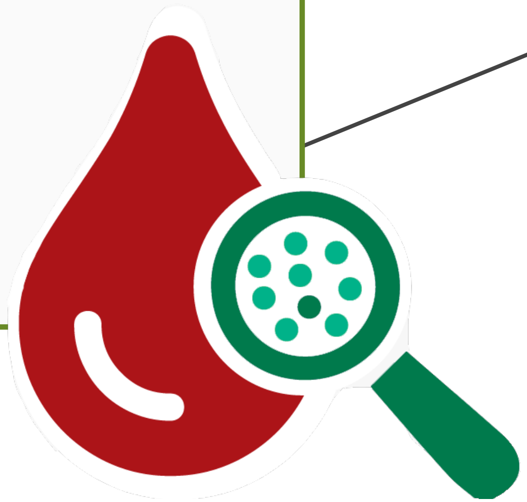
Blood Biochemistry BCH 471[Practical]

Lab (9) Complete Blood Count (CBC)



Objectives

- To estimate the number of RBC in blood sample
- To estimate the number of total WBC in blood sample
- To perform a differential count for a blood sample

Introduction

- **Complete blood count (CBC)** is a series of tests used to evaluate the composition and concentration of the cellular components of blood.

A CBC test usually includes:

I. **WBC count**

I. **WBC differential count**

II. **RBC count**

III. **HCT**

IV. **Hb**

V. **Red blood cell indices:** There are three red blood cell indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

VII. **Platelet count**

VIII. **Mean platelet volume (MPV)**

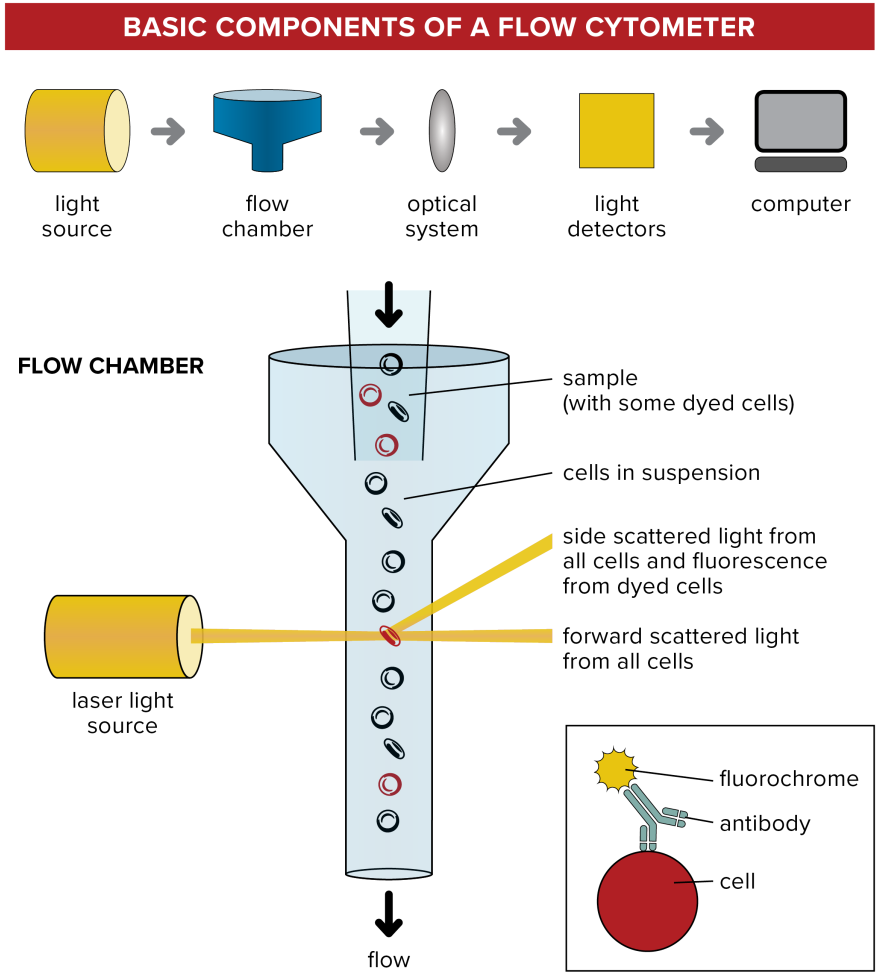
IX. **RBC distribution width (PDW)**

Complete Blood Count (CBC)

