Practical 1 – Blood Physiology: Full blood count (FBC)/ complete blood count(CBC)/ hemogram

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I. Erythrocyte parameters

1. red cell count
2. hematocrit
3. hemoglobin
4. erythrocyte indices :
   - i. Mean corpuscular volume (MCV)
   - ii. Mean corpuscular hemoglobin (MCH)
   - iii. Mean corpuscular hemoglobin concentration (MCHC)

(Note that I.2, I.3, I.4 will be discussed in a separate practical)

II. Leucocyte parameters

1. white cell count
2. leucocyte formula: Neutrophil granulocytes, Lymphocytes, Monocytes, Eosinophil granulocytes, Basophil granulocytes

III. Platelet parameters

1. platelet count
2. platelet size

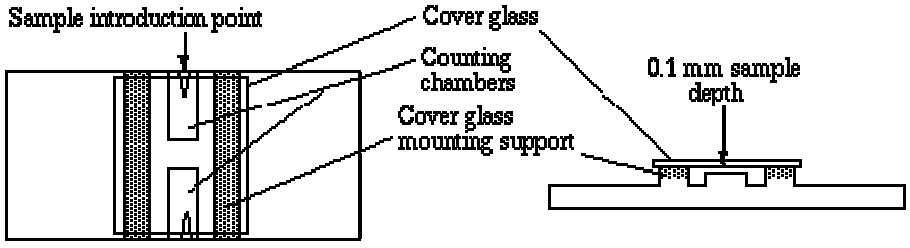
I. Erythrocyte parameters

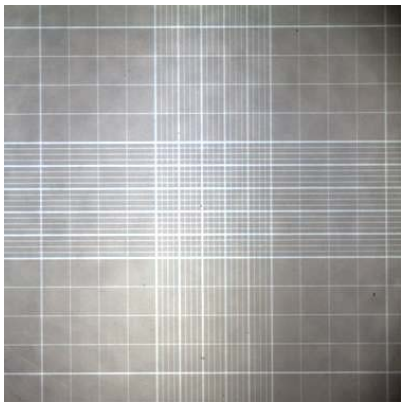
1. Red cell count can be automated (electronic count) or manual (visual count), the latter being less accurate.

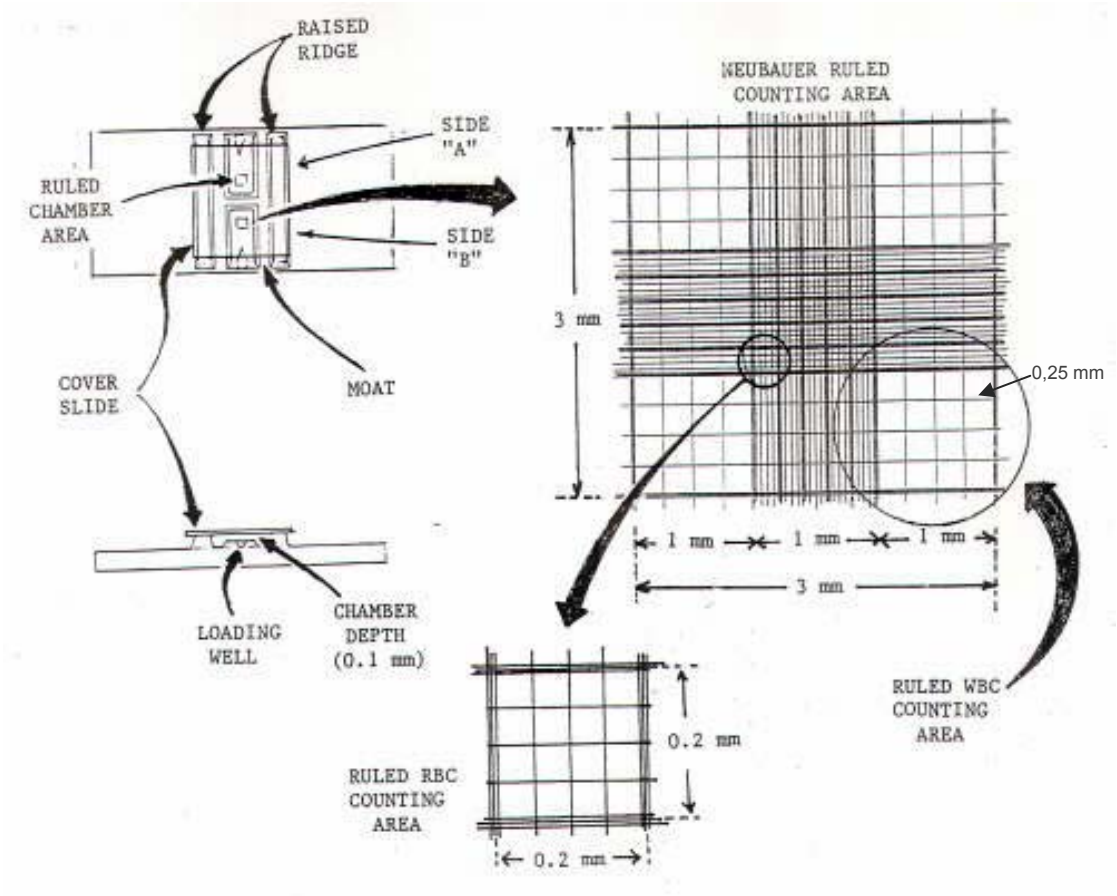
The manual manner uses **counting chambers / hemocytometer**. Counting chambers that hold a specified volume of diluted blood (as there are far too many cells if it is not diluted) are used to calculate the number of red and white cells per unit volume of blood.

