

# Physiology Lab 2

Differential Leukocyte count,  
Reticulocyte count, Packed cell  
volume, hemoglobin concentration  
and Erythrocyte Sedimentation rate

Dr. Tamara Alqudah

tamara.alqudah@ju.edu.jo

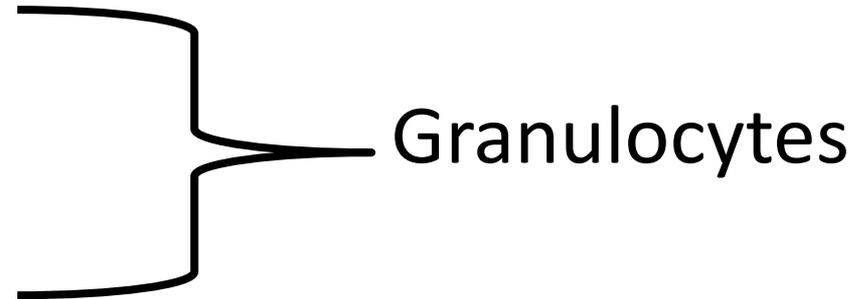
# Differential Leukocyte Count (DLC)

- The blood contains 5 different types of white blood cells:

1. Neutrophils: 40-80 %

2. Eosinophils: 1-4 %

3. Basophils: < 1-2%



4. Lymphocytes: 20-40%

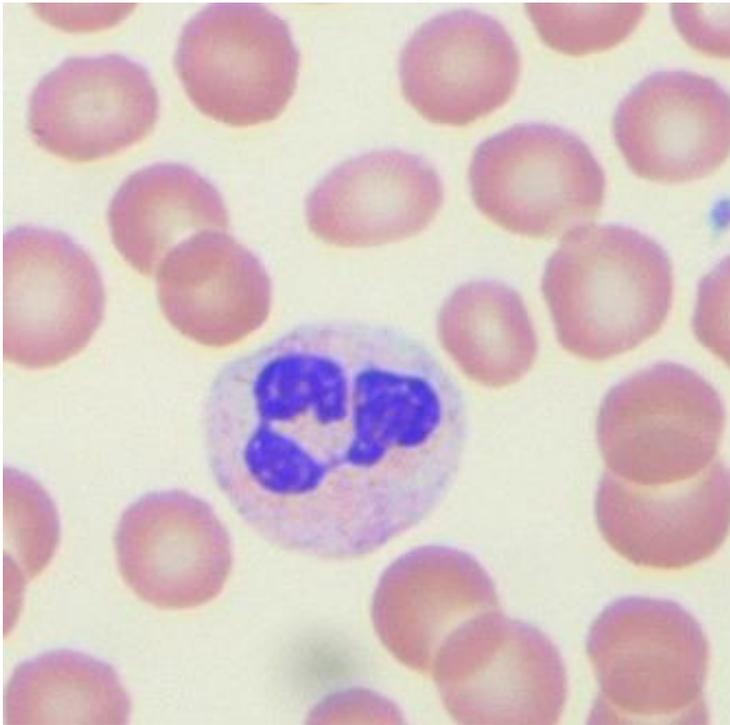
5. Monocytes: 2-8%



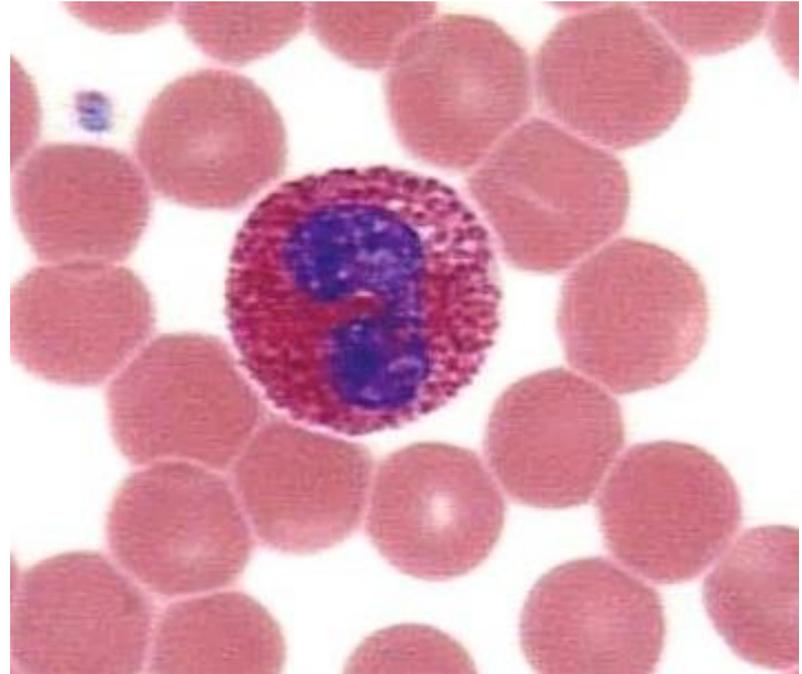
# The procedure

1. A drop of blood is thinly spread over a glass slide, air dried, and stained with an acidic dye (red) and a basic dye (blue-purple).
  2. Two hundred cells are then counted and classified.
  3. The number of each type of cells is expressed as a percentage.
- To do this one must be able to distinguish between the 5 types of WBCs based on the color and size of the cytoplasmic granules and the shape of the nucleus.

