CELL COUNT AND VIABILITY

MATERIALS:
Hemocytometer with coverslip
0.1% Trypan Blue solution

PROTOCOL:
Prepare a cell suspension in the appropriate plating media. Make certain the cells are well dispersed by vigorously pipetting the cell suspension.

1. In a 96 well plate, add **12 µL 0.1% Trypan Blue solution** to **12 µL** of the cell suspension and mix well.

2. Place coverslip on hemocytometer. Transfer **12 µL of trypan blue/cell suspension solution** into a chamber of the hemocytometer. This can be accomplished by touching the tip of the pipette tip to the edge of the coverslip and allowing the chamber to fill by capillary action.

3. Count all the cells in the four 1mm^2 corner squares. Nonviable cells stain blue. Keep separate counts of viable and nonviable cells. Count cells falling on the perimeter on only two adjacent sides (see diagram).

DIAGRAM II:
STANDARD HEMOCYTOMETER CHAMBER

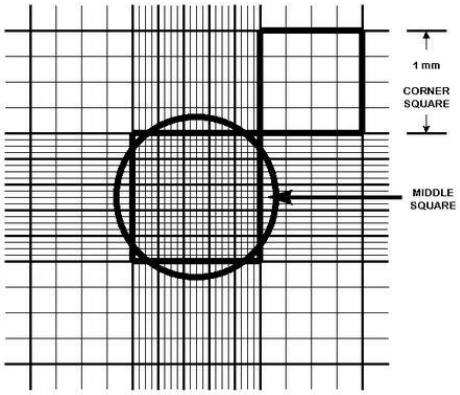
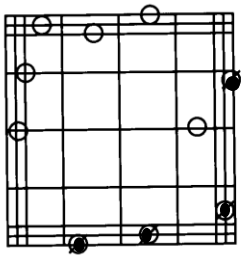


DIAGRAM III
CORNER SQUARE (ENLARGEMENT)



Count cells on top and left touching middle line ( ). Do not count cells touching middle line at bottom and right ( ).

4. If fewer than 20 cells/square or greater than 50 cells/square are observed, repeat the cell count after adjusting to an appropriate dilution.

5. Each square of the hemocytometer, with the coverslip in place, represents a total volume of 0.1 mm^3 or 10^{-4} cm^3. Since 1 cm^3 is equivalent to 1 mL, the subsequent cell concentration per mL (and the total number of cells) can be determined thusly:

CELLS PER ML = the average count per square x dilution factor (in this case 2) x 10^4

TOTAL CELLS = cells per mL x the original volume of fluid from which cell sample was removed.

6.**% CELL VIABILITY** = total viable cells (unstained) / by total cells (stained and unstained) x 100.