- **CBC can applied by two way:**
  1. <u>Automated</u> blood count
  2. <u>Manual</u> blood count

- **Automated blood count:**
  - CBC is performed by an automated analyzer that counts the **numbers** and **types** of <u>different cells within the blood.</u>
  - It aspirates a <u>very small amount</u> of the sample through the <u>narrow tubing</u>, within this tubing, there are <u>sensors</u> that count the <u>number of cells</u> going through it, and can identify the type of cell; this is called ***flow-cytometry***.

Automated Blood Count (Flow cytometer)



Mindray BC-2800 Auto Hematology Analyzer

Coulter Ac•T 5diff CP (Cap Pierce) Hematology Analyzer

Flow Cytometry Animation: https://www.youtube.com/watch?v=EQXPJ7eeesQ

Automated Blood Count (Flow cytometer)

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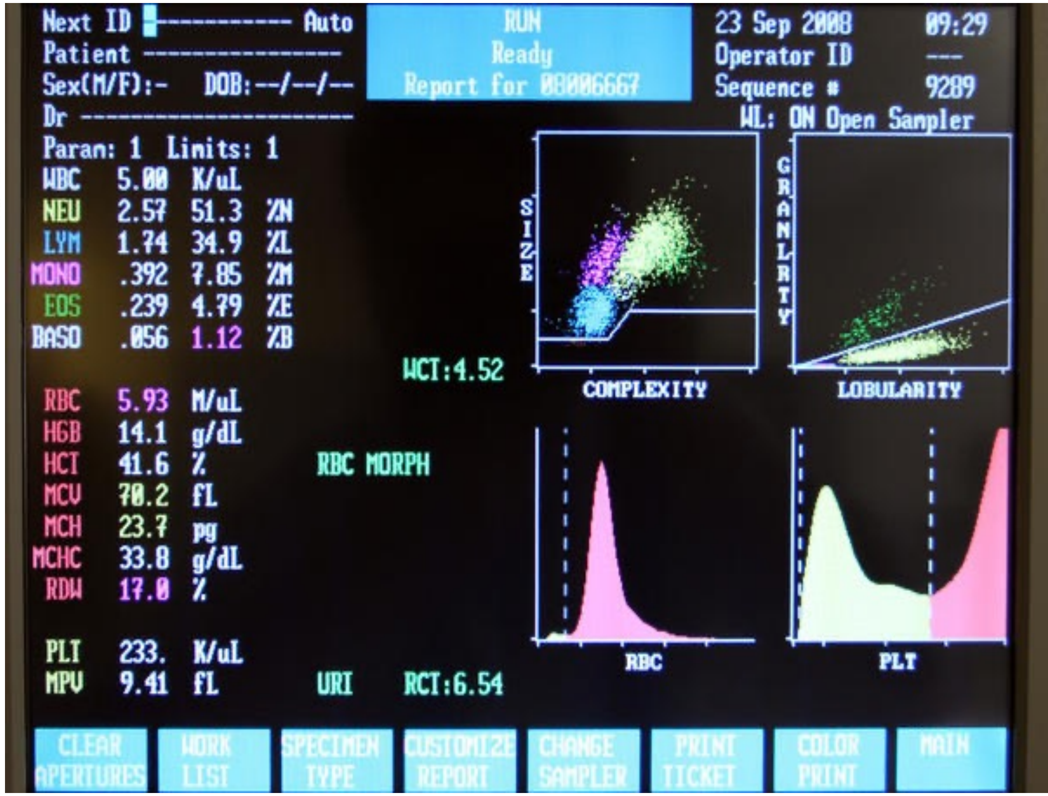
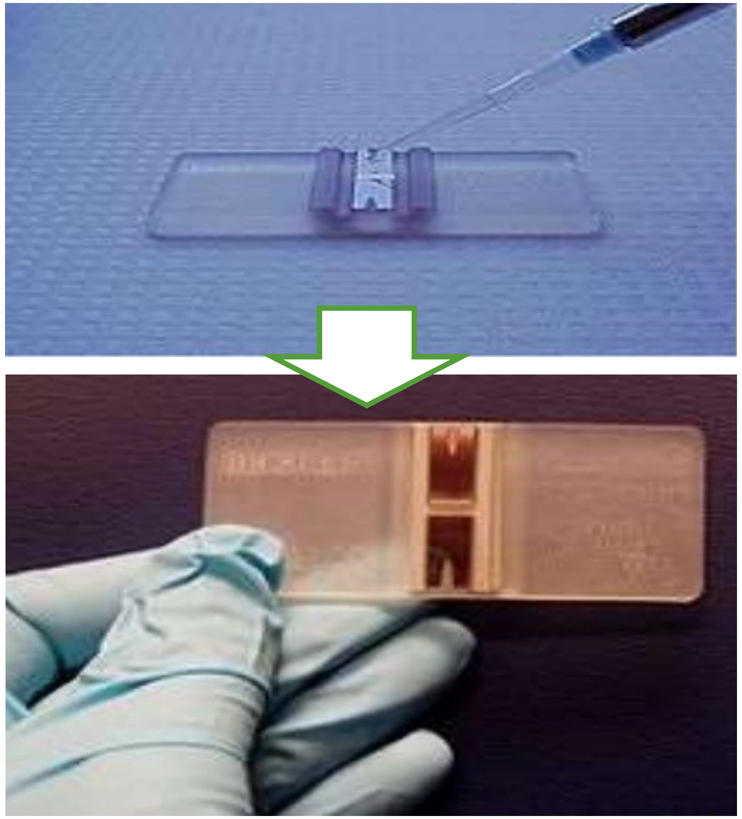
Patient:	**MALKIEWICZ, JUDITH A**			**Acc #:**	**55276**	
Patient #:	120850JM	Birth:	12/8/1950	Fasting:	UNKNOWN	
Doctor:	NON-STAFF	Age:	61 years	Collection Date:	2/22/2012 09:20	DZ
Home Phone:	(208)588-3977	Gender:	Female	Received in Lab:	2/22/2012 09:20	DZ
				Destination	OP	
				DR	SHULL/MYINT	

