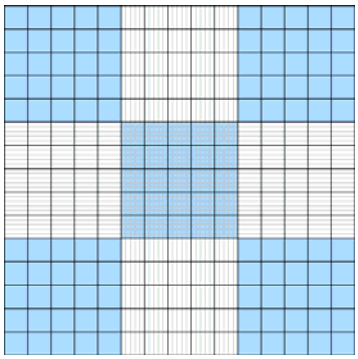
Creighton UNIVERSITY

Cell Counts using a Hemacytometer

A hemacytometer is a microscope chamber slide with a small (3mm x 3mm) square etched onto the surface. The slide has a coverslip, which rests exactly 0.1 mm above the slide. Cells in suspension are introduced into this area, and then counted.



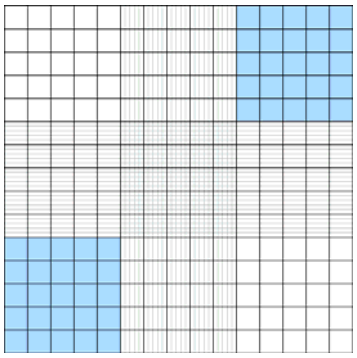
The etched square on the slide can be divided into 9 "large" areas of equal size. Each of these 9 "large areas" is 1mm square. Each box in the center area is further subdivided into 25 "smaller" areas.

As each of the "large" squares is 1mm x 1mm, and the area between the slide and the coverslip is 0.1mm, then the volume above each "large" square is 0.1 mm^3.

When you count the cells in one of these "large" squares, you are counting the number of cells in the Trypan Blue suspension in an area of 1mm x 1mm x 0.1mm (or 0.1mm^3). Ten (10) of these areas stacked on top of each other would be 1mm^3. Ten (10) of these lined up next to each other would be 1cm long x 1mm wide x 1mm tall. Ten (10) of these would be 1cm x 1cm x 1mm, and 10 of these would be 1cm x 1cm x 1cm (1cm^3), or 1ml. Thus to get from the number counted to the number of cells/ml, you need to multiply by 10,000.

In addition, you need to take into consideration any dilution of the cells. Generally cells are diluted 1:1 (vol:vol) with Trypan Blue (a dilution factor of 2). They are also usually diluted in PBS to get a countable number of cells in each grid. A "countable" number should be between 25 and 100 cells in each "large" square. These dilutions must also be accounted for.

To perform a cell count using the hemacytometer:



1. Combine 50µl of cells (at an unknown concentration) with 50µl of Trypan Blue Working Stock.
2. Fill the hemacytometer by capillary action. Place the pipette filled with Cells/Trypan Blue at notch at the edge of the hemacytometer and slowly pipette the cells out allowing the chamber to fill itself. Don't over or under fill the chamber.
3. Count the number of cells in 2 of the outer "large" squares as shown in the diagram at right.
4. Add these counts together and divide by 2 to get an average.
5. The cell concentration is then calculated as follows:

Cell concentration (in cells/ml) = average count x 2 x 10,000 x dilution factor of original cells

Example:

You have a 3ml of a cell suspension. You dilute it by adding 50ul of cells to 200ul of PBS. You then take 50µl of this dilution and combine it with 50µl of Trypan Blue and count it. You count two "large" squares (1mm x 1mm; 0.1mm tall) and get counts of 56 and 59 (average 57.5).

57.5	←	average counts per square
x 2	←	dilution with Trypan Blue
115	←	cells per 0.1 mm^3
x 10	←	to get to ….
1150	←	cells per 1.0 mm^3
x 1000	←	to get to ….
$1.15x10^6$	←	cells per cm^3 (= cells/ml)
x 5	←	dilution factor from original cell suspension
$5.75x10^6$	←	cells/ml in original suspension
x 3	←	volume of original cell suspension
$1.73x10^7$	←	total cells in original cell suspension