Cell Counting using a haemocytometer (Neubauer cell chamber) with fixer

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Abstract

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Protocol

Step 1.

Clean the haemocytometer using 70% ethanol before use

Step 2.

Moisten the raised glass rails with the tip of a moistened finger and affix the coverslip over the raised glass rails using gentle pressure thus the depth of the chamber is ensured.

Step 3.

Gently mix the cell suspension in the flask and transfer to 1ml of Eppendorf tube. Using micropipette, mix the cells in this sample again and take out 100 µl of cells and add 100 µl of 1X fixer and mix gently again

Step 4.

Carefully, add 20 µl of the mixed sample in the grid made between the coverslip and the counting chamber of haemocytometer, avoid overfill of the chamber, but allow the sample to reach the edges of the grooves. Re-load the pipette and fill the second chamber if required.

Step 5.

Focus the haemocytometer grid using the 10X objective of the microscope.

Step 6.

The chamber contains two identical squares. Each square has an area of 9 mm^2 (1 mm on each side). These squares are divided into nine primary squares with an area of 1mm^2. Count the cells in the 4 large squares commonly used for WBC cell counting. These 4 large corner squares contain 16 smaller secondary squares, each with an area of 0.04 mm^2. Calculate the number of cells counted / µL.

The total count from 4 sets of 16 corner = (cells / ml x 104) x 4 squares from one haemocytometer grid. Divide the count by 4. Then multiply by 2 to adjust for the 1:2 dilution in case of fixer.

To calculate cell concentration per ml:

Step 7.

Average number of cells in one large square x dilution factor* x 10^4

*dilution factor is usually 2 (1:1 dilution with trypan blue)

10^4 = conversion factor

Step 8.

Focus on the middle square consists of 16 squares further containing 25 small squares. Count all the live cells present in the middle square.The volume of one large corner square $1mm^2$x 0.1mm.

Percentage of viability

Step 9.

No. of Viable Cells Counted / Total Cells Counted (viable and dead) x 100 = % viable cells