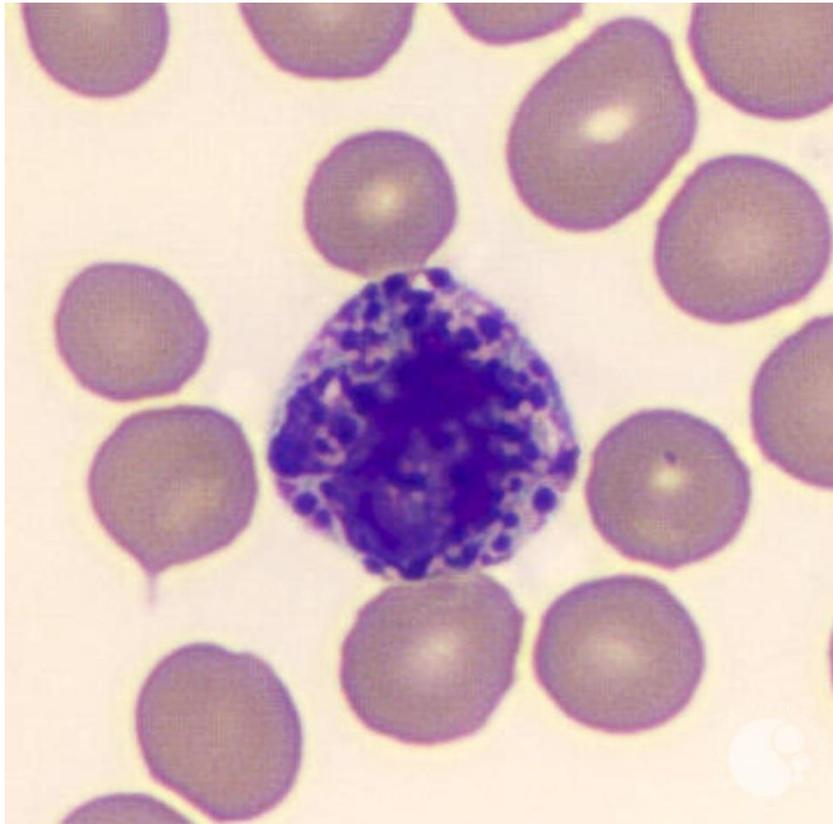
**Neutrophils** have nuclei with several lobes and fine pink granules in their cytoplasm. They are termed neutrophils, because their granules are not very amenable to staining with either acidic or basic dyes



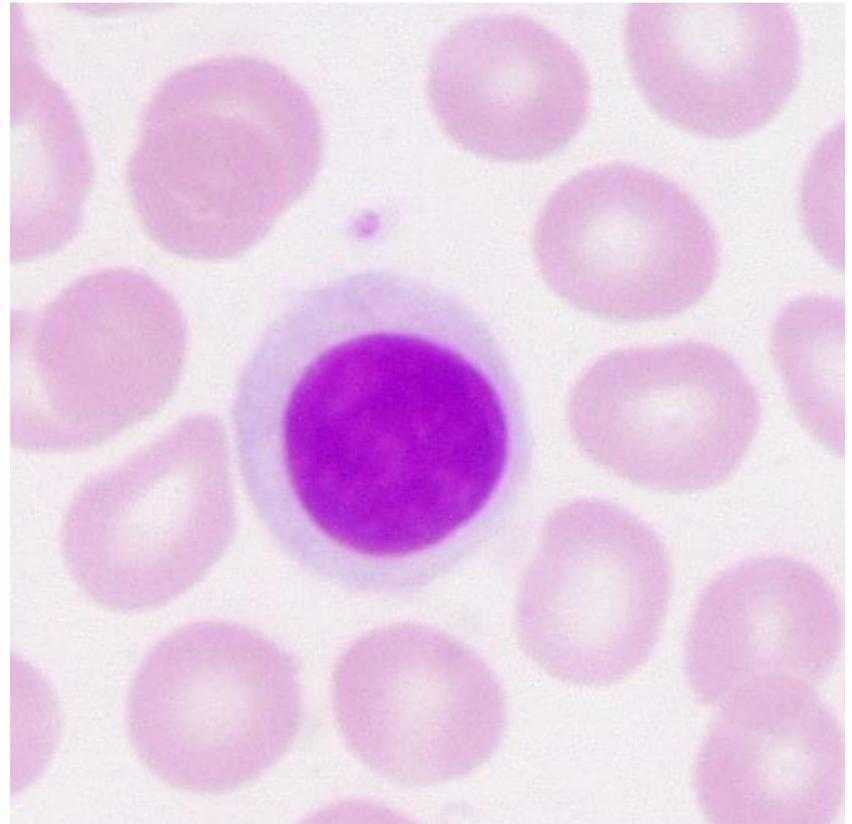
**Eosinophils** have bi-lobed nuclei and medium-sized granules that can be stained bright red with an acidic dye.



