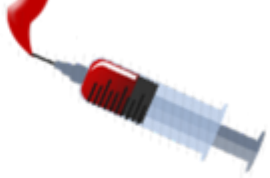
Hematology



Physiology lab

☐ Sheet ☐ Slide ☐ Handout

Number: 2

Subject: **WBCs, ESR,PCV and HB**

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Doctor:

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Date:

Price:

- Topics of the lab session:
  - ► WBC; their moroplolgy and count.
  - ► Reticlucytes.
  - ► ESR.
  - ► PCV.
  - ► Hemoglobin measuring.
- Normal values and percentages are imp to know.
- Some of the information mentioned in this lad may be a little bit different from what we had taken in histology.
- Figures that were drawn on the board in the lab are found in the last page in this sheet.

❖WBC:

- WBCs are classified according to their content of granules and their cytoplasm into:
  1- Granulocytes: which are neutorphils, basophils and eosinophils.
  2- Agranulocytes: monocytes and lymphocytes.

- We differentiate between granulocytes according to the **size** of their cytoplasmic granules:
  - ➢ Neutrophils: they have **fine granules** that look like the sand particles.
  - ➢ Eosinoplils: **medium sized pink granules** and their color becomes lighter as the cell grows. Granules are seen at one side of the cytoplasm (they appear like bubbles) .
  - ➢ Basophils: **dark blue large coarse granules**, distributed all over the cell **covering the nucleus**. Differentiated from lymphocytes by the presence of granules.

- ## Morphology of the nucleus:

  - ➢ Granulocytes: **bi or multi** lobed nucleus (2-5 lobes). The lobes are only connected by filaments, so you can recognize each lobe as a separate part. More specifically:
    - → In neutrophils: the nucleus has 2 or more lobes.
    - → Eosinophils: bi-lobed nucleus.
    - → Basophils: bi-lobed nucleus. The granules obscuring the nucleus and giving the appearance of spikes.

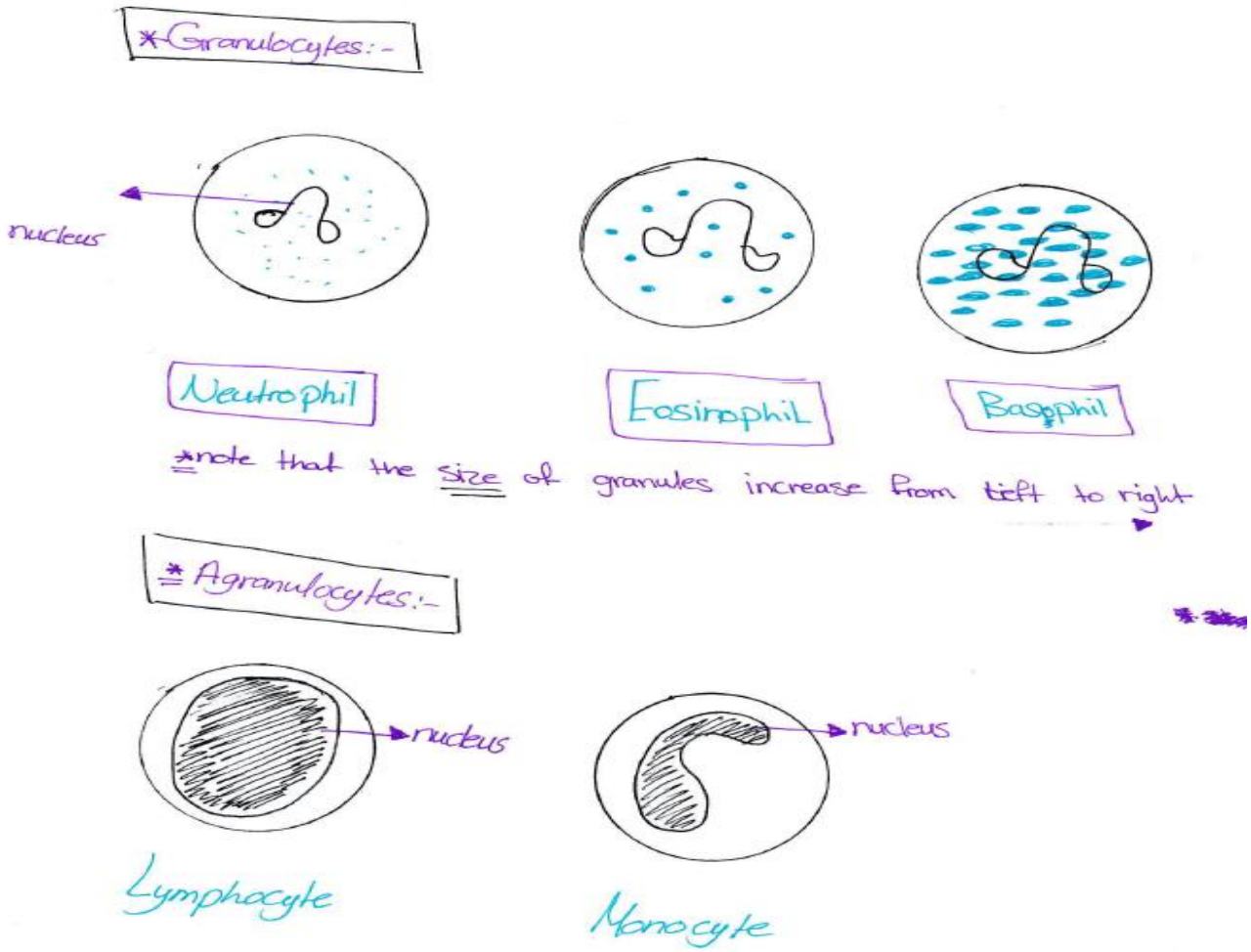
  - ➢ Agranulocytes: the nucleus has **NO** lobes and appears as one piece.
    - → Monocytes: kidney-shaped nucleus.
    - → Lymphocytes: large circular nucleus.

