HEMOCYTOMETER CELL COUNT AND TRYPAN BLUE CELL VIABILITY

Created by: Jason Fan
Date: January 28th, 2016
Bowdish Lab, McMaster University
Hamilton, ON, Canada
www.bowdish.ca

Background

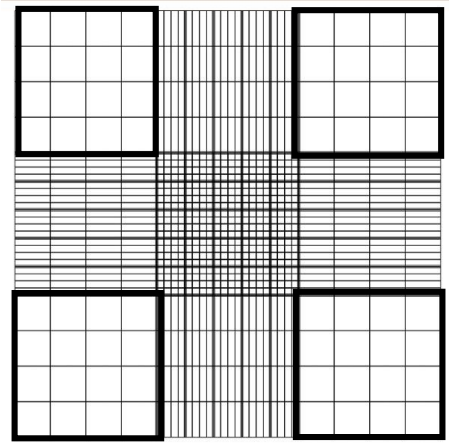
- Trypan blue staining is commonly described as a dye exclusion method. Live cells do not allow the dye to permeate the cell membrane and thus, the dye is excluded. Conversely, dead cells allow the dye to permeate and appear blue.

Materials

- Hemocytometer and coverslips
- Trypan Blue

Protocol

- Preparatory Work:
    - Cells must be collected and suspended in growth media
    - Aliquot 100 μl cell suspension into a separate Eppendorf tube
    - Add 10 μl trypan blue and mix well
        - Trypan blue is a carcinogen – work carefully

1. Add 10 μl of mixture into the groove of the hemocytometer, in between the coverslip
    a. By capillary action, the sample will fill the hemocytometer
2. 
    a. Count the number of **live** (cells not stained blue) in each outlined grid
    b. Do not count cells on the perimeter of the top and left side of each grid
3. Average the cell count from each grid
4. Multiple by 10^4 and 10 (to account for the 1:10 dilution) = cells/ml

Example Results

Below is an example of potential calculations:

$$\text{Average Cell Count} = 170 * 10^4 = 1.7 \text{ x } 10^6 \frac{\text{cells}}{\text{ml}} * 10\text{x dilution} = 1.7 \text{ x } 10^7 \frac{\text{cells}}{\text{ml}}$$

$$\text{Cell Viability \%} = \frac{\text{Number of Live Cells}}{(\text{Number of Live Cells} + \text{Number of Dead Cells})}$$

- The number of dead cells is estimated by the same fashion as live cells but **only cells stained blue** are counted