SOP: SP067.2

Updated: 7/8/22 KE

Counting cells with a Hemocytometer

Materials and Reagents:

1. Cell suspension
2. 10 µL pipet and filter tips
3. Hemocytometer
4. Light microscope
5. Manual cell counter
6. Ethanol 70%
7. Trypan blue (0.4%, SIGMA Cat# T8154)
8. Kim Wipes

Protocol:

1. _____ Re-suspend cell culture well and remove a small aliquot (~100-500 µL) for counting (in 0.65 mL tube).

2. _____ In a tube, mix 10 µL of aliquoted cell suspension (***mix cell suspension aliquot well beforehand***) and 10 µL of the 0.4% trypan blue dye (***vortex dye beforehand***) (Notes 1 & 2).

3. _____ Clean the hemocytometer with ethanol 70% and Kim Wipes.

4. _____ Place a coverslip on top of hemocytometer.

5. _____Fill one side of the chamber with the stained cell suspension. To do so, take 10 µL from the stained cell suspension and slowly dispense it in the sample introduction point (V-shaped place). Allowing that the chamber be filled by capillary action (Note 3).

6. _____Count the cells in the four corners of the hemocytometer (each of these contain 16 smaller square divisions). Use the light microscope 10X objective. Dead cells will take up the dye, appearing blue (Notes 4 & 5).

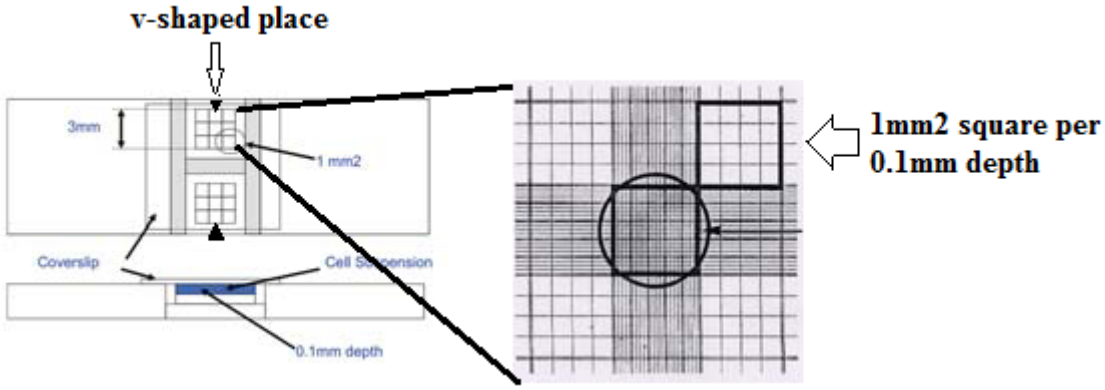
7. _____Count the number of dead and live cells (separately) (Notes 6 & 7).

8. _____Clean the hemocytometer with ethanol 70% and Kim Wipes and place back in the case for storage at room temperature.

Notes:

1. Trypan blue is very toxic and potentially carcinogenic. Always wear gloves, and a lab coat and goggles. Anything that comes in contact with the dye must go in biohazard trash. The EVE™ counting slides can be discarded in a biohazardous sharps containers.
2. Some protocols recommend incubating *cell suspension + trypan blue dye* mixture before counting. The old Dobos lab protocol (SOP: SP067.1) suggested to incubate for 2 min, room temperature. See references for more information/guidelines.

3.



4. If left incubating in the trypan blue dye for an extended period of time, live cells will also take up the dye.
5. Make sure to stay consistent with counting techniques. Read cells within squares and on top + right boarders. See references for more on how to discern which cells to count.
6. To calculate the ***average number*** of cells (live or dead) in each square, multiply by 2 (dilution factor (DF) from the ***cell mix suspension + trypan blue dye***), then multiply by 10,000. The final number will be in ***cells per milliliter***.

$$\frac{\text{Number of counted cells in the 4 corner squares}}{4} \times \text{DF} \times 10{,}000 = \text{Cells}/\text{ml}$$

7. The **live cell and *total* live cell counts** are important for continual growth (splitting, feeding, etc.) and freezing of cells. Make sure to note any additional dilutions (in addition to the 1:1 with the ***cell suspension + trypan blue dye mix).*** Make sure to note the total cell culture volume (that the cell suspension aliquot was taken from). Below are some important calculations:

   (Live cell count/Total cell count) x 100 = % viability

   Total cell count (live + dead cells/mL) x Total volume of cell culture (mL) = total cell count (cells)

   Live cell count (cells/mL) x Total volume (mL) = total LIVE cell count (cells)

References:

1. http://home.sandiego.edu/~josephprovost/Hemocytometer%20Cell%20Counting%20Protocol.pdf
2. https://med.wmich.edu/sites/default/files/Hemacytometer_Cell_Counting.pdf