

## Cell counting using a haemocytometer

### 1. Preparing haemocytometer

1. Ensure the haemocytometer is clean using 70% ethanol.
2. Moisten the shoulders of the haemocytometer and affix the coverslip using gentle pressure and small circular motions. The phenomenon of Newton's rings can be observed when the coverslip is correctly affixed, thus the depth of the chamber is ensured.

### 2. Preparing cell suspension

1. Make sure the cell suspension to be counted is well mixed by either gentle agitation of the flask containing the cells (or other appropriate container). A serological pipette may be used if required.
2. Before the cells have a chance to settle take out about 1 ml of cell suspension using a serological pipette and place in an eppendorf tube.
3. Using a 100  $\mu$ l pipette, mix the cells in this sample again (gently to avoid lysing them). Take out 100  $\mu$ l and place into a new eppendorf, add 100  $\mu$ l trypan blue and mix gently again.

### 3. Counting

1. Using the Gilson pipette, draw up some cell suspension containing trypan blue. Carefully fill the haemocytometer by gently resting the end of the Gilson tip at the edge of the chambers. Take care not to over-fill the chamber. Allow the sample to be drawn out of the pipette by capillary action, the fluid should run to the edges of the grooves only. Re-load the pipette and fill the second chamber if required.
2. Focus on the grid lines of the haemocytometer using the 10X objective of the microscope. Focus on one set of 16 corner square as indicated by the circle in Fig.1

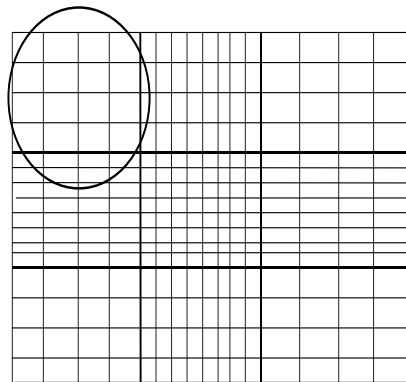


Figure 1: Gridlines on haemocytometer

3. Using a hand tally counter, count the number of cells in this area of 16 squares. When counting, always count only live cells that look healthy (unstained by Trypan Blue). Count cells that are within the square and any positioned on the right hand or bottom boundary line.

Dead cells stained blue with trypan blue can be counted separately for a viability count

4. Move the haemocytometer to another set of 16 corner squares and carry on counting until all 4 sets of 16 corner squares are counted.

5. The haemocytometer is designed so that the number of cells in one set of 16 corner squares is equivalent to the number of cells  $\times 10^4 / \text{ml}$

Therefore, to obtain the count:

The total count from 4 sets of 16 corner = (cells / ml  $\times 10^4$ )  $\times 4$  squares from one haemocytometer grid

1. Divide the count by 4

2. Then multiply by 2 to adjust for the 1:2 dilution in trypan blue

These two steps are equivalent to dividing the cell count by 2

As an example:

If the total cell count is 145

The cell density is  $\frac{145}{2} = 72.5 \times 10^4 / \text{ml}$

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#### 4. Viability

1. The trypan blue is used to stain any dead cells. Cells looking faint or dark blue within the grid being counted are counted as dead cells. To check the viability of the cells requires:

- **Live cell count** (not including trypan blue cells)
- **Total cell count** including those stained with trypan blue.

$\frac{\text{Live cell count}}{\text{Total cell count}} = \text{percentage viability}$

Example;  $\frac{45 \times 10^4 / \text{ml}}{46 \times 10^4 / \text{ml}} = 0.978 = \mathbf{97.8\% \text{ viability}}$