PROCEDURE:

1. Clean the Burcker chamber with 70% ethanol.
2. Take the Burcker chamber and place it on the flat surface of the work bench. Place the cover slip on the top of the chamber.
3. Place the Burcker chamber under the microscope and look for the grid as Figure 1 (left). Play with light and focus if you can not find.

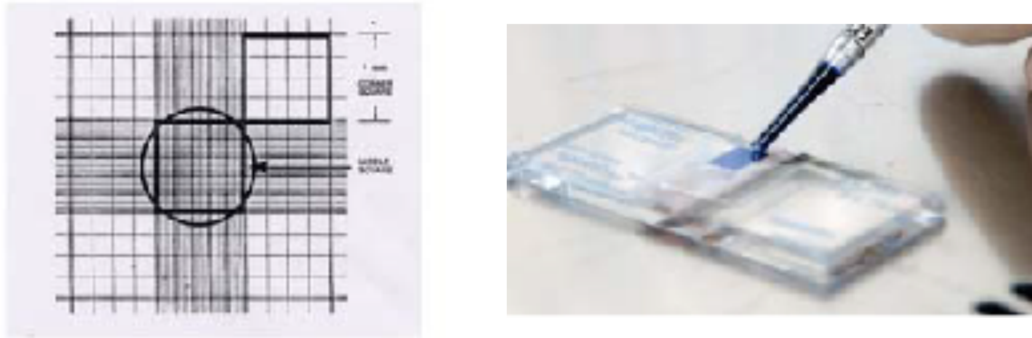

Figure 1. Counting chamber (left) and adding cells (right).

4. Mix 20µl of cells with an equal volume of tryphan blue in Eppendorf. Mix up and down couple of times.
5. Apply 10µl of cells to a Burcker chamber by pipetting from the edge of the cover slip and permitting diffusion by capillary action (Figure 1, right photo).
6. Make sure that there is no air bubble and there is no overfilling beyond the ruled area.
7. Leave the counting chamber for 2-3 minutes to allow the cells to settle.
8. Adjust the light (less light needed, hence close the aperture or lower the condenser) and focus on the wall of the counting chamber.
9. Count the number of cells seen on the small square of the upper left corner which is divided into 16 smaller squares to facilitate counting (Figure 2). Blue cells - death, White/transparent cells - vital.
10. Repeat the counting with three other corner squares.
11. Make a total of all the cells counted in 4 squares.

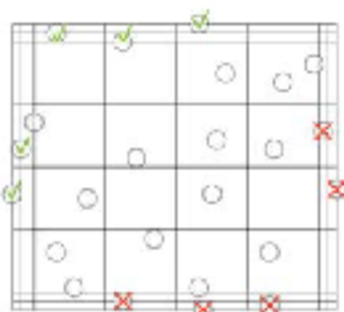

Figure 2. Cell counting

12.Calculate the concentration of viable cells and the percentage of viable cells using the equations below

Total (or viable) cells counted in 4 mm^2	*Divided by 4 = cells per mm^2*	*Divided by dilution*	*= cells/10^4 ml*	*x 10^4= Cells/ml*	*x total volume of cell suspension = Total (or viable) cells recovered*
101 (92)	25 (23)	½	50 (46)	5.0 (4.6) x 10^5 cells/ml	2.0 (1.84) x 10^6 cells