SOP NUMBER: 290
TITLE: Cell Counting and Viability Determination

Background:

Because the hemocytometer has an exact volume under the coverslip, one can determine the concentration (cells/ml) of live and dead cells in the chamber. The cell concentration of the original cell suspension will be the same as that of the chamber – except for any dilutions made. Dead cells take up the dye, trypan blue, and appear blue under the microscope. Living cells exclude tyrpan blue, and appear white. Thus, the percentage of viable cells can be calculated.

Objective:

To determine the cell concentration (cells/ml), the percentage of living and dead cells, and the total number of cells in a cell suspension. NOTE: Adherent cells must be trypsinized and resuspended prior to using this protocol.

I. Materials:

A. <u>Equipment</u>
Inverted microscope with 10X objective
Pipetman, 1-20 μl
Pipetman, 1-200 μl

B. <u>Supplies</u>
Hemacytometer with cover glass
Nitrile gloves
Plates, sterile 96-well
Pipette tips, 10-200 μl
1 ml sterile serological pipets

C. <u>Chemicals</u>
Trypan blue stain 0.4%

II. Method:

1. Prepare clean, dry hemocytometer with the cover slip.
2. Note the volume of the cell suspension in milliliters.
3. Mix the cell suspension thoroughly with a sterile serological pipet. Immediately proceed to step 4.
4. Transfer 100 μl (0.1 ml) of the cell suspension into one well of a 96 well plate using a 1ml sterile serological pipet.
5. Add 100 μl (0.1 ml) (an equal volume) of 0.4% (w/v) Trypan blue to the cell sample using a 1 ml serological pipet.
6. Mix cells and trypan blue solution thoroughly using an R-200 pipetman set for 100 μl by pipeting up and down five times. This is a 1:2 dilution of the cells.
7. Transfer ~20 μl of the cell mixture to one or both chambers of the hemocytometer using an R-20 pipetman. Allow the chamber to fill by capillary action. Do not under-fill or over-fill the chamber.

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8. Using the 10X objective, focus on the gridlines of the chamber. Count the viable cells (not blue) in four corner squares of one chamber. Cells that lie on the lines should only be counted if they are toughing the top and left-hand lines of each corner square. If the cell number in one square is vastly more than 50, take a new 0.1 ml sample of the cell suspension and add 0.9 ml of trypan blue. This is a 1:10 dilution of the cells. If the cell number per square is very low (<5) then the original suspension should be spun down and resuspended in a smaller volume.

9. Count the nonviable cells (blue) in four corner squares of one chamber.

10. Determine the viable cell density of the original mixture according to the following formula:

%Viability = $\frac{\text{Total number of viable cells}}{\text{Total number of viable and nonviable cells}}$ X 100

Cells/ml = $\frac{\text{Total viable cells counted in 4 squares}}{4}$ X 10,000

or

Cells/ml = $\frac{\text{Total viable cells counted in 5 squares}}{5}$ X 10,000

etc.

Total number of cells in the original solution = [Cells/ml] X [Number of mls in the original solution (step 1)] X [any dilution made]*

*A dilution of 2 is always made when an equal volume of trypan blue is added. Additional dilutions are made when the number of cells in one square is vastly more than 50.

Example						Total	
	L	28	30	26	29		L 113
	D	1	3	2	0		D 6

Determine "% viability" $= \frac{113}{113+6} = \frac{113}{119} = 0.95 \text{ x}100 = 95\%$

Determine "cells/ml" $= \frac{113}{4} = 28$ 28 x 2 x 10,000 = 560,000 = $5.6\text{x}10^5$ cells/ml

↑ Dilution made in trypan blue

Determine "total cells" = $5.6 \text{ x } 10^5$ cells/ml x 5 mls =$2.8 \text{ x}10^6$ cells/ml
Note: cells in total of 5 mls when counted

11. Clean the hemocytometer by rinsing with dH2O then 70% IPA and drying thoroughly with a soft wipe.

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