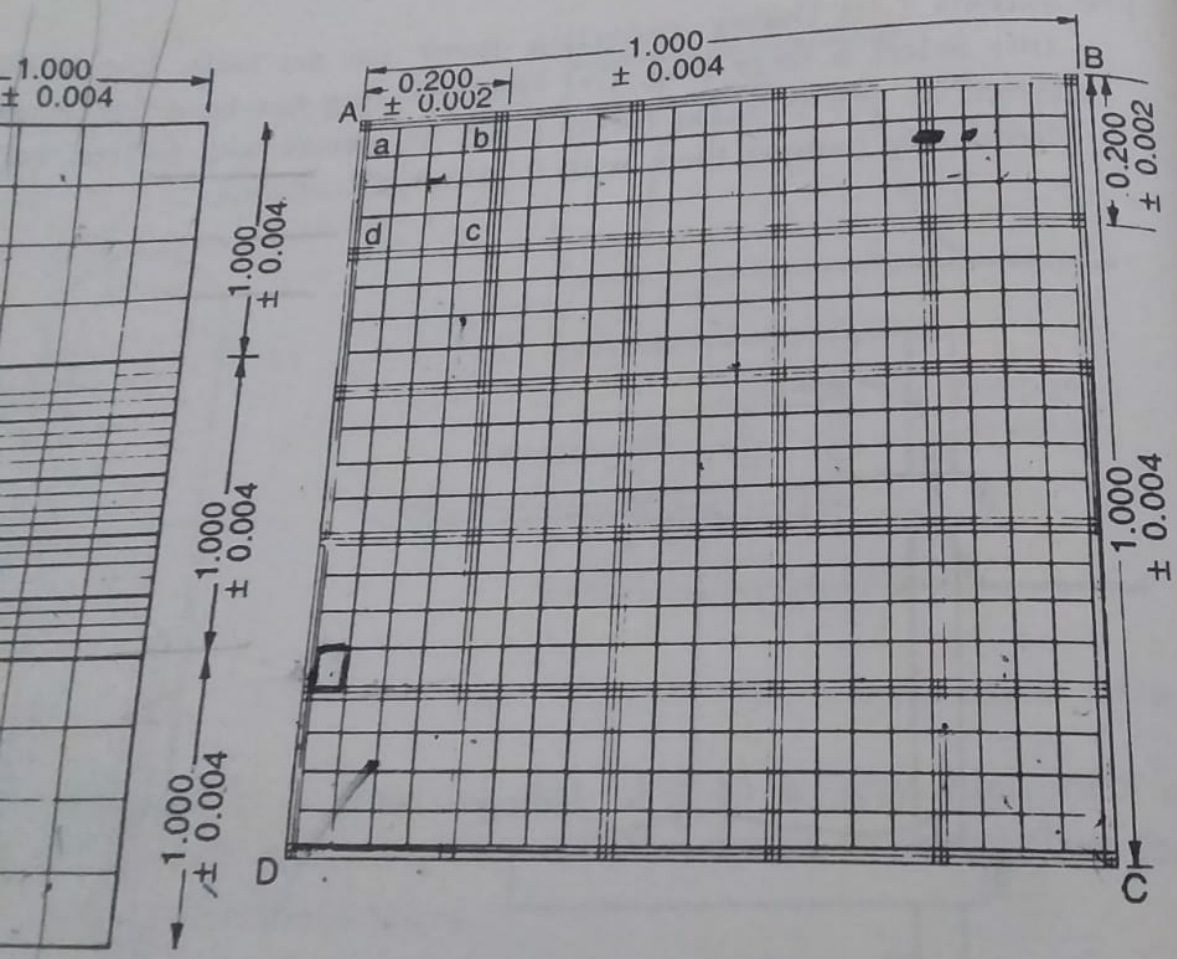

Fig. 13. Magnified view of central A B C D squares.

Fig. 11. The Burker Hawksley counting slide

Experiments of Blood : Blood Groups and Rh Factor

Experiment (1) **: Enumerate the total R.B.C. (red blood cells or erythrocytes) count of your own blood.**

Apparatus : Haemocytometer, sterilized pricking needle, R.B.C. pipette, compound microscope and **Hayem's diluting fluid** (NaCl - 1%, Na_2SO_4 - 2.5% and $HgCl_2$ - 2.5%).

Haemocytometer is a kind of slide containing counting chambers. The **Burker**, **Neubaur**, **Thoma** or any other haemocytometer may be used. The Neubaur haemocytometer consists of a double-cell slide with two sunk platforms in open cell type. Each platform has a ruling so that rapid duplicate counts

the junction of the two drops. Once an optimum drop has been placed, the cells should be allowed to settle for one minute and then counting should be done.

(7) Bring counting scale into the focus under the objective under a microscope. Count cells in 16 squares in five different parts of the field. The counting should be done exact replica of the squares drawn on your copy. Any corpuscle lying on lines should be moved either upwards or to right side.

Calculations : These may be done in the following manner :

$$\text{Number of R.B.C. per cubic mm} = \frac{\text{Number of cells counted} \times \text{Dilution} \times 400}{\text{Number of small squares counted}}$$

Suppose, five smaller squares or 80 smallest squares contain A+B+C+D+E R.B.Cs.

One smallest square will contain A+B+C+D+E //80 R.B.Cs.

400 smallest squares will contain A+B+C+D+E × 400//80 R.B.Cs.

But, height of chamber = 0.1 mm and dilution of blood = 200 times.

Therefore, one cubic mm of blood will contain :

$$\frac{A+B+C+D+E}{80} \times 400 \times 10 \times 200 \text{ R.B.Cs.}$$

$$= A+B+C+D+E \times 10000 \text{ R.B.Cs.}$$

Fig. 12. Ruling of improved Neubauer slide.

