Hematology

- Histology
- Biochemistry
- Pathology
- Pharmacology
- Physiology
- Microbiology

- Dr. name :
- lecture number : **Lab Notes**
- Done BY : **Lara Khoury**

- Handout
- Sheet
- Slide

By: Anwer Abu Shanab

Physiology lab #2 Notes

#1) Sahli's Method of Hemoglobin Determination:

Apparatus

1. micropipette (sahli's pipette)
2. Sahli's tube which is having red and yellow scales on two sides. Red scale is percentage scale and yellow scale is gram percentage or g/dl scale.
3. Heamometer ; with 2 colored rods to be used as a reference
4. HCL
5. Glass rod
6. Distilled water

Method

- Using the micropipette take 20 microliters of blood, add it to the tube
- Add 3 to 5 drops of HCL (why? HCL will cause the lysis of the blood cells and hemoglobin is released.) the blood's color will then be reddish brown (tan color)
- Add at first 3 drops of water then start mixing using the glass rod for a while then insert the tube into the Heamometer to compare the color of the sample with the colored rods
- Repeat step 3 but this time add one drop until the color of the sample is the same as the ones in the Heamometer
- When the colors are finally the same read from the yellow scale the level of the solution …

  … And that's gonna be our result!

Normal range

Males: 13.5-17.5 **g/dl** the units are ipm.

Females: 11.5-15.5 **g/dl**

#2) OSMOTIC FRAGILITY:

The osmotic fragility test determines the ability of the RBC membrane to resist rupturing in a hypotonic saline solution.

Remember: A 0.9% NaCl solution is said to be isotonic; when RBCs are immersed in a hypotonic solution the intracellular and extracellular fluids are in osmotic equilibrium across the cell membrane, and there is no net influx or efflux of water.

In a *hypotonic* solution (e.g. 0.2% NaCl or distilled water), an influx of water occurs: the cells swell, then burst (**hemolysis**) and hemoglobin escapes and dissolves in the external medium.

On the other hand in *a hypertonic* solution (e.g. 1.8% NaCl), the cells shrink and collapse (**crenation**) due to the rapid osmotic efflux of water.

Method

- Add the RBCs to tubes containing increasingly dilute saline solutions starting from 0.9% to 0.2% NaCl solution.
- The percentage of the first solution at which the cells swell and rupture is then noted.

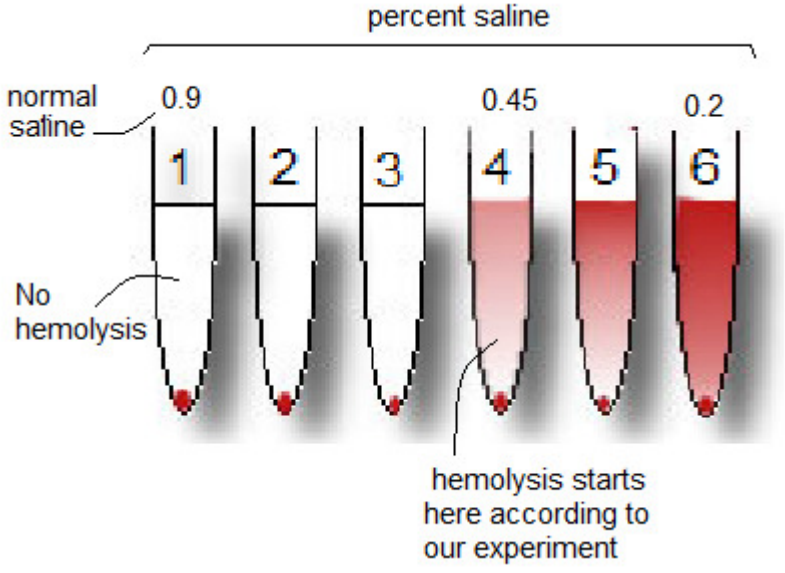
In our experiment in the lab the concentration at which RBCs started to lyse is 0.45% NaCl

Normal results:

Normal erythrocytes rupture in saline solutions of 0.30 to 0.45 percent.

