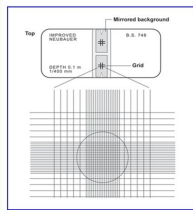


Cell Counting by Hemocytometer

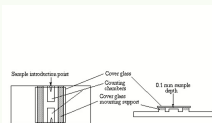
Cell counting by hemacytometer



cell count
= average of cell number
× dilution factor of cell suspension × 10 000

- Clean the chamber and cover slip with alcohol. Dry and fix the cover slip in position.
- Harvest the cells. Add 10 μL of the cells to the hemocytometer. Do not need to overfill.
- Place the chamber in the inverted microscope under a 10X objective. Use phase contrast to distinguish the cells.
- Count the cells in the large, central gridded square (1 mm²). The gridded square is circled in the graphic. Multiply by 10⁴ to estimate the number of cells per mL. Prepare duplicate samples and average the count.

Hemocytometer Counting Chamber



Procedure

-For an accurate cell count to be obtained, a uniform suspension containing single cells is necessary.

Obtain a uniform suspension of cells:

1. Follow the trypsinization/trypsin neutralization protocol for the specific cell type.
2. Place the cell suspension in a suitably-sized conical centrifuge tube.
3. a) Pipette the cell suspension up and down in the tube 5-7 times using a pipette with a small bore (5 ml or 10 ml pipette).
3. b) For cells thawed from cryopreservation (in 1ml cryopreservation medium), pipette up and down 7-10 times using a one ml pipette.

Prepare a 1:1 dilution of the cell suspension in trypan blue:

Approximately 10 microliters of cell suspension will be required to charge one chamber of the hemocytometer.

4. In a conical microfuge tube, add 10 microliters of 0.4% trypan blue solution.
5. Gently swirl (finger vortex) the cell suspension and remove 10 microliters of it using sterile technique.

Procedure (cont)

6. Combine the 10 microliters of cell suspension with the 10 microliters of trypan blue in the microfuge tube.
7. Pipette up and down several times to ensure a uniform cell suspension using the same pipette tip and allow to stand for 5-15 minutes.

Load the haemocytometer:

The chamber should not be overfilled OR underfilled.

8. Moisten and affix cover slip to the hemocytometer.
9. Ensure the cover slip and hemocytometer are clean and grease-free (use alcohol to clean).
10. A small amount of trypan blue-cell suspension is transferred to one of the chambers of the hemocytometer by carefully touching the cover slip at its edge with the pipette tip and allowing each chamber to fill by capillary action.

Determine the number of cells (total and viable):

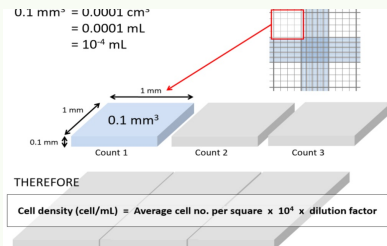
11. View the cells under a microscope at 100x magnification.
12. Under the microscope, you should see a grid of 9 squares.
13. Focus the microscope on one of the 4 outer squares in the grid.
14. The square should contain 16 smaller squares.
15. Count all the cells in the four 1 mm corner squares.
16. If there are too many or too few cells to count, repeat the procedure, either concentrating or diluting the original suspension as appropriate.

Types of Counting Method

1. Logical count
 - count the cells in the four corner squares and the middle square of the hemocytometer's grid
 - can predict the cell count of the rest of the squares
 - to make sure that the count is consistent and that cells aren't counted twice, you should only count cells on two of the square sides



Cell Density



Trypan Blue

- "vital stain", excluded dead cells from living cells

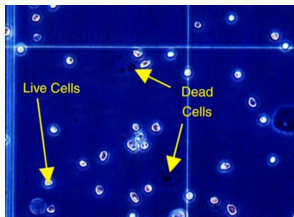
- living cells appear colourless and bright (refractile) under phase contrast.

-> charged trypan blue molecules do not penetrate into viable cells because of the integrity of the membrane

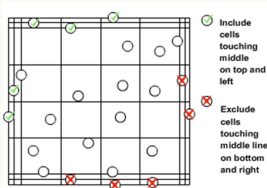
- dead cells stain blue and are non-refractile

-> non-viable cells, allow the free entry of trypan blue, thereby staining the cell interior blue

Living Cells and Dead Cells under the Microscope



Counting Cells



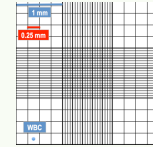
Corner Squares

- each of the 16 smaller squares will be $1 \text{ mm} / 4 = 0.25 \text{ mm}$ in width and $0.25 \text{ mm} \times 0.25 \text{ mm} = 0.0625 \text{ mm}^2$ in area (or $1 \text{ mm}^2 / 16 = 0.0625 \text{ mm}^2$)

- cells that are $10 \mu\text{m}$ or more should be counted in these corner squares

- i.e. white blood cells

When to Use Corner Squares



Central Square

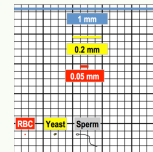
- each of the 25 smaller squares will be $1 \text{ mm} / 5 = 0.2 \text{ mm}$ in width and $0.2 \text{ mm} \times 0.2 \text{ mm} = 0.04 \text{ mm}^2$ in area (or $1 \text{ mm}^2 / 25 = 0.04 \text{ mm}^2$)

- each of the 25 smaller squares contains 16 even smaller squares which measure: $0.2 \text{ mm} / 4 = 0.05 \text{ mm}$ in width and $0.05 \text{ mm} \times 0.05 \text{ mm} = 0.0025 \text{ mm}^2 = 2500 \mu\text{m}^2$ (or $0.04 \text{ mm}^2 / 16 = 0.0025 \text{ mm}^2$)

- Cells that are $10 \mu\text{m}$ or smaller should be counted in the central square – sometimes even in one of the smaller squares inside the central square

- i.e. red blood cells, platelets, most types of yeast, and sperm cells

When to Use Central Squares



Calculation

- count 4 corner squares and calculate the average

- each large square of the haemocytometer, with cover slip in place, represents a total volume of 0.1 mm^3 ($1.0 \text{ mm} \times 1.0 \text{ mm} \times 0.1 \text{ mm}$) or 10^{-4} cm^3

- $1 \text{ cm}^3 = 1 \text{ mL}$

% Cell Viability:

$$= \left[\frac{\text{Total Viable cells (Unstained)}}{\text{Total cells (Viable + Dead)}} \right] \times 100\%$$

Viable Cells/ml:

$$= \text{Average viable cell count per square} \times \text{Dilution Factor} \times 10^4$$

Average viable cell count per square:

$$= \frac{\text{Total number of viable cells from the number of squares}}{\text{number of squares}}$$

Dilution Factor:

Calculation (cont)

= Total Volume (Volume of sample + Volume of diluting liquid) /
Volume of sample.

Total viable cells/Sample:

= Viable Cells/ml x The original volume of fluid from which the cell
sample was removed.

Volume of media needed:

= (Number of cells needed/Total number of viable cells) x 10^4

Cell density (cell/mL):

= average cell count per square x 10^4 x dilution factor

How to count: <https://www.youtube.com/watch?v=pP0xERLUhyc>



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cheatography.com/woozing/

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