**Basophils** have bi-lobed nuclei and large granules which stain dark blue with basic dyes and completely obscure the nucleus

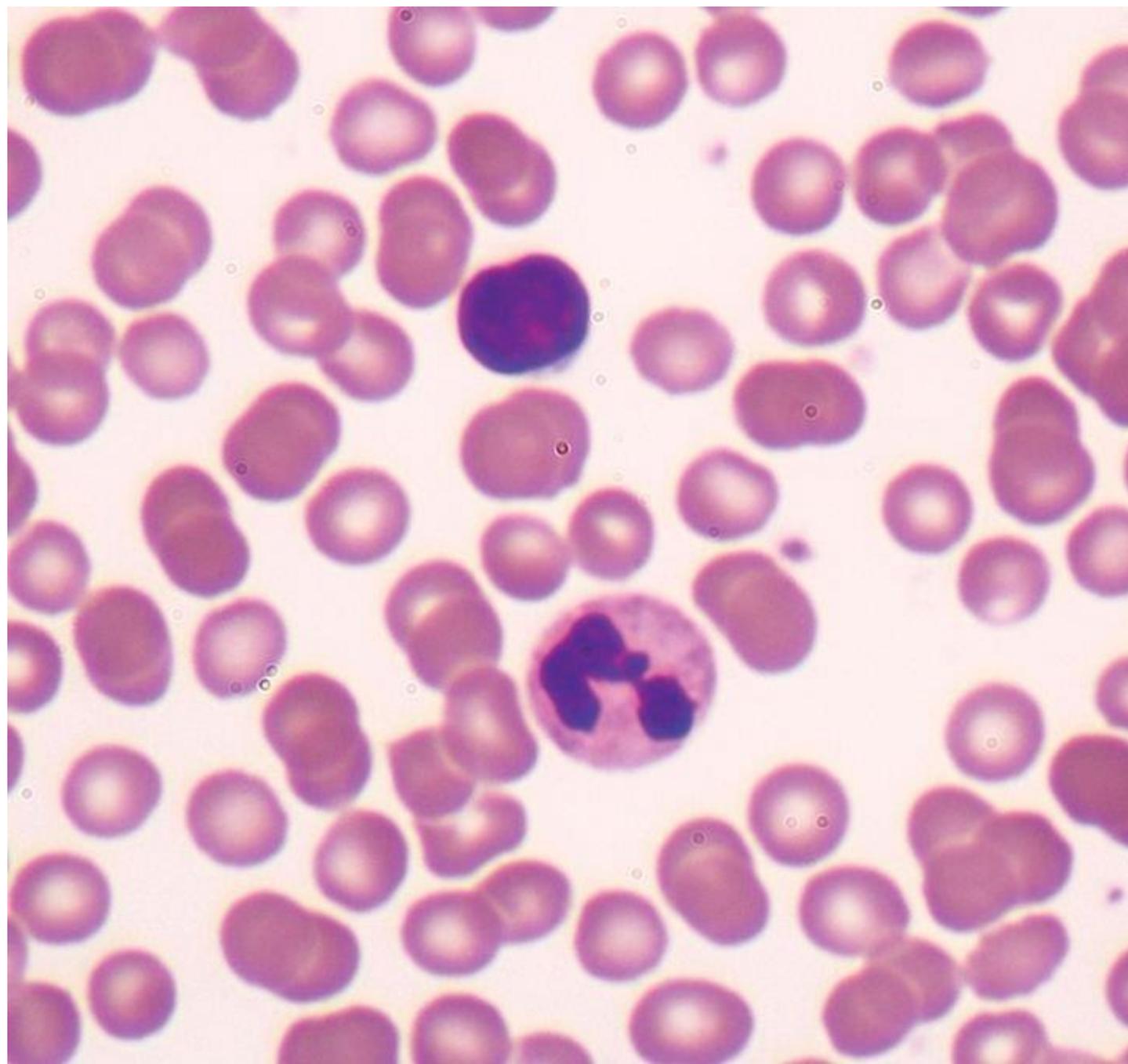


**Lymphocytes** can be large or small. They are spherical and have a very large nucleus taking up most of the cytoplasm. The cytoplasm has no granules



- **Monocytes** are large cells. They have large indented nuclei, often kidney-shaped





# Importance of DLC

- Gives relative percentage of each type of WBC
- Helps reveal the presence of abnormal WBCs like blasts or lymphoma cells.
- Used along with WBC count to generate an **absolute value** for each type of WBCs.
  - Relative percentages can be misleading
  - Absolute values are also useful for monitoring certain conditions.

# Absolute count calculation

- If the WBC count is 6000 cells/mm<sup>3</sup> and the lymphocytes make 30% of the DLC, the Absolute lymphocyte count (ALC) will be:

$$\text{WBC count} \times (\text{Lymphocyte}\%)/100 =$$
$$(6000 \times 30)/100 = 1800 \text{ cells/mm}^3$$

- Absolute neutrophil count (ANC) is the actual number of WBCs that are neutrophils.
  - $\text{ANC} = \text{WBC count} \times (\text{neutrophil}\% + \text{bands}\%)/100$ 
    - ✓ **Band cells** are an immature form of neutrophils

➤ Deviations of different white cell counts from the normal values often indicate a diseased state:

1. Neutrophilic leukocytosis: is defined as a total WBC above 11,000/ $\mu$ L along with an absolute neutrophil count (ANC) greater than 7700/ $\mu$ L
  - Bacterial infections, inflammatory conditions, stress.
  
2. Lymphocytic leukocytosis : is defined as a total WBC above 11,000/ $\mu$ L along with an absolute lymphocyte count greater than 4500/ $\mu$ L
  - Viral infections.
  