To identify the numbers of different red cells, a blood film is made, and a large number of red cells are counted. The advantage of manual counting is that blood cells that may be misidentified by an automated counter can be identified visually. It is, however, subject to human error and sampling error because so few cells are counted compared with automated analysis.

A Neubauer hemocytometer is an etched glass chamber with raised sides that will hold a quartz coverslip exactly 0.1 mm above the chamber floor. The counting chamber has a total surface area of 9 mm^2. One entire grid on standard hemacytometers with Neubauer rulings can be seen at 40x (4x objective).







Red cell count is done in the smallest squares (length = width = 1/20 mm). Calculation of cell concentration is based on the volume underneath the cover slip.

One square has a volume V = length x width x height.

A Potain pipette is filled in with blood up to division 0.5 and then up to division 101 with Hayem solution (preserves the RBCs and destroys the WBC) to obtain a dilution of 1/200. To fill the hemacytometer by capillary action place the Potain pipette containing a well-suspended mix of cells at the notch at the edge of the hemacytometer and then slowly expel some contents so that the fluid is drawn into the chamber by capillary action.

The number of squares to be counted is usually 80 small squares.

Number of red cells/mm^3= (number of cells counted in 80 squares /80) x 400 x 10 x 200

= X/80 x 4000 x 200

Volume of the square = surface x height = 1/400 x 1/10

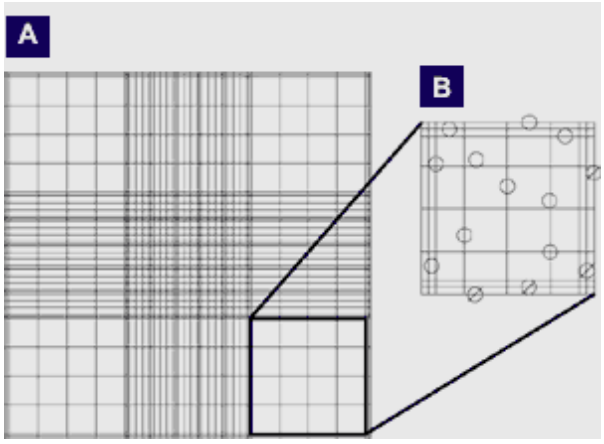
Dilution = 200 x

Normal values:

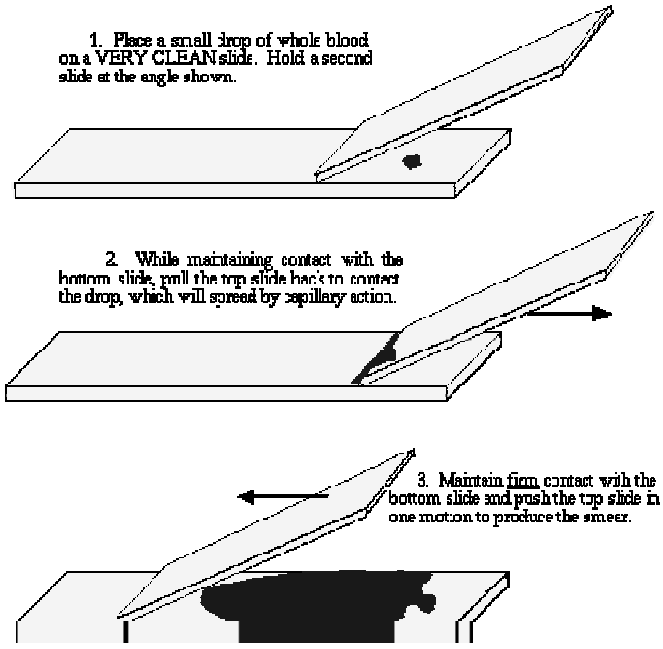
- *men – 5.200.000 (± 300.000)/mm^3*
- *women – 4.700.000 (±300.000)/mm^3*