  - ➢ *NOTE: it is discovered recently that the Agranulocytes are not devoid from granules, however the granules are few in number are hardly seen. In the lab we will follow the old classification.*

- NOTE: sometimes neutrophils' nuclei are thought to be kidney shaped as the monocyte's nucleus; however this is not true, it is bi-lobed.
- WBCs are seen under light microscope using the ***oil immersion lens*** that has a magnification of 100X.

- **Differential count of the WBCs:**

  - **Neutrophils:** around 60%, if more than the normal range this is called **neutrophila.** This percentage is increased in the case of bacterial infection. If less than the normal count this is called **neutropenia.**
  - **Eosinophils**: 1-6%, increases in parasitic infection or allergy.
  - **Basophils**: .5-1%, releases heparin and histamine.
  - **Lymphocytes**: 20-25%, increase in cases of viral infection (***lymphocytosis).***
  - **Monocytes:** 2-10%.



❖ Reticulocytes:

- Reticulocytes are cells of the last stage of RBCs maturation that are released into the blood stream from the bone marrow to allow further maturation. And once they complete maturation they become RBCs.
- *NOTE: The RBCs released from the BM has a nucleus, after 2 to 3 days nucleus will be degenerated. This was mentioned in the lab; however it is not totally accurate.*
- Reticulocytes have **remnants of the rRNA** that form like a net in the cell which can be seen under the microscope, using special stains, as a blue network.
- Normally reticulocytes present in the blood forming (**2%**) of the RBCs in adults. An increase in this percentage indicates a **more than normal active BM** due to underlining abnormality.
- This percentage is higher than 2% in newborns as they need higher RBCs production.

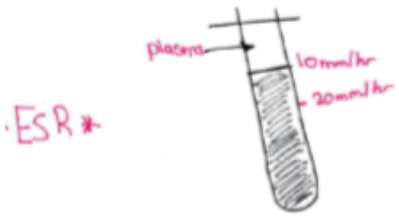
❖ Erythrocyte sedimentation rate (ESR): *معدل ترسيب كريات الدم الحمراء*

➢ The procedure:

I. In a narrow test tube containing an anti-coagulant, we add a blood until it is totally filled.

II. We place the tube vertically and let it for only 1 hour, to allow RBCs to settle by the action of the gravity.

III. When RBCs settle, the upper part of the tube will become transparent to yellow in color because it now contains plasma only.



IV. After the one hour, we measure the height of the plasma transparent layer that appeared. It will resemble the rate of the sedimentation of the RBCs. Measured in (mm/hour).