Clinical approach:

Spherocytes and cells with damaged membranes cannot tolerate the influx of water and burst before normal RBCs burst; that is in saline solutions only slightly less concentrated than normal saline; **more fragile** –eg. they'll lyse in tube no.2-. The opposite happens in thalassemia and sickle cell anemia; they burst in

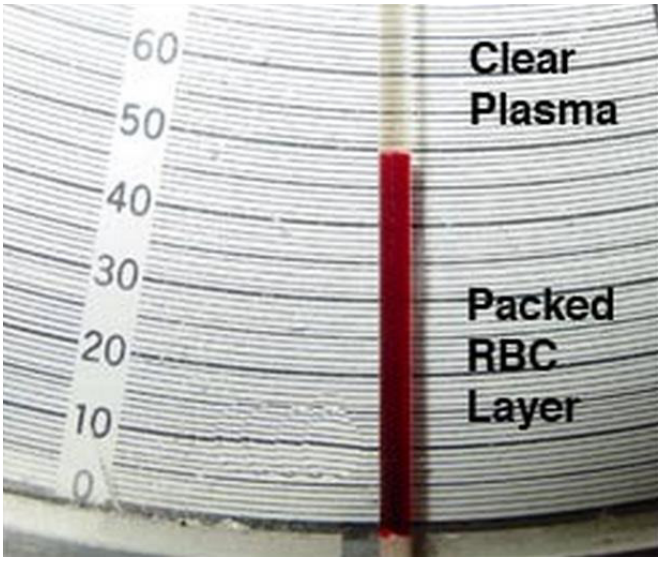


a solution that is even more hypotonic than what normal RBCs can tolerate; **less fragile** – eg.they'll lyse in tube no.6-.

→ Let us imagine the RBCs as a balloon half filed with air, we can add X more volume of air before it burst. If this balloon was nearly full of air (spherocyte) the amount of air that can be added before the balloon burst would be way more less than that of X. and if the balloon is not filled at all (crescent in shape RBCs and small RBCs) the amout of air we can add before the balloon burst will be way more higher than X.

#3) Packed Cell Volume (PCV):

Method:



- We put blood in a capillary tube that has an anticoagulant (marker: red line on the top) – unlike the one used in clotting time test that has a blue line on top –
- We put it in the microcentrifuge at full speed, the RBCs will settle at the bottom, WBCs and platlets on top of them –buffy coat- and on top of them all is the plasma.
- Using a ruler we measure the hight of the RBC column and the total hight
- To calculate the packed cell volume we apply the following formulae:

$$PCV = \frac{Hight\ of\ RBC}{total\ hight} \times 100\%$$

Normal results:

Males: 40 - 52%

Females: 36 - 42%

#4) Blood Group Determination Test:

We all know the ABO system for blood groups that is based on two types of antigens that are either present or absent on the RBC surface; antigen A and antigen B

- If antigen A is only present the blood group is A
- If antigen B is only present then the blood group is B
- If Both antigens are present then the blood group is AB
- If None of the antigens are present the blood group is O

The body will make antibodies against the antigen the blood cells don't express

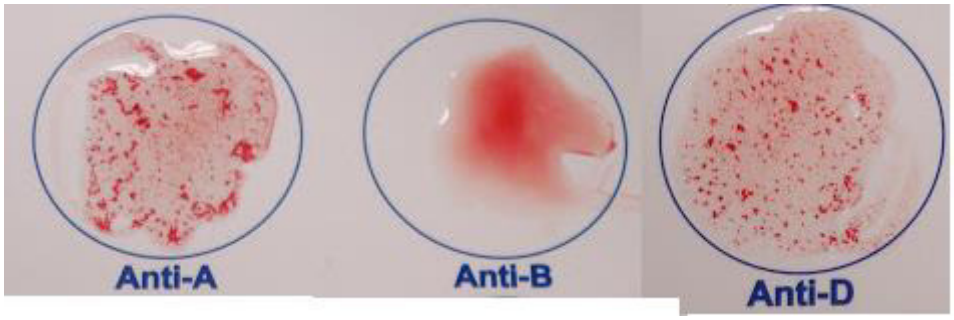
- In A blood group the antibodies present are anti-B
- In B blood group the antibodies present are anti-A
- In AB blood group NO antibodies are present because both antigens are present
- In O blood group Both antibodies are present because none of the antigens are present

