

Quantifying Viable Cells

Prepare Hemocytometer

Note grid pattern and keep your bearing and scale clearly in mind (i.e. what are the exact dimensions of the square you are looking at?)

Clean hemocytometer with EtOH and dry with lint free cloth. Helps to ensure minimum artifacts.

Load Hemocytometer:

Trypan blue is a vitality stain, and is excluded, so blue cells are dying or dead.

Add trypan blue solution (0.25 % (is this correct?) is physiological saline) into cells at a 1:10 dilution. Place pipette at edge of hemocytometer at groove near interface of hemocytometer and cover slip.

Techniques note: If cells are loaded onto hemocytometer, and not immediately counted, placed into petri dish with moist towel in petri dish.

Techniques note: After thawing, wait for 10-15 minutes, as cells immediately out of freezing may take up trypan and still be vital. MTT is a vital stain, and if mitochondria are healthy, color will increase.

Count cells microscopically and identify viable cells.

Identify optimum grid size and assess cell numbers (viable/not viable) Count only cells along two sides of square (be consistent) and count at least 4 squares (preferably 10) to get average and then calculate # of cells/ml.

Alternative counting methods.

1. Coulter Counting: As cells flow by an electrode, the voltage changes (as a result of the cell's conductivity) and registers on the counter.

Good for large number of samples/cells. Requires standards and calibration and cannot always discern between live and dead cells. Also, apparatus is comparatively expensive.

2. Flow Cytometer: Apparatus is similar to Coulter Counter in that voltage registers cell passage. Additional channels allow for flow to be diverted. As a result, cells can be both counted and separated. Cost big bucks and lots of experience to calibrate and resolve different cell features. Sophisticated models can actually check chromosomes (can actually separate x and y chromosome sperm!)