3. Monocytic leukocytosis:
  - Acute or chronic bacterial infection and chronic inflammation
  
4. Eosinophilic leukocytosis :
  - Parasitic infections & allergic conditions
  
5. Basophilic leukocytosis:
  - Allergic conditions

6. Neutropenia : absolute neutrophil count is less than 1,500 cells/ mm<sup>3</sup>

6. Certain infections like typhoid fever, HIV & CMV, chemotherapy, radiotherapy, autoimmune diseases

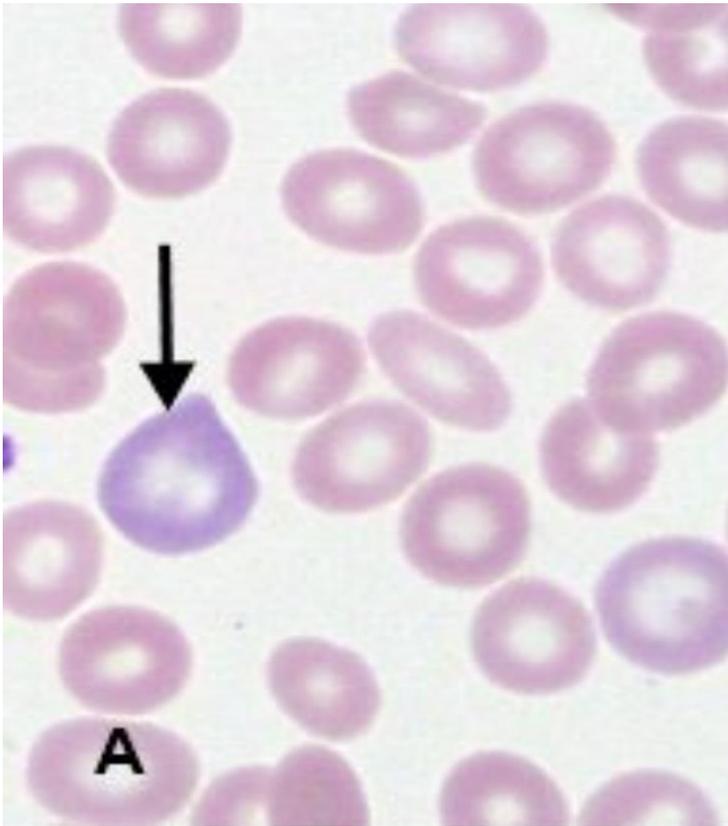
7. Lymphocytopenia:

– Persistent lymphopenia may occur in the normal elderly or be associated with chronic infection or malignancy.

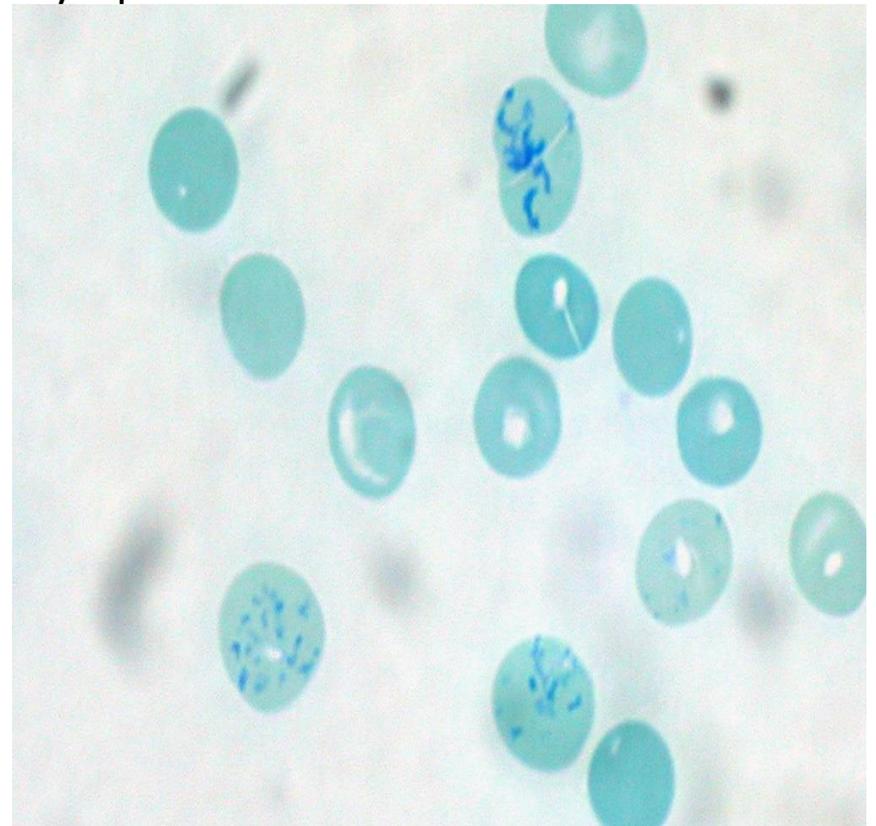
# Reticulocyte Count

- Reticulocytes are the immediate precursor of RBCs, following their release to the blood stream they mature within 1-2 days into RBCs.
- Contain a small amount of basophilic material, mainly remnants of the Golgi apparatus & mitochondria
- They normally make less than 1-2% of all RBCs
- Used to estimate the degree of effective erythropoiesis
- Their number increases in cases of bleeding and RBC hemolysis and decreases in cases of bone marrow failure

On a blood smear stained with Wright-Giemsa, reticulocytes appear larger than mature RBCs with irregular borders and are blue-grey in color. They often lack the central pallor.