Another antigen was later discovered; the RH-factor, if it was present on the surface of the RBCs then the blood group will be "+" and if it wasn't it will be "-"

Remember: If the antibody meets its antigen a coagulation rxn will happen

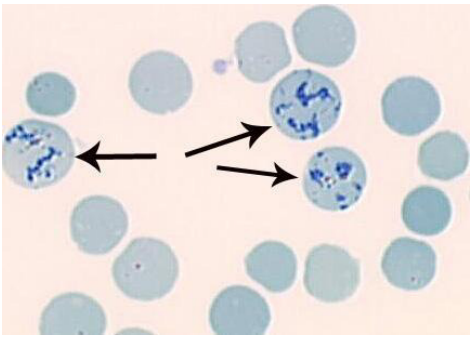
Accordingly we take 3 blood drops from the person whom blood group is going to be determined ,we put these 3 drops far away from each other on a glass slide and add to the first drop an anti-A, anti-B to the second, and anti-D (anti-Rh) to the third.

The droplets where coagulation takes place means that the RBCs have the antigen on their surface.



The blood group here is ??

#5) Reticulocyte count:



Measure the percentage of reticulocytes in the blood.

This can be used to monitor the function of the bone marrow and to determine the type pf anemia.

Method

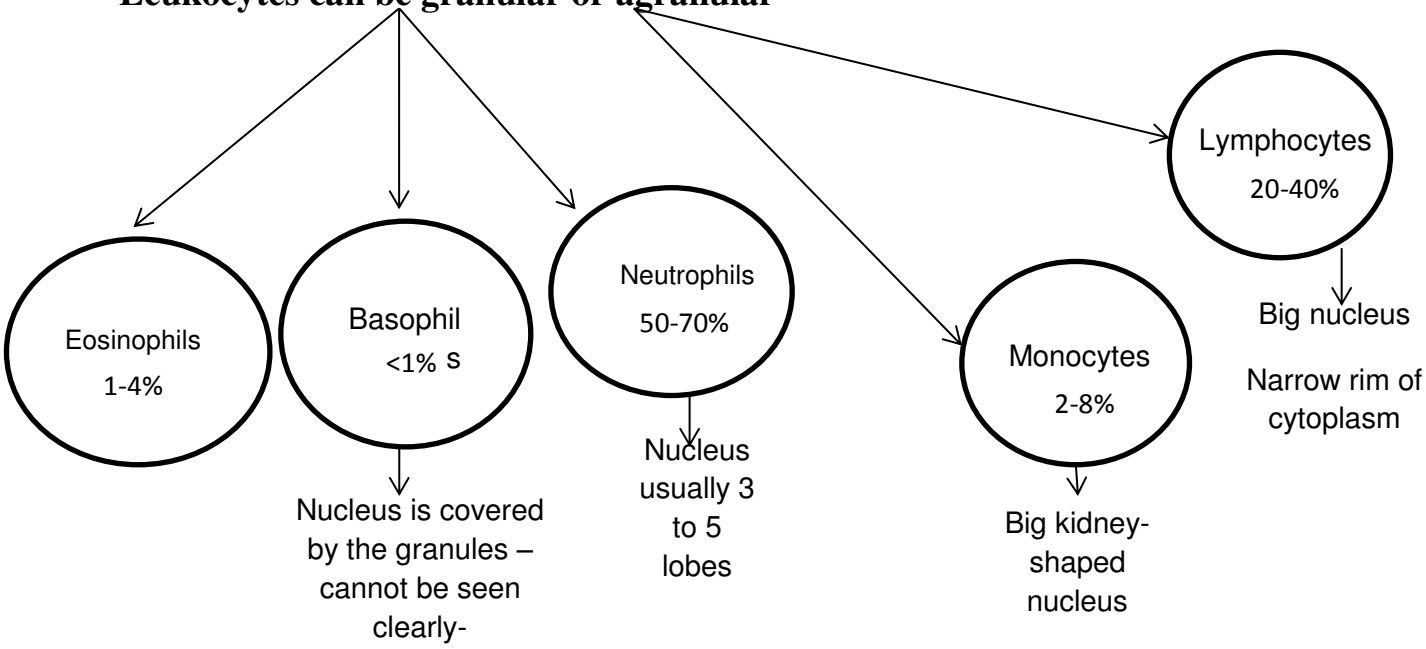
Make a blood smear, stain it and examine it under the microscope.

