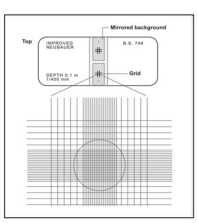
Cell Counting by Hemocytometer

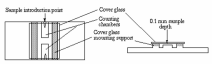
Cell counting by hemacytometer



- 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 hemacytometer. 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 mm^2). The gridded square is circled in the graphic. Multiply by 10^4 to estimate the number of cells per mL. Prepare duplicate samples and average the count.

$$\text{cell count} = \text{avarage of cell number} \times \text{dilution factor of cell suspension} \times 10\,000$$

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 typsinization/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.

6. Combine the 10 microliters of cell suspension with the 10 microliters of trypan blue in the microfuge tube.

Procedure (cont)

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

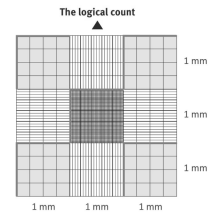
1. Logical count	-count the cells in the four corner squares and the middle square of the hemocytometer's grid
	-can predict the cell count of the rest of the squares
	-to make sure that the count is consistent and that cells aren't counted twice, you should only count cells on two of the square sides
	-i.e. choose the cells on the left and top borders of the square while the ones on the right and top aren't included or the other way round

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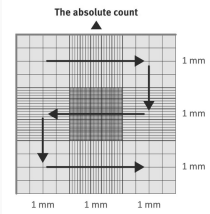
Types of Counting Method (cont)

	-be consistent throughout your counting procedure and to apply the same strategy every time, so you can compare your data
2. Absolute count	-count all the 9 squares in the hemocytometer
	-ensures that you count everything and avoid error from the generalization-assumption mentioned above
	-count the cells in the squares while following a zig-zag pattern
	-useful when there's a high cell concentration in the sample because of a pattern that's easy to follow
3. Quick count	-in a rush to do a cell count
	-only count the cells in the top left square as well as the cells in the bottom right square
	-won't get a result that is as representative as the other two methods mentioned above, but it can be a good way to spot-check your cell culture when in a hurry

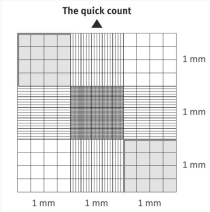
The Logical Count



The Absolute Count



The Quick Count



Cell Counting Accuracy

The total number of cells overlying one 1 mm2 should be between 15 and 50

-If the number of cells per 1 mm2 exceeds 50, dilute the sample and count again

-If the number of cells per 1 mm2 is less than 15, use a less diluted sample

-If less dilute samples are not available, count cells on both sides of the hemocytometer (8 x 1 mm2 areas)

Keep a separate count of viable and non-viable cells

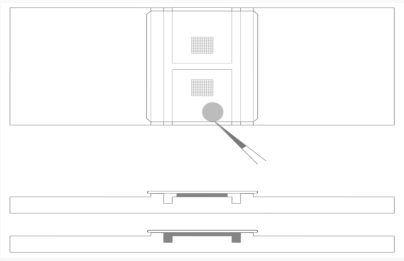
-If > 25% of cells are non-viable, the culture is not being maintained on the appropriate amount of media.

-Reincubate the culture and adjust the volume of media according to the confluency of the cells and the appearance of the media.

-Include cells on top and left touching middle line.

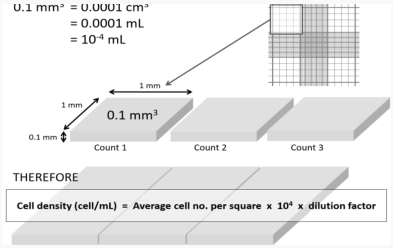
-The cells touching middle line at bottom and right are not counted.

Loading Cell Suspension Mixture



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Cell Density



Trypan Blue

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

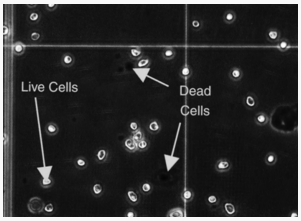
-living cells appear colourless and bright (refractile) under phase contrast.

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

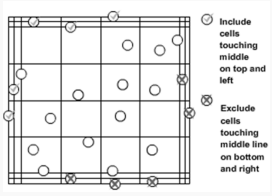
-dead cells stain blue and are non-refractile

->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



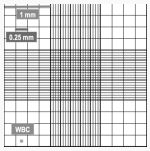
Corner Squares

-each of the 16 smaller squares will be 1 mm/4 = 0.25 mm in width and 0.25 mm x 0.25 mm = 0.0625 mm2 in area (or 1 mm2/16 = 0.0625 mm2)

-cells that are 10 µm or more should be counted in these corner squares

-i.e. white blood cells

When to Use Corner Squares



Central Square

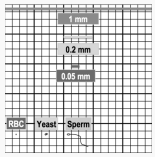
-each of the 25 smaller squares will be 1 mm/5 = 0.2 mm in width and 0.2 mm x 0.2 mm = 0.04 mm2 in area (or 1 mm 2/25 = 0.04 mm2)

-each of the 25 smaller squares contains 16 even smaller squares which measure: 0.2 mm/4 = 0.05 mm in width and 0.05 mm x 0.05 mm = 0.0025 mm2 = 2500 µm2 (or 0.04 mm2/16 = 0.0025 mm2)

-Cells that are 10 µm 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 mm3 (1.0mm X1.0mmX 0.1mm) or 10-4 cm3

-1 cm3 = 1 mL

% Cell Viability:

= [Total Viable cells (Unstained) / Total cells (Viable + Dead)] X 100%

Viable Cells/ml:

= Average viable cell count per square x Dilution Factor x 10^4

Average viable cell count per square:

= Total number of viable cells from the number of squares / number of squares

Dilution Factor:

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

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Calculation (cont)

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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