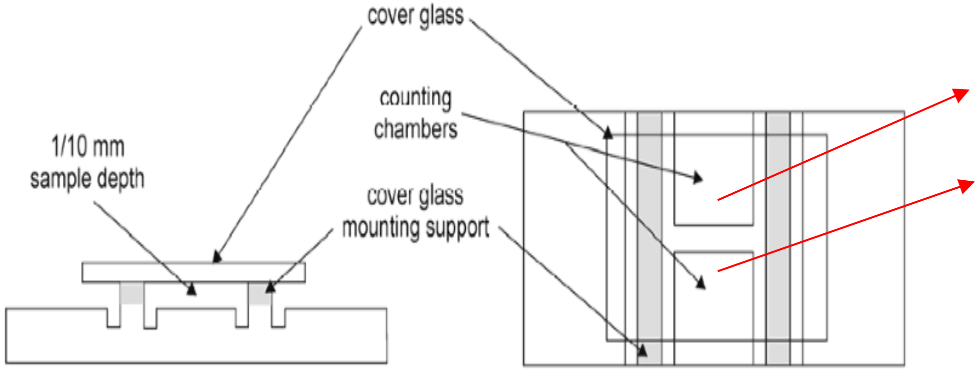
Test Name	Result	Units	Flag	Reference Range
CBC W/ 5 PART DIFF. (X6)				*Run by: TB on 2/22/2012 09:33*
WBC	2.1	K/uL		4.0 - 11.2
RBC	4.15	M/uL		4.00 - 5.60
HGB	13.5	gm/dL		12.0 - 16.0
HCT	39.5	%VOL		35.0 - 50.0
MCV	95	fl		82 - 98
PLATELETS	172	K/uL		140 - 440
MCH	32.6	pg		26.0 - 36.0
MCHC	34.3	g/dL		27.0 - 36.0
RDW	13.2	%		9.0 - 18.0
MPV	7.3	fl		6.0 - 12.0
NEU%	55.2	%		45.0 - 65.0
LYMPH%	30.9	%		20.0 - 50.0
MONO%	9.0	%		0.0 - 11.0
EOS%	4.0	%		0.0 - 7.0
BASO%	0.9	%		0.0 - 3.0
NEUT#	1.17	K/uL		2.00 - 8.00
LYMPH#	0.65	K/uL		1.80 - 4.80
MONO#	0.19	K/uL		0.10 - 1.10
EOS#	0.08	K/dl		0.00 - 0.80
BASO#	0.02	K/dl		0.00 - 0.30



