

Manual Cell / Particle Counts

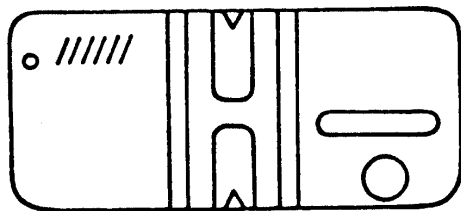
Even in this wonderful age of automated cell / particle counters, it may be necessary to perform a manual count. Reasons why manual counts may be needed include:

1. The specimen is too viscous for the instrument or may have clots, etc that could damage the equipment.
2. The number of cells is too high or too low to be within the instruments linearity.
3. The lab does not have a cell counting instrument designed for specimens other than EDTA whole blood.
4. The instrument is down / not available.

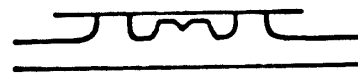
The Neubauer Hemacytometer

The hemacytometer is a glass counting chamber with two central raised platforms. Each of the platforms contains a ruled area / grid composed of nine large squares of equal size (1 mm x 1 mm)

On each side of the platforms are raised ridges on which a cover glass is placed. The space between the top of the platform and the cover glass over it is 0.1 mm.

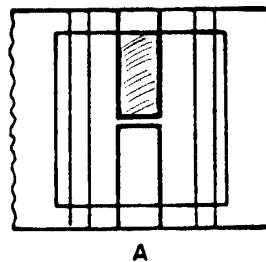


Neubauer hemacytometer

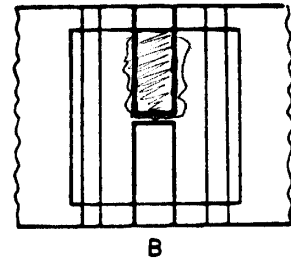


Neubauer hemacytometer (side view)

The well mixed fluid (or a dilution) is loaded into the hemacytometer, between the base and the cover glass. Figure A shows a properly filled chamber; the chamber labeled Figure B was improperly filled by being overfilled. It is also possible to underfill a chamber or introduce air bubbles. Chambers that are improperly filled must be recleaned and refilled.

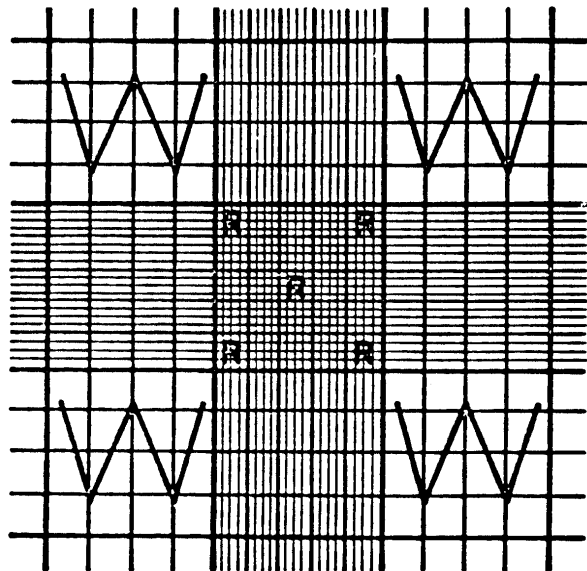


A



B

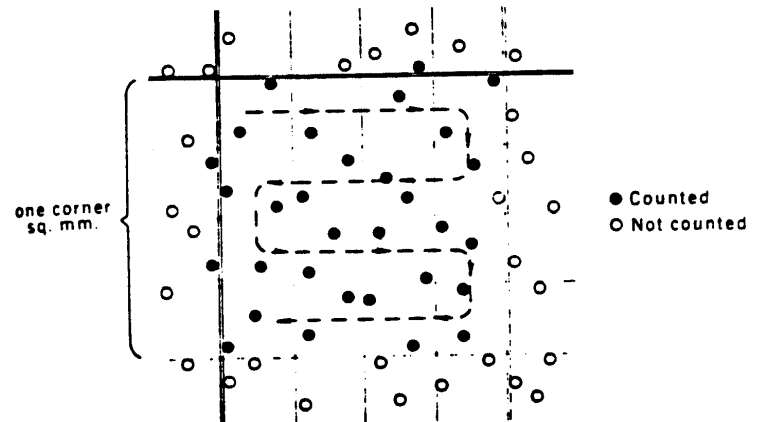
The entire ruled area of the platform (9 large squares) is 3 mm wide and 3 mm long, each of the nine large squares measures 1 mm by 1 mm and has a volume of 0.1 μL . In this drawing, the center square (the one that has 'R's in it) is divided into 25 small squares (5 columns across X 5 rows deep). These are called the small squares; they each have a volume of 0.004 μL . They are used when the expected count is high.



After loading the hemacytometer, allow it to sit in a dampened, covered petri dish for approximately five (5) minutes to allow the cells to settle onto one plane. Do not jar the hemacytometer or move the cover glass.

After the cells have settled into one plane the hemacytometer is placed on the microscope stage. Use the low power objective to locate the ruled / grid area. Once you are oriented, use the high power objective to count the cells in a methodical fashion as illustrated in the picture below. You should count any cell that touches the left side line or upper line, but never count those that touch a right side line or bottom line.

The number of squares you count (and whether you count large squares or small squares) is depended upon the number of cells present. For example, if there are not many cells present, count more cells, or even all of the 9 cells to improve accuracy. If there are many cells, count a few of the small squares - most commonly count the five small squares (ie the "R" squares).



Examples of white blood cells that are counted in a representative area.

Checking validity of the chamber counts.