Fig. 13. Magnified view of central A B C D squares.

can be made. When a special cover glass is kept in position, a depth of 1/10 mm is maintained over the rulings. The diluted blood is run on by capillary force after the cover glass has been fixed. The counting chambers are nine large squares having sides of 1 mm each. The central large square ABCD is subdivided in 25 medium squares and each medium square in turn contains 16 tiny squares having sides 0.05 mm long.

Hayem's diluting fluid may be kept as stock solution and a small amount of it may be taken in watch-glass, so that entire solution may not be mixed with the blood.

Procedure : (1) Clean and dry mixing pipette (dilution 1 : 200).

(2) Sterilise your middle finger and the puncturing needle (preferably over a flame) with a small pad of cotton wool dipped in 90% alcohol.

(3) Pick the middle finger with the puncturing needle so that blood flows freely. Don't squeeze.

(4) Wipe away cleanly the first few drops, then suck the blood with R.B.C. pipette upto the 0.5 mark slowly and carefully. If the blood has been drawn up too far, the excess may be removed by wiping the end of the pipette suitably drying on blotting paper.

(5) Wipe the excess blood from the tip of the pipette and immediately mix it with Hayem's diluting fluid and suck in R.B.C. pipette the diluting blood upto 101 mark. Hayem's fluid prevents haemolysis, rouleaux formation, coagulation and bacterial growth. Disconnect the rubber tube, grip the ends of the pipette between forefinger and thumb and shake thoroughly for a minute.

(6) Re-attach the rubber tube to the pipette, reject the unused diluting fluid in the stem of the pipette. Avoid drying and quickly run the diluted blood under the cover glass on to each of the central platforms. If unable to do so, wash the slide and the cover glass and repeat it until you become expert. The acquirement of skill in performing procedure No. 3 and 6 is very essential and otherwise the counting will be valueless. The correct size of the blood drop and speed is obligatory, in order to avoid uneven distribution of the cells. Very large drop will result in low count because the cells tend to be sucked down into the trough away from the counting grid. On the contrary, a very small drop, so that a second drop is to be added, results in high count due to the uneven distribution of cells at

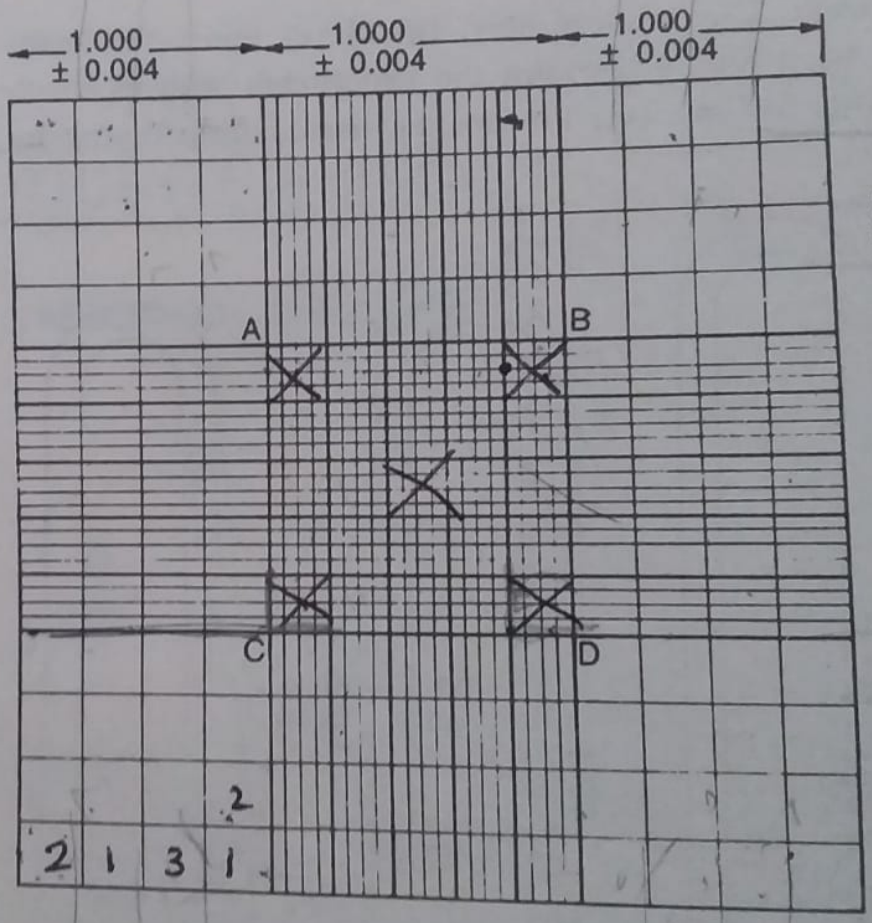

Fig. 12. Ruling of improved Neubaus slide.

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Calculations :-

Number of RBC per cubic mm $= \frac{\text{No. of cells counted} \times \text{Dilution} \times 400}{\text{No. of small squares counted}}$

Suppose, five smaller squares or 80 smallest squares contain A+B+C+D+E RBC(s)

1 smallest square will contain $\frac{A+B+C+D+E}{80}$ RBC (s)

400 smallest squares will contain $\frac{A+B+C+D+E}{80} \times 400$ (RBC's)

But height of chamber $= 0.1$ mm
and dilution of blood $= 200$ times.

$\Rightarrow$ One cubic mm of blood will contain:

$$\frac{A+B+C+D+E}{80} \times 400 \times 10 \times 200 \text{ R.B.C's}$$

$$= A+B+C+D+E \times 10000 \text{ RBC's.}$$
$$= (100+98+92+104+89) \times 10000$$
$$= 483 \times 10000$$
$$= 4.83 \text{ million R.B.C's}$$