If supravital staining (new methelene blue) is performed on a blood smear, the reticulocytes appear larger than RBCs and contain dark blue dots and curved linear structures in their cytoplasm.



# The procedure

- 500-1000 RBCs should be counted and the number of reticulocytes noted. The count is expressed as a percentage which can be used to calculate the absolute reticulocyte count (ARC) .
- ARC accurately reflects the degree of reticulocytosis regardless of the degree of anemia. The normal absolute reticulocyte count is between 25,000 to 75,000/mm<sup>3</sup>
- $ARC = (RBC \text{ count} \times \text{reticulocyte}\%) / 100$

# Packed Cell Volume (PCV) Hematocrit (HCT)

- PCV is the ratio of the volume of packed red cells to the total blood volume.
  - Males: 40%- 54%
  - Females: 36% - 46%
- It decreases in cases of anemia and increases in polycythemia.

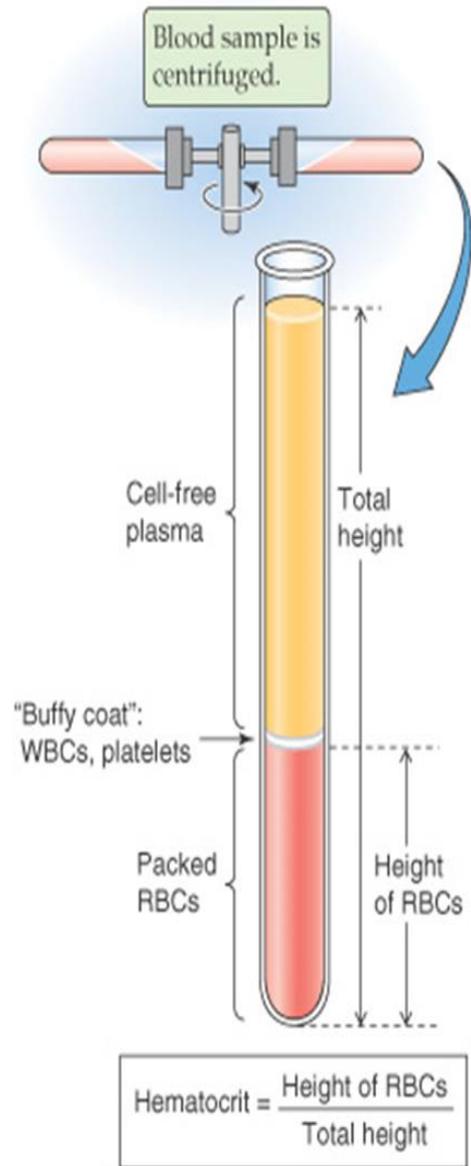


Figure 18-1 Determination of the hematocrit.

## The procedure

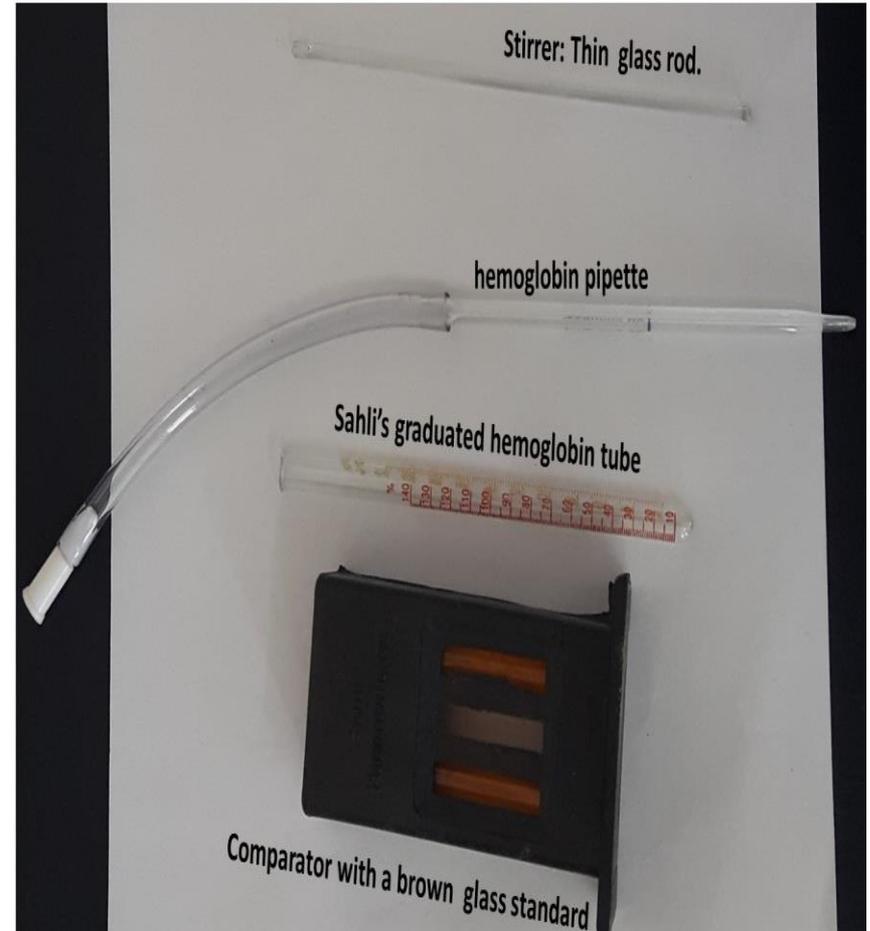
- A blood sample is centrifuged in a heparinized capillary tube (red tip ),
- The RBCs become packed at the bottom of the tube.
- The PCV is then calculated according to the following formula:
- $PCV = \frac{\text{RBC height}}{\text{Total height}} \times 100$
- Beware not to include the buffy coat