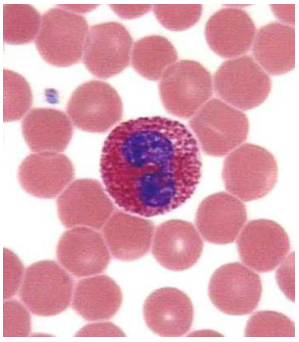
Normal Results:

Normal reticulocyte count must not exceed 2%.

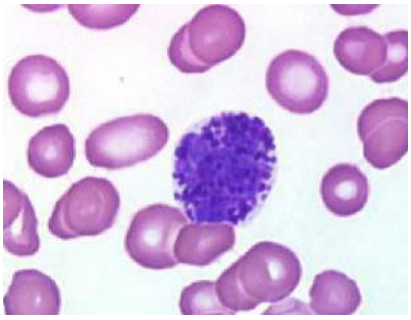
#6) Differential leukocytes count:

Leukocytes can be granular or agranular

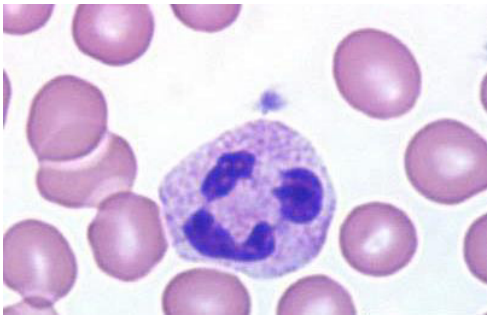




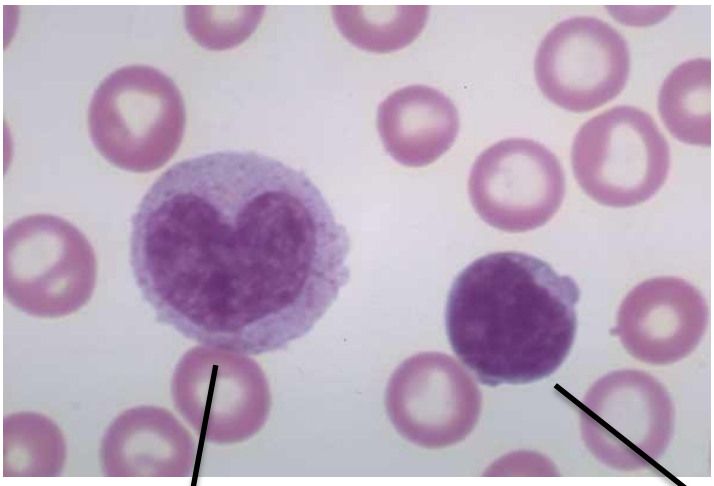
Eosinophil



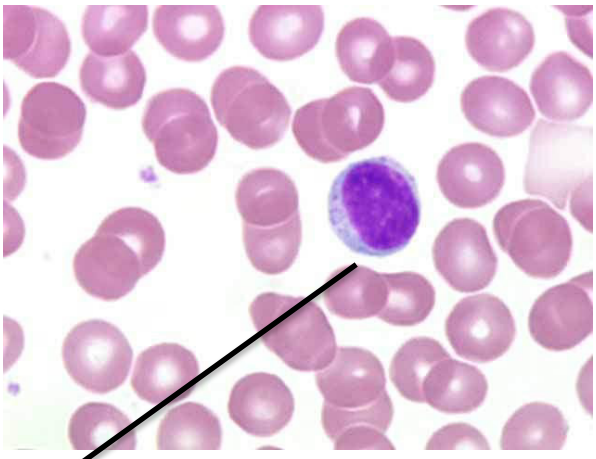
Basophil



Neutrophil



Monocyte



Lymphocyte

Best of luck!!

Tala Khoury