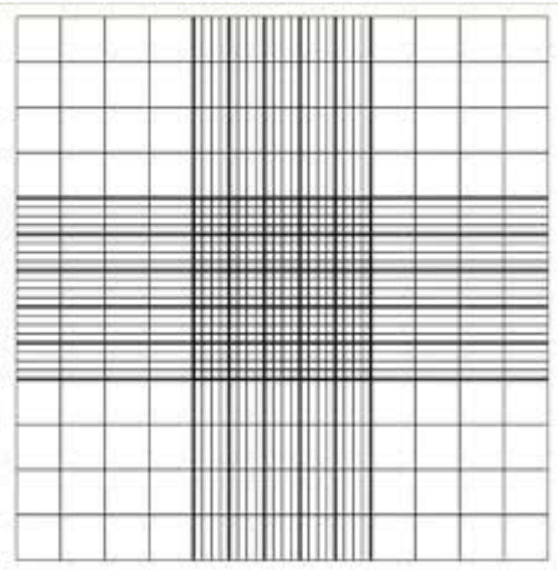
Manual Blood Count

- This measurement is made with a **microscope** and a specially ruled chamber (***Hemocytometer***) using diluted blood.





Counting Grid



(A) RBC Count

- It is test done to determine the **number of RBC** in a sample of blood, also it evaluate the **size** and **shape of RBC.**
- It is range from **4.2 – 5.5** million RBC per cubic millimeter (mm^3).
- It is considered a very important indicator of a patient's health.

Low RBC count

- Anemia
- Acute or chronic blood loss
- Malnutrition
- Chronic inflammation

High RBC count

- Polycythemia
- Congenital heart disease
- Renal problem.

Normally high (RBC count)

- People who live at high altitudes
- Smokers

} Oxygen is low→ RBC synthesis increases

(B) WBC Count

- Total leukocytes count shows the **number of WBC** in a sample of blood.
- A normal WBC count is between **4,500-11,000** cells per cubic millimeter (mm^3).
- The number of WBC is sometimes used to identify an infection or to monitor the body's response to treatment.

Low WBC count → Leukopenia

- A Condition in which the number of leukocytes is abnormally low and which is most commonly due to sever infections (such as **HIV**) and **radiation poisoning**.

High WBC count → Leukocytosis

- A condition characterized by an elevated the number of WBC occur as a result of an infection, or cancer (Leukemia).

