with depth 0.1 mm the counted volume is 0.020 µL. Red-cell concentration alone does not describe haemoglobin content or identify the cause of anaemia.

Requirements

- Improved Neubauer chamber and specified coverslip.
- Instructor-supplied control or specimen, calibrated pipettes and approved RBC diluent.
- Microscope and count sheet.

Procedure

1. Confirm dilution

Record the assigned dilution, commonly 1:200 in the teaching calculation. Use the laboratory-approved diluent and procedure.

2. Prepare the chamber

Seat the special coverslip and load the well-mixed dilution without bubbles or overflow. Allow the specified settling time.

3. Select counting groups

Identify the four corner and one central group squares within the central 1 mm^2 area. Each group is 0.04 mm^2 and contains sixteen small squares.

4. Count

Count all cells in the five selected groups with the same border rule. Record group totals and assess the second chamber as required.

5. Calculate and interpret

Use N × D ÷ 0.020. Report units and compare with the provided interval alongside Hb, Hct and red-cell indices.

Observations and Record

Group square	Cells counted	Record
Central-grid corner groups	____; ____; ____; ____	Subtotal: ____
Central group	____	Total N: ____
Dilution / concentration	D: ____	____ cells/µL

Worked Example

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

- Illustrative count: N = 480, D = 200. RBC = 480 × 200 ÷ 0.020 = 4,800,000 cells/µL.
- Thus the usual factor for this exact area and dilution is 10,000. A different area or dilution needs a different factor.
- 4,800,000/µL = 4.8 million/µL = 4.8 × 10^{12}/L.

Result

N = ____ and D = ____. RBC concentration = ____ cells/µL, equivalent to ____ × 10^{12}/L.

Precautions

- Do not count five large 1 mm^2 squares when the method calls for five central group squares.
- Mix before loading because cells can settle in the dilution.
- Do not equate an RBC count with a haemoglobin concentration or a confirmed diagnosis.

Viva Questions and Answers

1. Why use an isotonic diluent?

To help preserve red-cell integrity for counting.

2. What is the counted volume for five groups?

0.020 µL at a 0.1 mm chamber depth.

3. What makes the count factor change?

Dilution, counted area or chamber depth.

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