II. Leucocyte parameters

1. White cell count can also be done in an automated or manual manner.
Using the counting chamber to identify the numbers of different white cells, a blood film is made, and a large number of white cells (at least 100) are counted. This gives the percentage of cells of each type. By multiplying the percentage with the total number of white blood cells, the absolute number of each type of white cell can be obtained.
The dilution used is 1/20 with Turk solution.
Normal value : 7000/mm^3

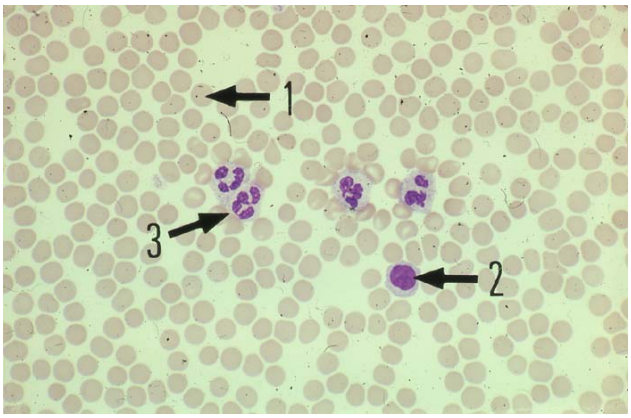


2. Leucocyte formula is done by a blood smear stained with May-Grumwald-Giemsa.
How to obtain a blood smear:

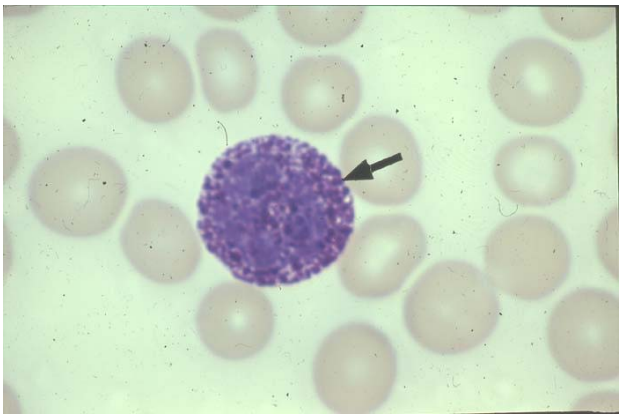


May-Grumwald-Giemsa staining method is based on the electrostatic interaction between dye and target molecules.
The basic dyes (methylene blue, related azures) carry net positive charges, thus staining in blue nuclei (as DNA and RNA molecules have negative charges of phosphate groups), granules of basophil granulocytes, and RNA molecules of the leukocytes cytoplasm. However, the leukocytes' cytoplasm appears light blue, because of the low RNA concentration.
The acid dye (eosin) carries net negative charge and stains in red erythrocytes and granules of eosinophil granulocytes.

The normal formula:
neutrophils 60-70%; lymphocytes 25 -32%; eosynophils 1-4%; basophils 0.5-1%, monocytes 6-10%.