Principle

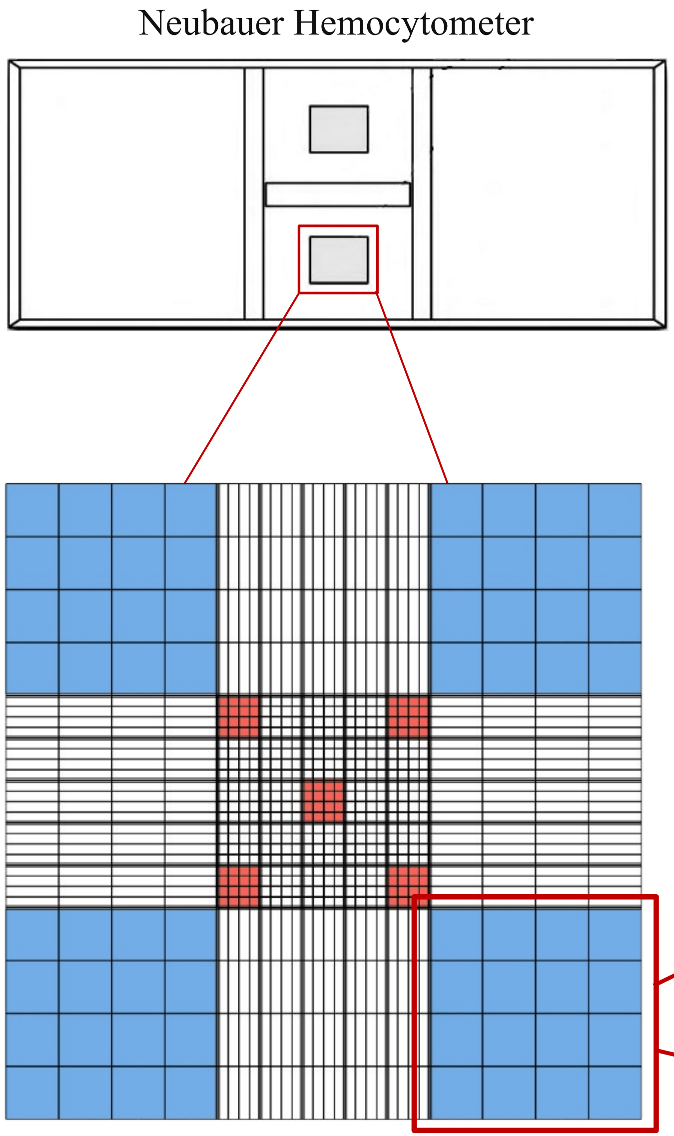
A. Regarding RBC

- The process involves by **counting cells** in <u>several squares</u> of the grid and obtain an average number.
- This number is multiply by a factor that compensates the amount of dilution.
- The final results expresses the number of **RBC /mm³** of original blood sample.

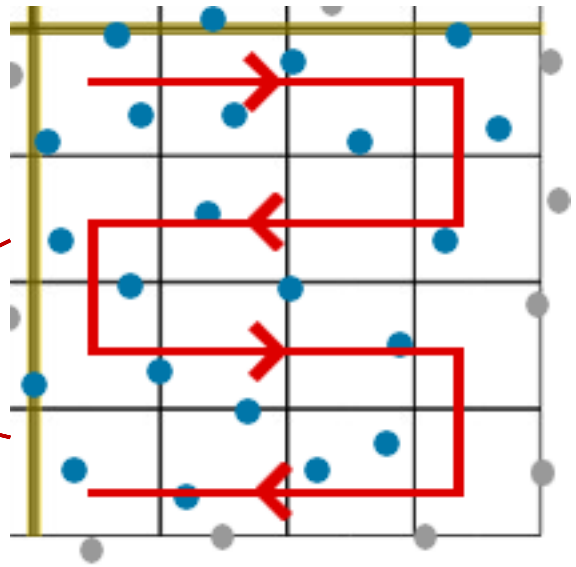
B. Regarding WBC

- It is necessary to obtain **RBC free** preparation of WBC from blood.
- Suspension of the red blood cell in a very **hypotonic solution** will lead to the destruction of RBC.

How To Count Blood Cells ?



- Areas where WBCs are counted
- Areas where RBCs are counted



Counting **2 sides** in L shape

(i.e. count the cells settled on the top and left sides)