➢ Notes regarding ESR:

- → The tube that is used is graduated into mm, to allow easy measuring.
- → The rate in **females is higher than in male** because of the higher RBCs count in male than females.
- → ESR is **NOT a specific test**, which means an abnormal ESR indicate that the patient has an abnormality without telling what it is, it does not provide an definitive diagnosis.
- → ESR is elevate in most cases of **inflammation** because in inflammation there will be an increase in the in plasma proteins specially fibrinogen and immunoglobins as they will make RBCs stick to each other, forming *rouleoaux,* more easily.
- → ESR is affected by technical factors such as the temperature and the position of the tube.
- → **ESR is measured in the first hour only,** which means you can't for example let the tube for 2 hours and then measure the rate by dividing the number you have on 2. This is incorrect.
- → There is no automated way to measure ESR, only manual.

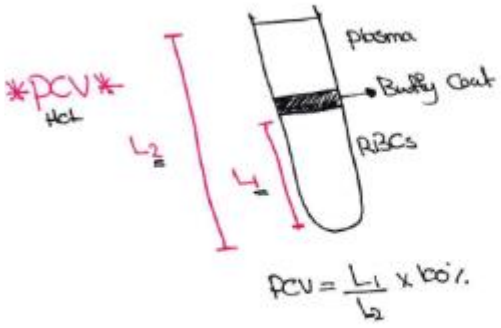
- → Normal values:
  - ► Males: <15mm/hr
  - ► Females: <20mm/hr

- → *Remember that values differ according the method used.*

❖Packed cell volume:

- **The procedure:**

1. We fill three quarters of the tube with blood (e.g. capillary tube).
2. Place the tube in the micro-centrifugation machine.
3. The sample will be separated by centrifugation into two layers: lower RBCs layer and upper plasma layer, and between the two layers there is the Buffy coat.



4. Using a ruler we measure the height of the RBCs layer ***(L1)*** and the height of the whole sample ***(L2)*** and then we use the formula to calculate PCV : $\boldsymbol{PCV = \frac{L1}{L1} * 100\%}$.

   → **Notes:** The PCV in males in higher than females because RBCs count is higher in males.

   → The used tube is a special capillary tube whose tip is marked with **specific color**. A **red** tip means that an anticoagulant is add while when the tip is **blue** that means that there is no anticoagulant -it is used in clotting tests-.

   → PCV is the same as hematocrit.

   → Normal values:

   - Males: 40-52%
   - Females: 36-48%

❖Hemoglobin measuring:

- The manual method used is known as Sahli method. In this method we have a comparator with two tubes, known as ***standard columns***, that have a specific color. We use them to compare the color in the sample with.
- Hemoglobin is measured in g/dl which equals g/100ml.

• The procedure:

1. In a specific graduated tube, 20 µl of blood is added and then 3 drops of HCl are add one by one with stirring the mixture when adding each HCl drop.
2. HCl will convert hemoglobin into hematin which is brown.
3. We dilute the mixture with distilled water until we have a mixture whose color is **exactly the same** as the color in the standard columns.
4. Once the sample's color becomes the same as the standard columns, we stop diluting and we read the number we reached in the tube.
5. This number will give us, ***directly,*** the hemoglobin concentration in the original sample.

Note that hemoglobin color is red while the hematin is brown.

• Notes:

→ There are automated methods that are used nowadays, in which we just place the tube containing the sample in the machine and the machine by itself will compare the wave length of the sample, compare it with the standard wave length and give the hemoglobin concentration directly. However, it is important to know the manual method because it is needed in the calibration of the automated machine. In other words, the automated machine sometimes is programmed according to the manual one.

→ It is important that after each drop of HCl or water to stir the sample in order to have a homogenous mixture.

- → If more than enough water is added, the sample will become **lighter** than the standard columns. In such case we need to repeat the whole procedure again.
- → If 20 to 25 drops of water were added and the sample's color still **darker** than the standard columns then we add two more drops of HCl one by one. So in total we are allowed to add 5 drops of HCl (3 at the beginning and 2 in the end).
- → When comparing the color of the sample with the standard columns, the comparator must be placed in a way that allows the light to pass through it. So you must not place your hand behind it for example.
- → Once again the normal values here are different between males and females, and the values are:
  - ► Males: 13.5-17.5 g/dl.
  - ► Females: 11.5-15.5 g/dl.

Sorry for any mistakes,

Wish you all best of luck.