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Haemocytometer Instructions For Use

To Obtain Blood

Using a lancet make a comparatively small puncture; this will cause a large drop of blood to ensue.

For Red Corpuscles

Under normal blood conditions a dilution of 1:200 is suitable. A dilution of 1:100 may be used in cases of anaemia. In the first instance the blood would be drawn up to the mark '0.5' on the stem of the red pipette and in the second instance to '1' otherwise the procedure is the same.

To Fill the Pipette

Dip the point of the pipette quickly into the drop of blood, obtained in the manner described and by means of suction through the rubber tube, using a pipette holder, draw blood into the stem of the pipette up to the line marked '0.5' or '1', depending on the dilution required.

Remove any blood that may adhere externally to the point of the pipette, and at once dip the pipette into the diluting fluid, which should be placed ready to hand.

Draw up the diluting fluid to the line above the bulb marked '101'. Close the pipette by holding both ends between the finger and thumb, and then thoroughly shake the solution in the bulb for three minutes to ensure a uniform mixture. The glass bead inserted in the bulb of both red and white pipettes is to facilitate this. Since the fluid in the stem of the pipette does not enter the bulb it will be seen that the latter, when full, contains a mixture of 99 parts by volume of diluting fluid and one part of blood. Expel the diluting fluid from the stem of the pipette.

For White Corpuscles.

Use the pipette, which provides for a dilution of 1:10 and follow a similar procedure, noting that '101' has become '11' on the white cell pipette. Fill to '0.5' for a 1:20 dilution; fill to '1' for a 1:10 dilution.

To Fill the Counting Chamber

The surface of the counting chamber bridges to either side of the ruled counting platform should be lightly wetted with distilled water. The cover glass is placed in position half way across the ruled counting platform with Newton rings visible on the bridges, indicating a proper fit. A drop of blood from the pipette is then placed on one end of the counting platform just beyond the edge of the cover glass and the blood will run under the cover glass and over the ruling by capillary action. Slide the cover glass all the way over the ruled counting platform. The chamber is now ready for counting.

Continued:

Counting

In making blood counts a microscope with a mechanical stage should, if possible, be used. The illumination of the microscope is important, as too much light prevents the rulings from being easily seen. The light can be reduced by means of the iris diaphragm until the rulings stand out clearly against the background.

Counting the Cells

It is advisable to count 100 squares as this obviates a fractional result. The counted squares should be easily distributed over the ruled area employed, and this is facilitated by the squares being framed off into blocks by means of triple rulings.

Having counted 100 squares divide the sum by 100 (the number of squares counted), to obtain the average per square. Multiply by the dilution and divide by 1/4000 (the cubic capacity of each small square being 1/20 x 1/20 x 1/10 – 1/4000mm³).

The number arrived at by this calculation is the number of cells per cubic millimetre of the original undiluted fluid.

The following formula gives a convenient form to the method described.

(D x N) / (S x K)
Where:
D = Dilution Factor
N = Number of Corpuscles/Cells counted
K = Volume for each small square
(1/4000mm³ for an Improved Neubauer 0.1mm deep chamber)
S = Number of squares counted

Example:

Where D = 100
N = 1350
S = 100

Then (100 x 1350)/(100 x 4000) = 5,400,000 cells per cubic mm

Counting Larger Cells (e.g. Leucocytes)

In counting larger cells it is common to count all the cells contained in the large squares which measure 1mm x 1mm. Having made the count, proceed as before, but in this case the value "K" would be 1/10 as the capacity of each large square is 1x1x1/10 = 1/10mm³.

The K values for one small square of various ruling types is given below:

Improved Neubauer (0.1mm cell depth)	K = 1/4000
Neubauer (0.1mm cell depth)	K = 1/4000
Burker (0.1mm cell depth)	K = 1/250
Thoma (0.1mm cell depth)	K = 1/4000
Modified Fuchs Rosenthal (0.2mm cell depth)	K = 1/80
Helber Bacteria Z30000 (0.2mm cell depth)	K = 1/20000
Malassez (0.2mm cell depth)	K = 1/2000
Semen Fertility Z3BC1B (0.01 cell depth)	K = 1/10000