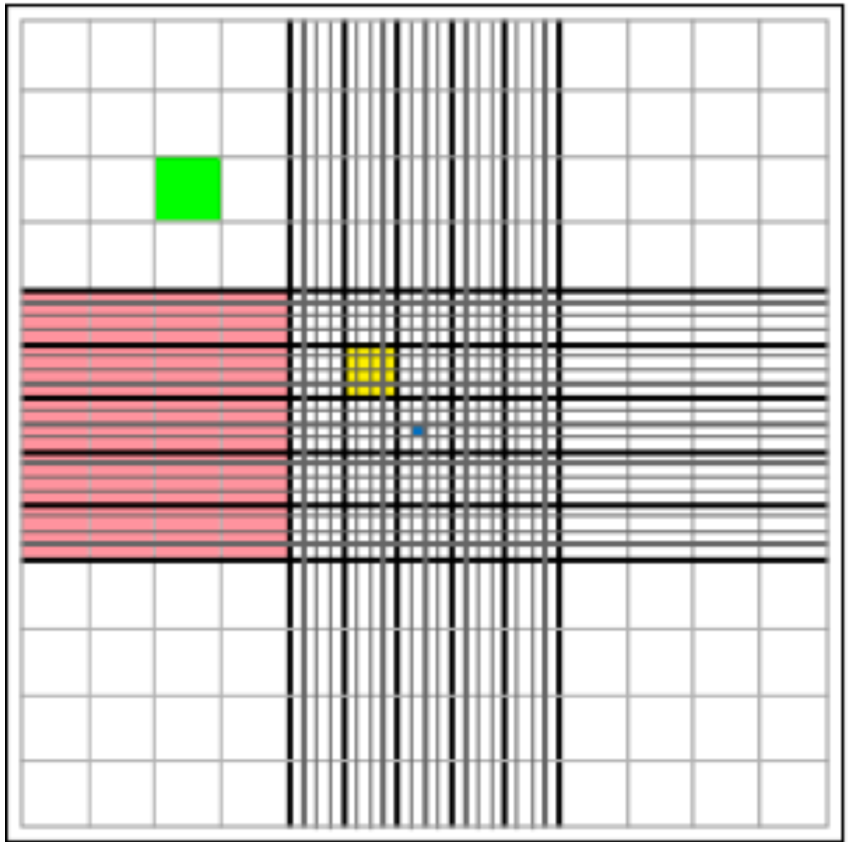
(Yellow sides)

And (exclude the cells on the bottom and right sides)

Counting in zigzag in all squares

Blue cells are counted
Grey cells aren't counted

How To Count Blood Cells ?



square = 1 mm2

square = 0.0625 mm2

square = 0.04 mm2

square = 0.0025 mm2

at a depth of 0.1 mm

The result is obtained as cell / mm3:

The Cubic Millimeters (mm3) = area per 1 square (mm2) X depth (mm)

RBC Calculations

- **RBC blood cell count (5 squares)**

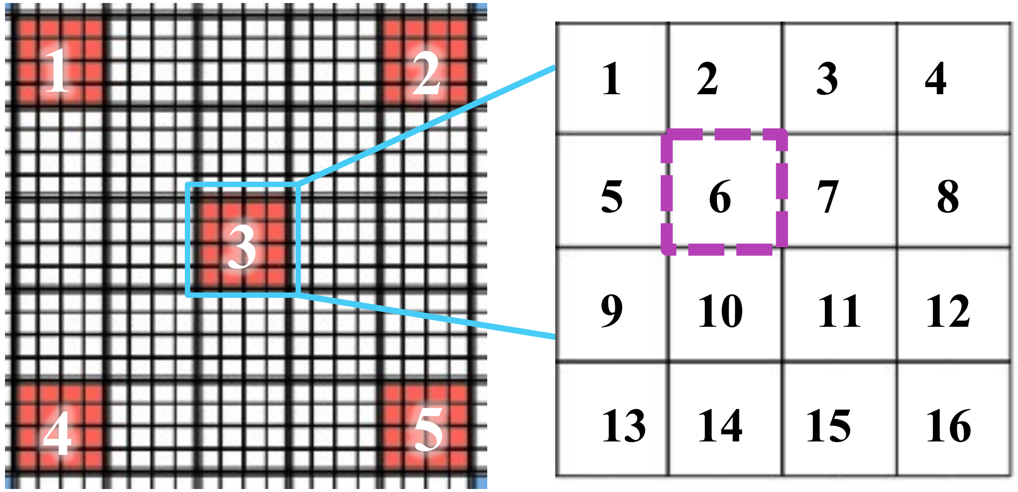
  - Find the sum of RBCs in **5 large squares**, and **divide it with 80** (5 x 16) small squares to find the average in one square, **multiply it by 200** to allow for the dilution and then **multiply by 4000** to obtain the number per cubic milliliter.

  - The sum of RBCs in 5 large squares = (84) + (71) + (63) + (93) + (83) = 394 cells.

  - The average of RBCs in one square= $\frac{394}{5x16} = \frac{394}{80}$= 4.9 cells.