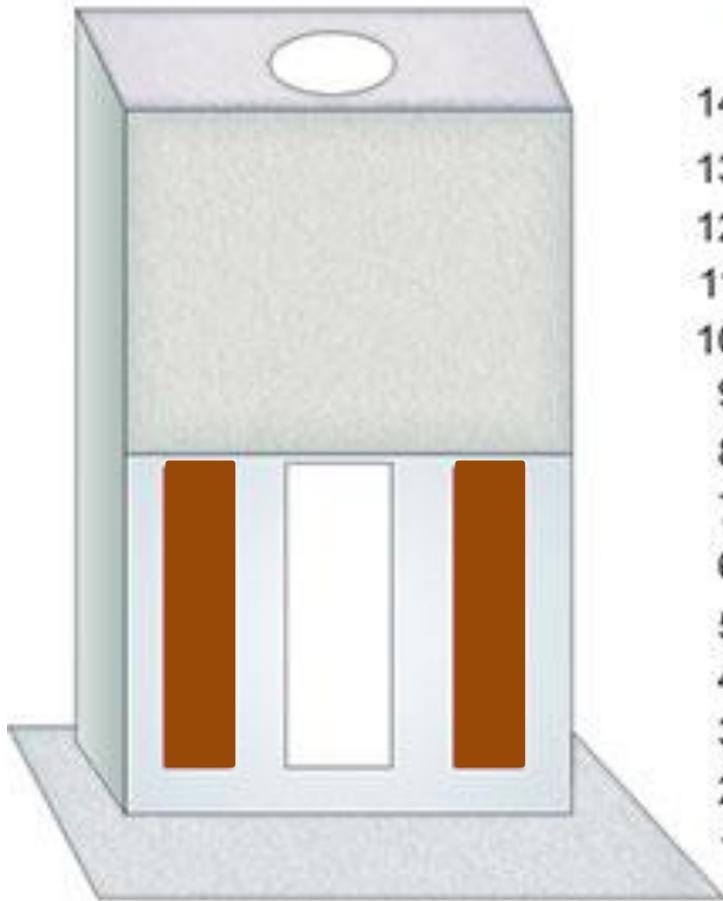
# Hemoglobin Concentration

- Hemoglobin is a globular protein made up of four subunits. Each subunit contains a **heme** group conjugated to a polypeptide. Heme is an iron-containing porphyrin derivative. The polypeptides are referred to collectively as the **globin** portion of the hemoglobin molecule.
- Heme has the ability to bind oxygen reversibly and carry it to tissues.
- Normal values of hemoglobin
  - 14-17.5 g/ 100 ml in males
  - 12-15 g/ 100 ml of in females
- Different methods can be used to find the hemoglobin concentration one of them is Sahli's method.
- Based on the fact that when blood is mixed with HCl, hemoglobin is converted to acid hematin which is brown in color