A large variation in the number of cells counted on each side of the hemacytometer chamber would indicate possible error in dilution or technique. To determine whether there is too much variation, perform the following procedure.

- 1) Record the number of cells in each chamber (A and B).
- 2) Obtain difference (C) between A and B. Set that number aside.
- 3) Now calculate 2 S.D. as follows:

$$2 \text{ S.D.} = 2\sqrt{A + B}$$

- 4) If C is less than 2 S.D., the results are acceptable. If greater, then the counts must be repeated.

Example:

A = 430
B = 470
C = 40

$$2 \text{ S.D.} = 2\sqrt{470 + 430} = 2\sqrt{900} = 2 \times 30 = 60$$

C (40) is less than 2 S.D. (60) and therefore is acceptable.

Calculate the cells/ μL .

WBCs (counted in 4 corner large squares)

$$\text{WBC}/\mu\text{L} = \frac{\text{Average of cells} \times \text{Correction for dilution}}{\text{No. of squares counted} \times \text{Volume of one square}}$$

Average of Cells: Total the cells in the four large squares on each side. Average the two totals.

Correction for Dilution: Since the blood was initially diluted 1/100, the correction factor for dilution is 100.

of squares counted = 4

Volume of Square: One large square on the hemacytometer is 0.1 μL .

Example

Sum Side #1 = 27

Sum Side #2 = 29

Average Side #1 and #2 = 28

$$\text{WBC}/\mu\text{L} = \frac{28 \times 100}{4 \times .1} = 7,000/\mu\text{L} \text{ or } 7.0 \times 10^3/\mu\text{L}$$

RBCs (counted in 5 small "R" squares located in the center square)

$$\text{RBC}/\mu\text{L} = \frac{\text{Average of cells} \times \text{Correction for dilution}}{\text{No. of squares counted} \times \text{Volume of one square}}$$

For example:

Average number of cells counted = 152

Dilution = 1/200

Number of squares counted = 5

Volume of one of the 5 squares = 0.004 μL

$$\text{RBC}/\mu\text{L} = \frac{152 \times 200}{5 \times 0.004} = 1,520,000/\mu\text{L} \text{ or } 1.52 \times 10^6/\mu\text{L}$$

Platelets

Platelets are difficult to count. They are small and are hard to distinguish from dirt. They readily adhere to each other and also become easily attached to any foreign body. The use of EDTA as an anticoagulant helps to decrease the clumping of platelets. Capillary blood is less satisfactory.

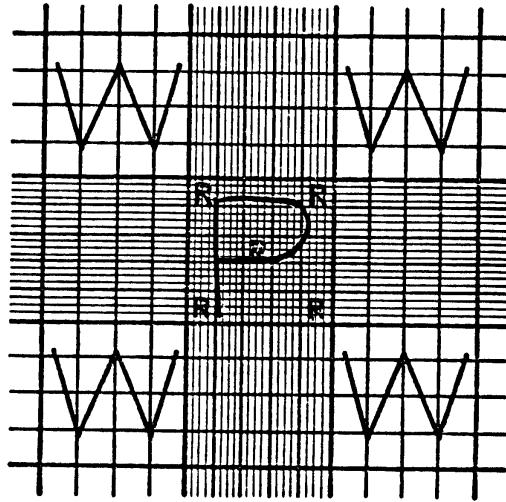
1 % ammonium oxalate can be used for the dilution. 20 μL of blood is diluted in 1.98 ml of ammonium oxalate for a 1/100 dilution. The red cells are lysed, and the number of platelets in the large center square of the hemacytometer (vol = 0.1/ μL) are counted. Results should be verified by performing a platelet estimation on the Wright-stained blood smear.

$$\text{Platelets}/\mu\text{l} = \frac{\text{average x dilution}}{\# \text{ of squares counted x volume of one square}}$$

dilution = 100

squares counted = 1

volume of one square = 0.1 μL



Neubauer Hemocytometer, counting area.

Study Questions

1. Calculate the following white cell count if the dilution 1/100. Four large corner squares are counted. Show your calculations.

Side #1

Square 1 = 30 white cells
 Square 2 = 28 white cells
 Square 3 = 25 white cells
 Square 4 = 27 white cells

Side #2

Square 1 = 27 white cells
 Square 2 = 23 white cells
 Square 3 = 24 white cells
 Square 4 = 32 white cells

2. Calculate the following white cell count if the dilution is 1/100. Four large corner squares are counted. Show your calculations.
 Total cells counted on one side = 36
 Total cells counted on the other side = 32
3. Calculate the following white cell count if the dilution is 1 / 20, and all 9 large squares are counted.
 Total cells counted on one side = 52
 Total cells counted on the other side = 60

4. You have performed a manual WBC and the counts from the two sides of the chamber are 232 and 268. Are the counts close enough to accept? Explain your reasoning and show your math calculations.

5. Once the diluted blood is plated onto the hemacytometer, it must set for five minutes before reading. Why?

6. What is the purpose of the dampened, covered petri dish?

7. Calculate the following manual RBC count, using the following information. Show your calculations.
Dilution = $1/200$
Five small (the "R" squares) were counted.
Number of RBC counted first side = 250
Number of RBC counted second side = 260

8. Calculate the following manual RBC count: Show your calculations.
Dilution = $1/200$
Five small (the "R" squares) were counted.
Number of RBC counted first side = 386
Number of RBC counted second side = 402

9. Calculate the number of platelets / μL using the following information: Show your calculations.
Dilution = $1/100$
Twenty-five small squares in the center large square were counted. ("P" area)
Side #1 = 207
Side #2 = 195

10. How can you double-check the manual platelet count?