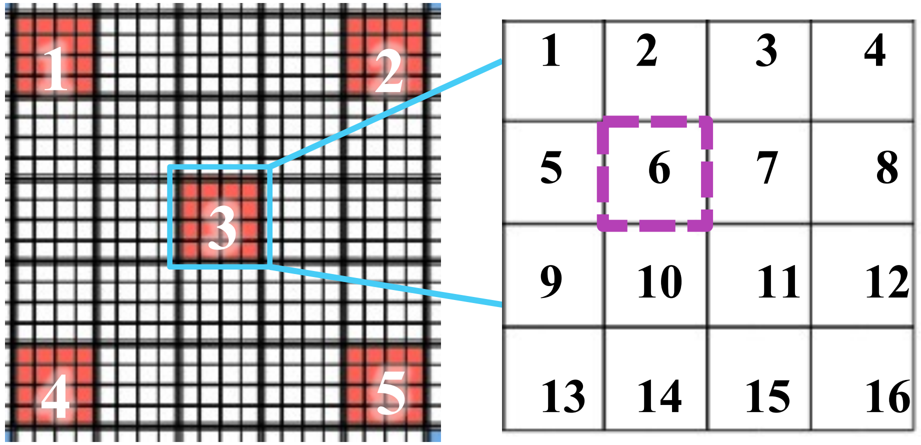
  - RBC count= 4.9 x 200 x 4000 = 4 million/mm^3.

- **Normal range= 4.2-5 million/mm^3**



RBC Calculations: Step by Step

- **RBC count**= The average of RBCs in one square (1) x Dilution factor (2) x Reciprocal of volume (3)
- The sum of RBCs in 5 large squares = (84) + (71) + (63) + (93) + (83) = 394 cells.
- **(1) The average of RBCs in one square**= $\frac{\text{No. of cells in 5 squares}}{\text{No. of small squares}} = \frac{394}{(16 \times 5) = 80} = 4.9$ cells.
- **(2) Dilution factor**= $\frac{\text{Final volume (RBC solution)}}{\text{aliquot volume (blood sample)}} = \frac{200}{1} = 200$
- **(3) Reciprocal of volume**= $\frac{1}{\text{cubic volume}} = \frac{1}{0.00025 \text{ mm}^3} = 4000$
- **RBC count**= 4.9 x 200 x 4000 = 4 million/mm^3