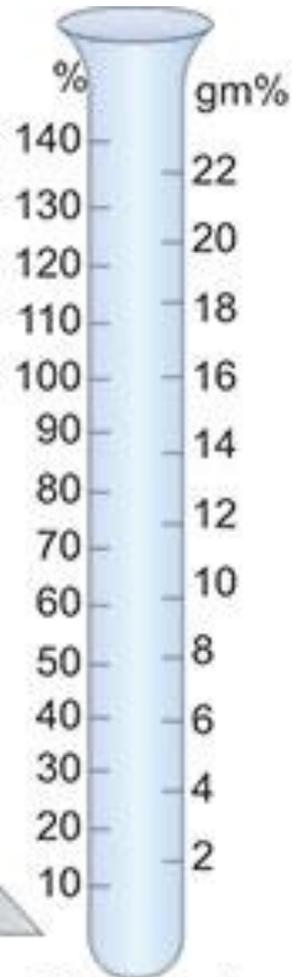
# Sahli's apparatus

Sahli's haemoglobinometer

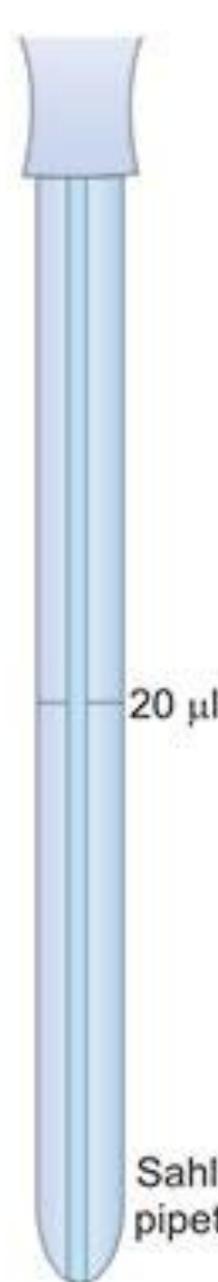




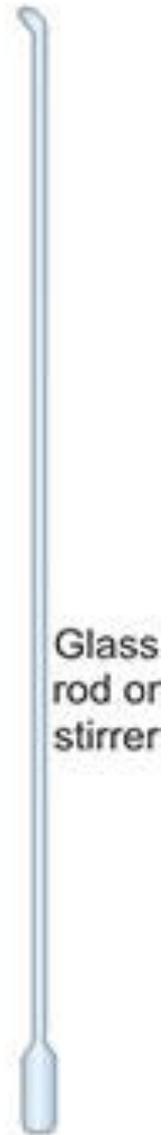
Comparator with a brown glass standard



Graduated hemoglobin tube



Sahli's pipette

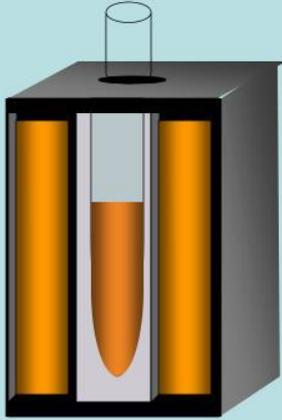


Glass rod or stirrer

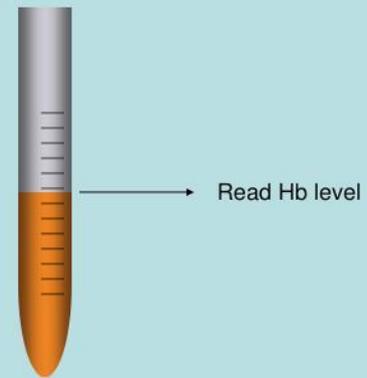
# The procedure

1. Add HCl into the tube up to 2g% mark
  2. Mix the EDTA sample gently and fill the pipette with 0.02ml blood.
  3. Wipe the external surface of the pipette to remove any excess blood.
  4. Add the blood into the tube containing HCl. Wash out the contents of the hemoglobin pipette by drawing in and blowing out the acid few times so that the blood is mixed with the acid thoroughly.
  5. Allow to stand undisturbed for 10min. (This is because, maximum conversion of hemoglobin to acid hematin, occurs in the first ten minutes)
  6. Place the hemoglobinometer tube in the comparator and add distilled water to the solution drop by drop stirring with the glass rod until it's colour matches that of the comparator glass.
  7. Remove the stirrer and take the reading directly
- Hemoglobin concentration is read directly from the graduated scale on the dilution tube.

Continue adding, stirring until colour matches with standard



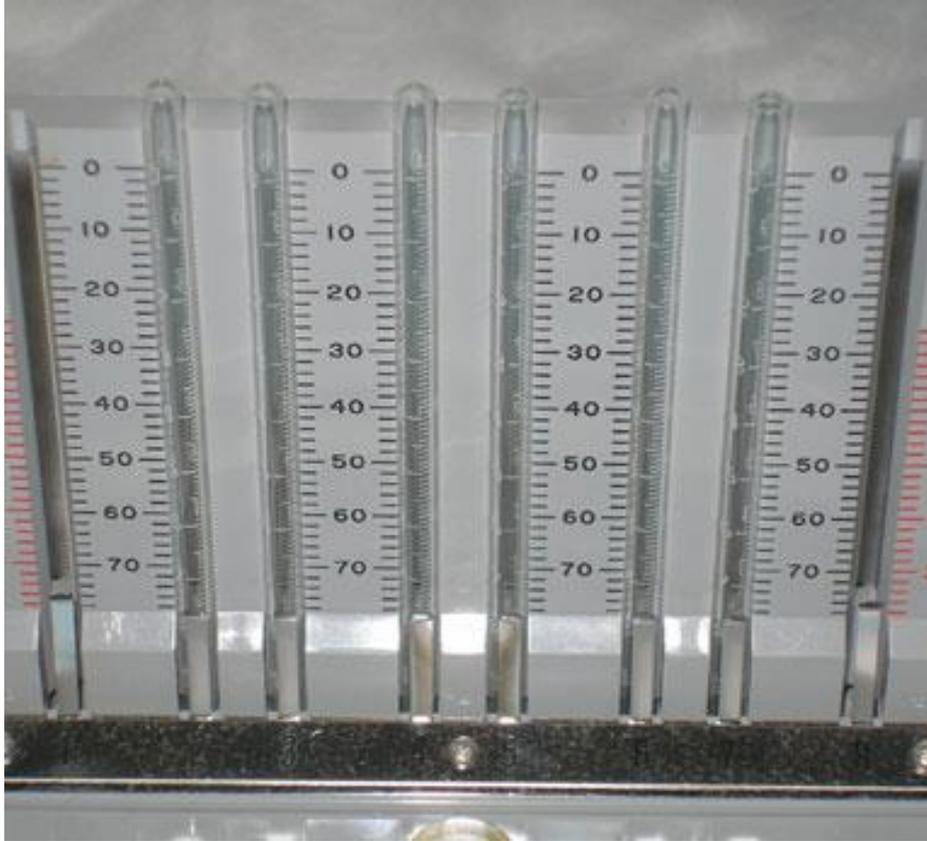
Read Hb from lower meniscus,  
express as g/dl



# Erythrocyte Sedimentation Rate (ESR)

- The rate at which RBCs sediment in a period of one hour.
- The ESR is a simple non-specific screening test that indirectly measures the presence of inflammation in the body.
- It reflects the tendency of red blood cells to settle more rapidly in the presence of some disease states, usually because of increases in plasma fibrinogen, immunoglobulins, and other acute-phase reaction proteins. Changes in red cell shape or numbers may also affect the ESR.

