

Manual Cell / Particle Counts

Points 40

Purpose

This clinical session is a review of manual cell or particle counts. Students should review related course materials and review reference resources in preparation for this clinical session.

Objectives

By the completion of this exercise, the student will be able to:

1. Describe or demonstrate the proper method of loading the hemacytometer chamber.
2. Describe or demonstrate the procedure for counting cells / particles in the hemacytometer.
3. Calculate cell / particle concentrations using appropriate recording format to report results.
4. Use quality control results to determine the acceptability of test results.
5. Answer study questions and submit the results to the instructor by the due date.

Supplemental References

Current hematology course textbook

Estridge, B.H., Reynolds, A.P., Basic Clinical Laboratory Techniques, 6th ed. Delmar Publishing

MLAB 1311 course Lecture and Lab Guides and course textbook(s)

Web resources:

YouTube: Loading a hemacytometer correctly - this is the old glass hemacytometer, in case you have these in your clinical lab setting

<http://www.youtube.com/watch?v=PPhE8I-dpp4&feature=related>

You Tube: Loading a disposable hemacytometer

<https://www.youtube.com/watch?v=ZBezFBaFwhk>

YouTube: Counting Cells

<http://www.youtube.com/watch?v=89SB1tAa6kg&feature=fvwrel>

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 instrument's 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.

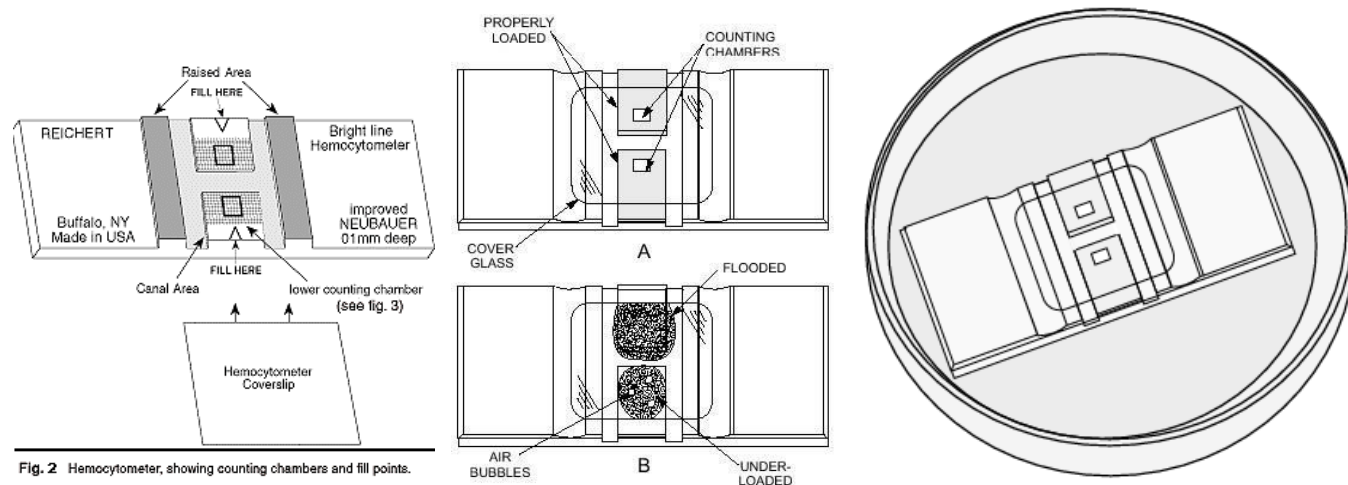


Fig. 2 Hemocytometer, showing counting chambers and fill points.

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.

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 re-cleaned and refilled.

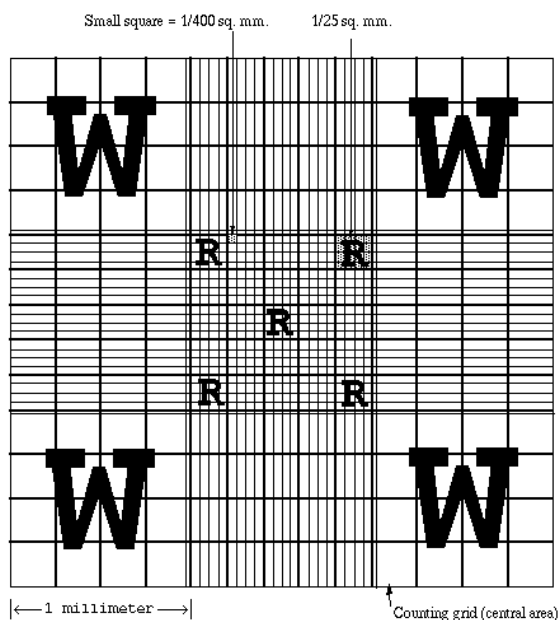
The entire ruled area of the platform (9 large squares) is 3 mm wide and 3 mm long, each of the nine large squares measures 1 mm by 1 mm and has a volume of 0.1 μ L. In this drawing, the center square (the one that has 'R's in it) is divided into 25 small squares (5 columns across X 5 rows deep). These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

The number of squares you count (and whether you count large squares or small squares) is dependent 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).