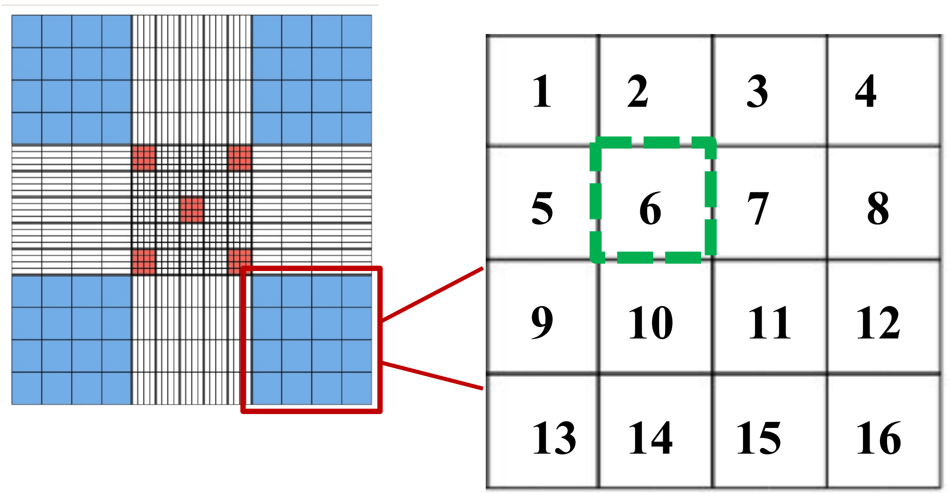


WBC Calculations

- **WBC blood cell count (4 squares)**

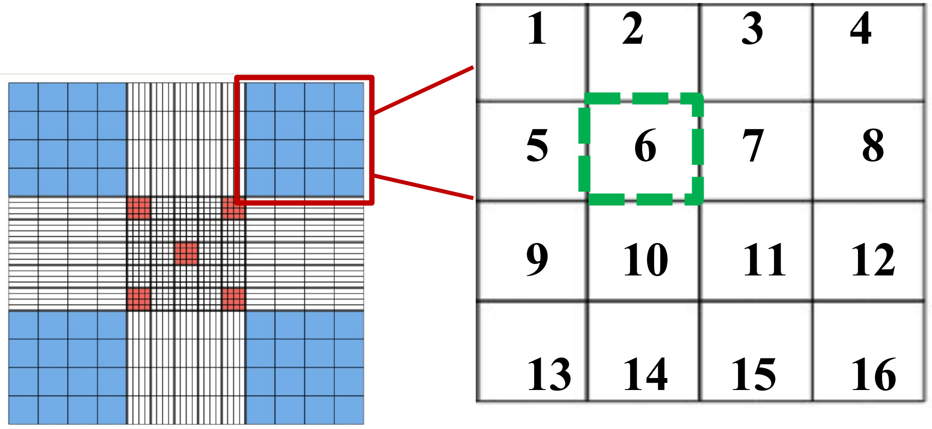
  - Find the sum of WBCs in **4 large squares**, and **divide it with 64** (4 X 16) small squares to find the average in one square, **multiply it by 20** to allow for the dilution and then **multiply by 160** to obtain the number per cubic milliliter.
  - The sum of WBCs in 4 large squares = (16) + (21) + (17) + (15) = 69 cells.
  - The average of WBCs in one square= $\frac{69}{64}$ = 1.07 cells.
  - WBC count= 1 x 20 x 160 = 3200 Cells/mm^3.

- **Normal range= 4500-11000 cells /mm^3**



WBC Calculations: Step by Step

- **WBC count**= The average of WBCs in one square (1) x Dilution factor (2) x Reciprocal of volume (3)
- The sum of WBCs in 4 large squares = (16) + (21) + (17) + (15) = 69 cells.
- **(1) The average of WBCs in one square**= $\frac{\text{No. of cells in 4 squares}}{\text{No. of small squares}} = \frac{69}{(16 \times 4) = 64} = 1.07$ cells.
- **(2) Dilution factor**= $\frac{\text{Final volume (WBC solution)}}{\text{aliquot volume (blood sample)}} = \frac{20}{1} = 20$
- **(3) Reciprocal of volume**= $\frac{1}{\text{cubic volume}} = \frac{1}{0.00625\ mm^3} = 160$
- **WBC count**= $1 \times 20 \times 160 = 3200$ Cells/mm^3.

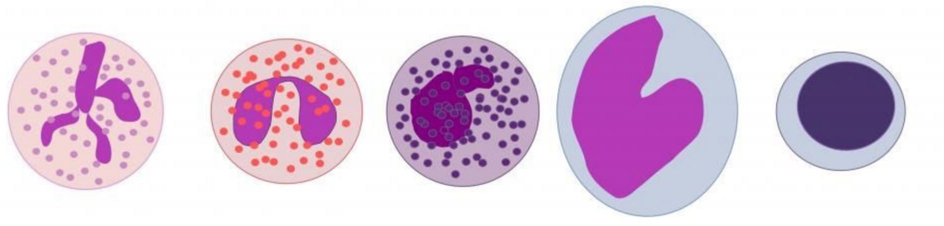


(C) Differential Count

- It determines the **number** of each **type of WBC** present in the blood.

Class of white cells	White cell type		% of total white cell population
Granulocytes	**Polymorphonuclear**	**Neutrophils**	**40 - 75**
		Eosinophils	**1 - 6**
		Basophils	**Approx. 1**
Non-granulocytes (agranulocytes)	Mononuclear	Monocytes	2 - 10
		Lymphocytes	20 - 45
		Plasma Cells	*

*: Rarely seen in blood, but present in the tissues.



neutrophil eosinophil basophil monocyte lymphocyte

Principle

- Classification of polymorphonuclear granulocytes (PMN) is based on the **size**, **shape**, **number** and **staining characteristics** of their granules .
- ***Leishman's stain***
  - It is based on a mixture of methylene blue and eosin.
  - It **differentiates between WBC** as indicated in the following table:

Type of cell	Color of the stain
Neutrophils nuclei	Purple
Eosinophils granules	Orange - Red
Basophils granules	Dark Blue
Lymphocytes nuclei	Dark Purple
Monocytes cytoplasm	Grey blue
Platelets granules	Violet
RBC	Pink