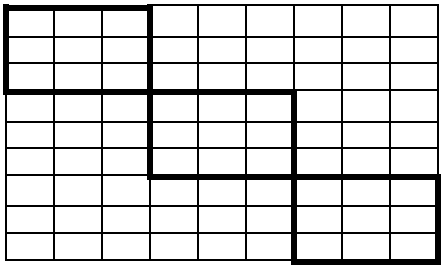
Institut für HIV Forschung

Cell Counting SOP

Counting Using a Hemacytometer:

1. Resuspend cells in a flask or tube, making sure they are thoroughly mixed and there are no clumps of cells.
2. After mixing, expel all liquid in the pipet back into the flask or tube. Take a new fresh aliquot (~200-500 µl) of the cells and place in a clean 1.7mL Eppendorf tube.
3. Use a second Eppendorf tube for dilution in Trypan Blue Solution *(Trypan Blue Solution = 1 part Trypan Blue and 8 parts RBC Lysis Solution or PBS)*.
4. Common dilutions:
   a. 1:10 90 µl Trypan Blue Solution, 10 µl cells
   b. 1:20 190 µl Trypan Blue Solution, 10 µl cells
   c. 1:50 490 µl Trypan Blue Solution, 10 µl cells
   d. 1:100 990 µl Trypan Blue Solution, 10 µl cells
5. Vortex the cell aliquot vigorously for 4-5 seconds, and immediately add 10 µl cells to tube with Trypan Blue Solution.
6. Vortex the dilution tube vigorously and immediately add 10 µl to hemacytometer.
7. Load the counting chamber slowly. The liquid should flow easily from the pipet tip into the chamber. Make sure there are no air pockets in the slide.
8. Count at least 3 large 9x9 squares to get an accurate measurement.



$$\frac{\text{(counted cells) x 90 x dilution factor}}{\text{(\# of small squares counted)}} = \text{cells/µl}$$

Counting Using Nucleocounter:

1. See steps #1-2 above.
2. Use a second eppendorf for dilution in solutions A and B. Common dilutions:
   a. 1:5 100 µl Buffer A, 100 µl Buffer B, 50 µl cells
   b. 1:10 225 µl Buffer A, 225 µl Buffer B, 50 µl cells
   c. 1:200 995 µl Buffer A, 995 µl Buffer B, 10 µl cells
3. Add cells, then add Lysing Buffer A. Vortex. Add Stabilizing Buffer B. Vortex again.
4. Load nucleocassette by submerging tip into eppendorf and pressing down on white piston.
5. Inactivate tip for 3 minutes in expose.

6. Load nucleocassette into reader. Close lid. Press run.
7. The machine only accurately measures concentrations between $5 \times 10^3 – 2 \times 10^6$ cells/ml.
8. For a non-viable cell count, only dilute in stabilizing buffer B.