A blood smear stained with May-Grumwald-Giemsa:
1 – erythrocytes, 2 – mononuclear cells (agranulocytes),
3 – polymorphonuclear leukocytes (granulocytes)



A polymorphonuclear basophil

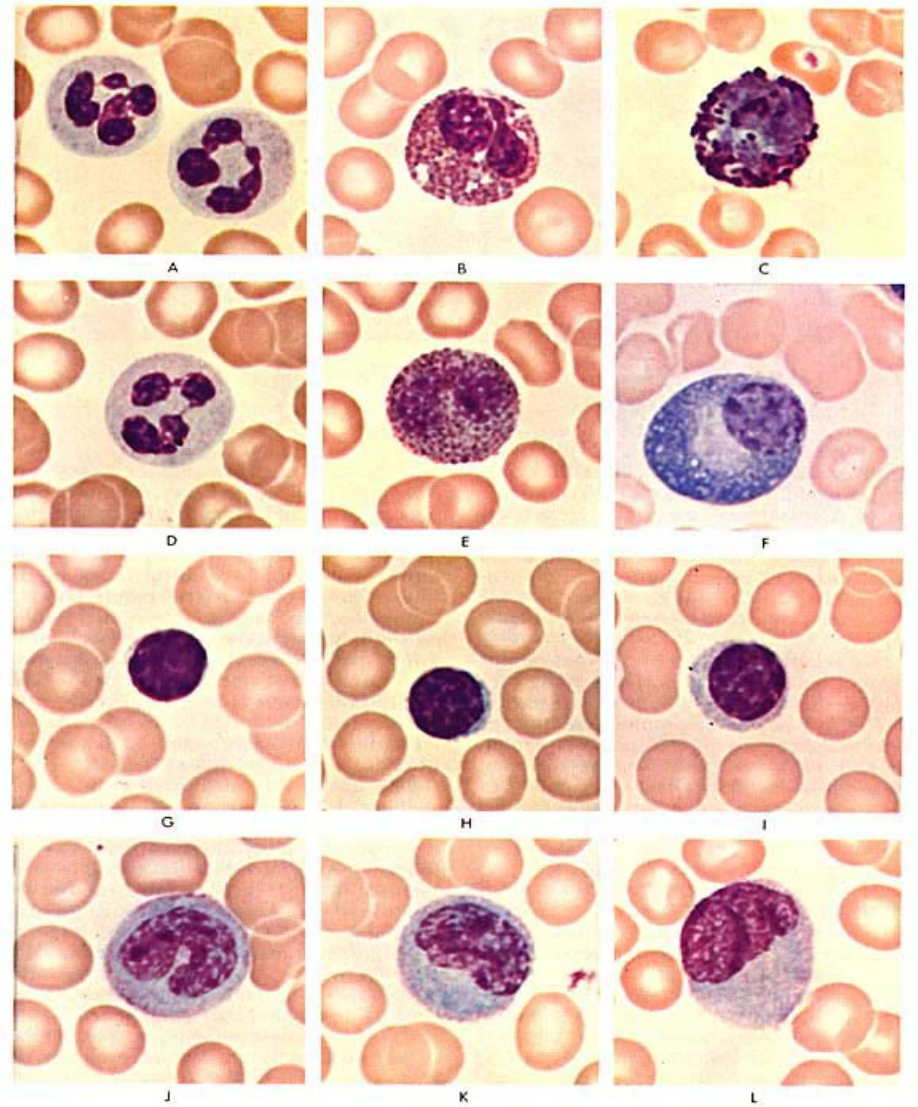


Figure 4–7. Human blood cells form a smear after Wright's stain. *A* and *D*, Neutrophilic leukocytes. *B* and *E*, Eosinophilic leukocytes. *C*, Basophilic leukocyte. *F*, Plasma cell; this is not a normal constituent of the peripheral blood but is included here for comparison with the nongranular leukocytes. *G* and *H*, Small lymphocytes. *I*, Medium lymphocytes. *J*, *K*, and *L*, Monocytes.

(Photomicrographs from Bloom & Fawcett, Textbook of Histology, 11th Edition)

III. Platelet parameters

Platelet count is done in the same manner as the red cell count, and the same dilution as in the white cell count.

Normal value -300.000/ mm^3

Note that plateles will be discussed in detail in Hemostasis practical)

Discuss the different physio-pathologic conditions, using the table below:

Type of Cell	Increase	Decrease
Red Blood Cells (RBC)	erythrocytosis or polycythemia	anemia or erythroblastopenia
White Blood Cells (WBC):	leukocytosis	leukopenia
-- lymphocytes	-- lymphocytosis	-- lymphocytopenia
-- granulocytes:	-- granulocytosis	-- granulocytopenia or agranulocytosis
--neutrophils	-- neutrophilia	-- neutropenia
-- eosinophils	-- eosinophilia	-- eosinopenia
-- basophils	-- basophilia	-- basopenia
Platelets	thrombocytosis	thrombocytopenia
All cell lines	---	pancytopenia