

EXERCISE # 7**CELL COUNTING BY HEMOCYTOMETER**

The Hemocytometer is a thick crystal slide with the size of a glass slide. (30 x 70 mm and 4 mm thickness). In a simple counting chamber, the central area is where cell counts are performed. The chamber has three parts. The central part, where the counting grid has been set on the glass. Double chambers are most common than simple chamber. In this case, the chamber has two counting areas than can be loaded independently.

Despite the fact of the recent technical development of scientific laboratories, the hemocytometer remains the most common method used for cell counting around the world.

MATERIALS

- Microscope
- Hemocytometer and coverslip
- Suspension of bacterial cell or fungal spores

PROCEDURE**STEP 1. SAMPLE PREPARATION**

1. Depending on the type of sample, a preparation of a dilution with a suitable concentration should be prepared for cell counting.
2. Typically, the concentration range for a cell count with hemocytometer is between 250,000 cells / mL and 2.5 million cells / mL.
3. It is recommended for the dilution concentration to be around 10^6 cells / mL (1 millón cells / mL) applying the required dilutions.
4. With concentrations below 250,000 cells per mL, ($2.5 * 10^5$ cells / mL) the amount of cells counted will not be enough to obtain a fair estimation of the original concentration.
5. Above 2.5 million cells / mL ($2.5 * 10^6$), the probability of counting errors increases as well as the time and effort required to achieve a reliable cell count.
6. Above 2.5 million, it is preferable diluting the sample to obtain a final concentration closer to the optimum 1 million per mL. It is important to write down the dilution performed to the original sample.

STEP 2. INTRODUCING THE SAMPLE INTO THE HEMOCYTOMETER

Take 10 μ L of dilution prepare in STEP 1 with the micropipette.

1. Put the glass cover on the hemocytometer central area. Use a flat surface to place the chamber, like a table or a workbench.
2. Put a disposable tip at the end of the micropipette.
3. Adjust the micropipette to suck 10 μ L. You can adjust it by turning the upper plunger roulette to select the required pipetting volume.
4. Introduce the micropipette tip on the dilution previously prepared (STEP 1)
5. Push the pipette plunger slowly until you feel it has arrived to the end of its travel.
6. Remove the pipette tip from the dilution, and bring it to the hemocytometer.

When the pipette is loaded, it must always be held in vertical position.

7. Place pipette tip close to the glass cover edge, right at the center of the hemocytometer.
8. Release the plunger slowly watching how the liquid enters the chamber uniformly, being absorbed by capillarity.
9. In case of the appearance of bubbles, or that the glass cover has moved, repeat the operation.

STEP 3. MICROSCOPE SET UP AND FOCUS

1. Place the hemocytometer on the microscope stage. If the microscope has a fixing clamp, fix the hemocytometer.
2. Turn on the microscope light.
3. Focus the microscope until you can see a sharp image of the cells looking through the eyepiece and adjusting the stage.
4. Look for the first counting grid square where the cell count will start. In this example, 5 big squares from hemocytometer will be counted.
5. Start counting the cells in the first square. Different laboratories have different counting protocols, but there is a popular unwritten rule that states: "Cells touching the upper and left limits should be counted, unlike cells touching the lower and right limits which should not be taken into account"

In case of high cell concentration, it will become very easy to get lost when counting cells. In this case, a counting technique in zigzag is used.

6. Write down the amount of cells counted in the first square.

7. Repeat the process for the remaining squares, writing down the counting results from all of them. The higher the number of cells counted, the higher the accuracy of the measurement.

STEP 4: CONCENTRATION CALCULATION

- We apply the formula for the calculation of the concentration
- $\text{Concentration (cell / mL)} = \text{Number of cells} / \text{Volume (in mL)}$
- The number of cells will be the sum of all the counted cells in all squares counted. The volume will be the total volume of all the squares counted. Since the volume of 1 big square is:
 - $\text{cm} \times 0.1 \text{ cm} = 0.01 \text{ cm}^2$ of area counted.
- Since the depth of the chamber is 0.1mm
 - $\text{mm} = 0.01 \text{ cm}$
 - $\text{cm}^2 \times 0.01 \text{ cm} = 0.0001 \text{ cm}^3 = 0.0001 \text{ mL} = 0.1 \mu\text{L}$
- So, for the Hemocytometer, the formula used when counting in the big squares.
- $\text{Concentration} = \text{Number of cells} \times 10,000 / \text{Number of squares}$
- In case a dilution was applied, the concentration obtained should be converted to the original concentration before the dilution. In this case, the concentration should be divided by the dilution applied.
- The formula will be:
- $\text{Concentration} = \text{Number of Cells} \times 10,000 / \text{Number of square} \times \text{dilution}$
- Example: For a 1:10 dilution. Dilution = 0.1 for a 1:100. Dilution = 0.01

ERROR

Errors in the range of 20%-30% are common in this method due to pipetting errors, statistical errors, chamber volume errors, and errors from volume of sample introduced into the chamber. Even though, the hemocytometer remains the most widely used cell counting method in the world.

QUESTIONS

1. What is the use of hemocytometer?
2. How do you use a hemocytometer?
3. How many squares are in a hemocytometer?