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Title: **Standard Method for Cell Counting using the Improved Neubauer Haemocytometer**

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SOP History		
Number	Date	Reason for Change
v1	01/01/2013	Original
V2	01/01/2018	Update

1.0 Purpose –

The purpose of this SOP is to outline the principles for cell counting using the improved Neubauer haemocytometer in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to the standard method for cell counting using the improved Neubauer haemocytometer within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users of an improved Neubauer haemocytometer within the SASoM to comply with this SOP.

4.0 Procedure –

Principles of Operation:

The principal use of the Improved Neubauer Haemocytometer is to measure the density of cells in suspension.

Cells should be in single cell suspension prior to counting. Adherent cell cultures should be trypsinised and syringed as necessary to achieve this. (NB trypsin should be inhibited with FCS-containing media prior to counting).

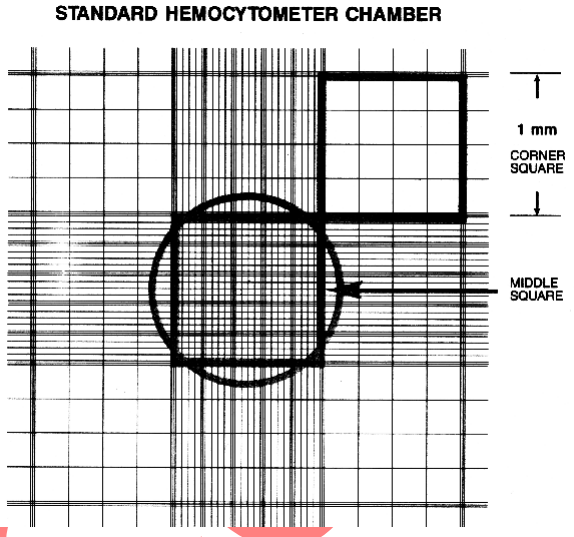
The easiest way to set up the haemocytometer is to breathe on the cold surface in order to produce a thin film of condensation. Place the cover-slip on this film and move gently with thumbs using a to-and-fro motion until you feel the cover-slip adhering to the haemocytometer. Good contact is confirmed by the appearance of Newton's rings (a rainbow effect) between the cover-slip and the slide.

After mixing your cell suspension transfer a small volume (approx 10ul) to one of the counting chambers (each haemocytometer has 2). Holding the tip of the pipette / pastette against the edge of the cover-slip will allow the chamber to fill by capillary action.

View the haemocytometer under the microscope, focussing on the gridlines.

If the cell density is very high, counting will be difficult so a dilution should be made and this can be added to the remaining chamber.

The haemocytometer grid is laid out as follows:



If cell counts are high (>100) count the cells in the middle square only. If cell counts are low count the cells in all four corner squares and combine counts.

When counting cells that fall on the grid lines count only the cells that lie on the top and right-hand side of each square. Do not count cells that lie on the bottom or left-hand side grid lines.

Calculations:
For cells counted in middle square only: multiply cell count by dilution factor and by 10^4 (10,000) to give cell density in cells / ml.

For cells counted in the four corner squares: divide combined count by 4 and then multiply by dilution factor and by 10^4 (10,000) to give cell density in cells / ml.

Identifying dead / dying cells:
To differentiate between live and dead cells during counting (or where cell visualisation is difficult) cell suspensions may be made up in 0.4% Trypan Blue dye in PBS prior to counting (1:10 dilution of cell suspension into dye solution). The darkly coloured cells that readily absorb dye are leaky and are dead or dying whereas cells that exclude the dye are generally viable.
NB. Trypan blue is toxic to cells so counting should be done immediately after cell suspensions are made up in this solution.

Cleaning:
Rinse the haemocytometer and cover-slip under cold running water and dry before returning to storage. Haemocytometer and cover slips are stored in 70% ETOH.

5.0 Personal protection –
Howie coat must be worn at all times.

6.0 Spillages –
Always clean up any spills immediately after use.

7.0 Training –
All users have to be trained before using the Haemocytometer.

8.0 Approval and sign off –

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Signature: Date:

Management Approval:

Name: Mary Wilson

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