# The procedure



- In our lab we use the Wintrobe tube which is 100 mm long.
- EDTA anticoagulated blood is drawn into the Wintrobe tube
- The tube is placed in its rack in a strictly vertical position for 1 hour at room temperature
- the RBCs – under the influence of gravity - settle out from the plasma.
- The rate at which they settle is measured as the number of millimeters of clear plasma present at the top of the column after one hour (mm/hr).

At the beginning of the experiment

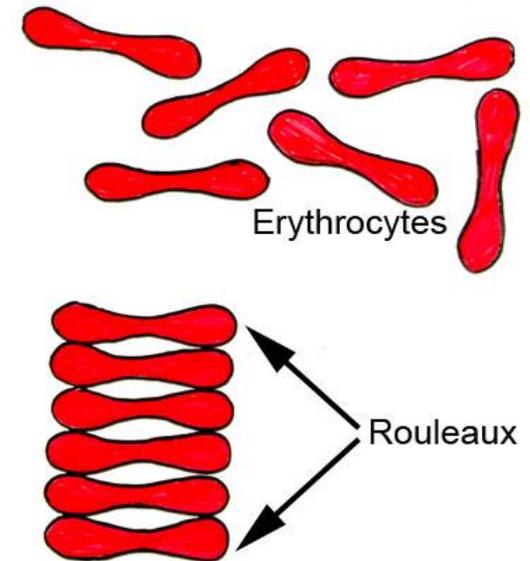


After one hour



# Why do RBCs sediment

- The RBCs sediment because their density is greater than that of plasma.
- This sedimentation increases when there is an alteration in the distribution of charges on the surface of the RBC (which normally keeps them separate from each other) resulting in their coming together to form large aggregates known as rouleaux.
- Rouleaux formation is determined largely by increased levels of plasma fibrinogen and globulins, and so the ESR reflects mainly changes in the plasma proteins that accompany acute and chronic infections,



- Normal ESR values
  - Men < 15mm/hr
  - Women < 20mm/hr
- High ESR
  - Inflammation
  - Anemia
  - Old age
  - Technical factors: tilted ESR tube, high room temperature.
- **Some interferences which decrease ESR:**
- Abnormally shaped RBC (sickle cells and spherocytosis)
- Technical factors: low room temperature, delay in test performance (>2 hours), clotted blood sample, excess anticoagulant, bubbles in tube.
  - ✓ Polycythemia decreases ESR

Pa [REDACTED]

Patient No. : **19/899500**

Age : **38 Year(s)**

Sample No. : **BA0120/1983778**

Sex : **Female**

Sample Date / Time : **15-Aug-2020 1:14 PM**

**Routine Haematology**

			<u>Reference limit</u>
Haemoglobin	: 127	g/L	120 - 160
Haematocrit	: 36.8	%	37.0 - 48.0
RBC	: 4.8	x10 <sup>12</sup> /L	4.2 - 5.2
MCV	: 76.8	fL	80.0 - 99.0
MCH	: 27.0	pg	27.0 - 32.0
MCHC	: 34.5	g/dL	32.0 - 36.0
RDW	: 14.3	%	11.7 - 15.2
Platelets	: 292	x10 <sup>9</sup> /L	150 - 450
MPV	: 9.2	fL	7.2 - 11.7
WBC	: 6.080	x10 <sup>9</sup> /L	4.0 - 11.0

<b>Differential</b>				<u>Reference limit</u>
Neutrophils	: 49	%	2.979 x10 <sup>9</sup> /L	1.800 - 7.500
Lymphocytes	: 43	%	2.614 x10 <sup>9</sup> /L	1.200 - 4.000
Monocytes	: 7	%	0.426 x10 <sup>9</sup> /L	0.200 - 1.000
Eosinophils	: 1	%	0.061 x10 <sup>9</sup> /L	0.040 - 0.500
Basophils	:	%	x10 <sup>9</sup> /L	0.015 - 0.100

Blood Film : The red blood cells are mainly normochromic normocytic.  
The white blood cells are normal in total count and differential.  
The platelets are adequate with normal size.

- Serum iron profile is recommended.

<b>Doctor:</b>	[REDACTED]	<b>Age:</b>	5 Years
<b>D.B:</b>	04-12-2012	<b>Gender:</b>	Male
<b>Date/Time:</b>	26-06-2018	:	

## Hematology Report

Test	Value	Unit	Expected Value
Hb	12.4	g/dL	10.3 - 14.9
PCV	36.6	%	30.0 - 44.0
RBC	4.93	X10 <sup>12</sup> /L	4.0 - 5.2
MCV	74.24	fL	75 - 85
MCH	25.15	pg	25.0 - 29.0
MCHC	33.88	g/dL	32.0 - 36.0
RDW	14.2	%	11.0 - 16.0
<b>Platelet count</b>	325	X10 <sup>9</sup> /L	150 - 450
<b>Leukocytes</b>	<b>14.5</b>	X10 <sup>9</sup> /L	6.0 - 15.0
<b>Differential:</b>			
Neutrophils	80	%	11,600
Lymphocytes	15	%	2,175
Monocytes	4	%	580
Eosinophils	1	%	145
Basophils	0	%	0

**Notes:**