ACC MLT Program uses the disposable hemocytometer. This device does not require any equilibration. Use a 10 μ L pipette to load the sample to both sides of the plastic hemocytometer and place it on the microscope. Focus at 10x and read at 40x.



Counting the cells

To obtain valid reproducible results, it is important to count the fields in a prescribed order. When counting large squares (such as those marked "W" in the picture drawing above) The count should start at the upper most left square. After you have located this square using lpf, you will need to switch to hpf magnification. Use the pattern shown in the picture above to determine which cells are to be counted.

All cells that lie with the square and those touching the left or upper boundary are to be counted. Cells touching a right or lower boundary are not to be counted, even if they lie mostly in the square. Strict adherence to this pattern of counting is necessary to avoid double counting of cells or not counting those cells that should be counted.

Note: Depending on the manufacturer, you may find double or triple lines separating areas on the grid. When double lines separate the areas, the outer line is considered the boundary. When triple lines divide the areas, the middle line is considered the boundary.

As you move from square to square you must continuously, but gently, focus up and down using the microscope's fine adjustment knob to see the details of each cell.

- RBC – smooth, shiny surface, highly refractile, and may have a yellowish or reddish tinge to it. It may be a round shape or may be crenated (spiky) but its surface will still be smooth and shiny.
- WBC – have a rough or grainy surface, not very refractile. May have grayish or bluish tinge. Shape is generally round, but may have rougher or more irregular outer edges.

Platelets

Platelets are difficult to count. They are small shiny refractile objects; 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 produces less satisfactory results. 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. This diluent will also lyse the red cells making it easier to see the platelets. Standard protocol calls for manual counting of platelets to be performed in which the entire large center square of the hemacytometer (vol = 0.1/ μL) is counted. Platelet counts can be performed using a bright-field microscope, but are best counted using phase microscopy. Regardless of type of microscope, results should be verified by performing a platelet estimation on a Wright-stained blood smear.

Checking the validity of the chamber counts.

Each count (side 1 and side 2) must be within 10% of each other. The method may vary somewhat from laboratory to laboratory, but the consistency among the professionals is what is important. Here, at ACC, we will use the higher number for obtaining the ten percent.

Example:

Side 1 = 546

Side 2 = 650

$$650 \times 0.10 = 65$$

Subtract 65 from 650, which equals 585. This is the lowest count acceptable. The side one was 546; therefore, these values cannot be used and the count must be repeated on both sides. This is the same for all types of cells being counted.

Calculating the (RBCs or WBCs) cells / μL

Basic Cell/Particle counting formula

$$\# \text{ Cells} / \mu\text{L} = \frac{\text{AVE \# cells} \times \text{Correction for dilution}}{\# \text{ Squares counted} \times \text{Volume of one of those squares}}$$

EXAMPLE 1 – WBCs counted in 4 corner large squares. Blood sample diluted 1/100.

Side 1 = 27

Side 2 = 29

Average = 28

$$WBC/\mu L = \frac{28 \times 100}{4 \times 0.1} = WBC/\mu L = \frac{28 \times 100}{4 \times 0.1} = 7000 \text{ WBCs/uL or } 7.0 \times 10^3 \text{ WBCs/uL}$$

EXAMPLE 2 – RBCs counted in 5 small (R) squares.

Dilution 1/200.

Average number of cells counted in the two chambers = 152

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

EXAMPLE 3 – Platelets counted in the 1 large center square (P) square. Dilution 1/100. Average number of platelets counted in the two chambers = 252

$$Platelets/\mu L = \frac{252 \times 100}{1 \times 0.1} = Platelets/\mu L = \frac{252 \times 100}{1 \times 0.1} = 252,000 \text{ platelets/uL or } 2.52 \times 10^5 \text{ platelets/uL}$$

Manual Cell / Particle Counts Study Questions**Student Name** _____ **Date** _____

____ / 40 points (5 points each)

Instructions: Answer the questions as appropriate; unless otherwise stated, each question is worth three points. Study Questions are due by the end of the following lab period. Using lecture notes, reading assignments and information presented in this clinical session, answer the following questions.

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

<u>Side 1</u>	<u>Side 2</u>
Square 1 = 30 white blood cells	Square 1 = 27 white blood cells
Square 2 = 28 white blood cells	Square 2 = 23 white blood cells
Square 3 = 25 white blood cells	Square 3 = 24 white blood cells
Square 4 = 27 white blood cells	Square 4 = 32 white blood cells

2. Calculate the following white blood 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 side 1 = 160 Side 2 = 158

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. Calculate the following manual RBC count, using the following information. Show your calculations.

Dilution = No dilution
Five small (the "R" squares) were counted.
Number of RBC counted first side = 250
Number of RBC counted second side = 260

6. 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

7. 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

8. Explain the procedure to double-check the manual platelet count?