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WBC Count and Differential Count

Experiment 8 - Practical Notes

Name: _____ Roll no.: _____ Date: _____

Aim

To calculate a manual white-cell count and identify the major white-cell types on an instructor-prepared stained smear.

Principle

A validated WBC diluent allows leukocytes to be counted while reducing interference from red cells. In an improved Neubauer chamber, four corner squares have volume 0.4 μL . A differential describes the proportions of white-cell types; an absolute count combines the percentage with the total WBC count.

Requirements

- Improved Neubauer chamber, special coverslip, pipettes and laboratory-approved WBC diluent.
- Instructor-prepared blood control or supplied specimen and prepared stained smear.
- Microscope and counting record.

Procedure

1. Record the dilution

Use the supplied dilution or prepare the assigned dilution under instructor supervision. For $D = 20$, one original volume is included in twenty total volumes.

2. Load and count

Mix the diluted material, load the chamber correctly and allow the prescribed settling. Count the four large corner squares using one border rule. Count the second chamber as directed.

3. Calculate total WBC

Sum the cells and use $N \times D \div 0.4$ to obtain cells/ μL . Review chamber agreement before reporting.

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4. Examine the smear

Find a suitable monolayer region on the instructor-prepared smear. Use the prescribed microscope objective and a systematic path that avoids repeatedly counting the same cells.

5. Record the differential

Classify the assigned number of WBCs, commonly 100 for a teaching exercise. Sum the type counts and calculate percentages.

6. Interpret cautiously

Calculate selected absolute counts and compare with the supplied laboratory intervals. Explain that a small manual differential has counting uncertainty.

White-cell Recognition Guide

Cell type	Typical stained appearance	Broad role
Neutrophil	Segmented nucleus; fine granules	Innate defence
Lymphocyte	Dense rounded nucleus; variable cytoplasm	Adaptive immune functions
Monocyte	Larger cell; folded nucleus	Phagocytic and immune functions
Eosinophil	Often bilobed; conspicuous eosinophilic granules	Allergy and parasite-related responses
Basophil	Dark granules may obscure the nucleus	Allergic and inflammatory responses

Observations and Record

Count category	Number observed	Calculated result
Corner squares 1-4	___; ___; ___; ___	Total N = ___
Neutrophils / lymphocytes	___ / ___	___% / ___%
Monocytes / eosinophils / basophils	___ / ___ / ___	___% / ___% / ___%

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Worked Example

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

- Total WBC: $N = 136$, $D = 20$, counted volume = $0.4 \mu\text{L}$. $\text{WBC} = 136 \times 20 \div 0.4 = 6800/\mu\text{L}$.
- Illustrative differential of 100 cells: neutrophils 60, lymphocytes 30, monocytes 6, eosinophils 3, basophils 1; total = 100.
- Absolute neutrophils = $6800 \times 60/100 = 4080/\mu\text{L}$. A percentage alone can mislead when the total WBC count changes.

Result

Total WBC = ____ cells/ μL . Differential total = ____ cells. Absolute ____ count = ____ cells/ μL ; interpretation uses the supplied laboratory interval.

Precautions

- Do not confuse the four WBC corner squares with the five smaller RBC counting groups.
- Do not count in a thick smear region where cells overlap excessively.
- Apply specimen precautions and instructor disposal procedures; do not undertake unsupervised blood collection.

Viva Questions and Answers

1. Why calculate an absolute differential count?

It expresses the number of a particular WBC type per volume, rather than only its proportion.

2. What is the count factor at $D = 20$?

For four large squares, $\text{WBC} = N \times 50/\mu\text{L}$.

3. Can a differential alone diagnose infection?

No; the count must be considered with clinical assessment and other findings.

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