



# Hematology



---

## PHYSIOLOGY

---

☒ Sheet

☐ Slide

☐ Handout

Number: **3**

Subject: **Physiology Lab**

Done By: **Rawan Abudawood**

Corrected by: **Wahib Zehlawi**

Doctor:

Date: **00/9/2016**

Price:

## بسم الله الرحمن الرحيم

Well, this sheet is the easiest in the whole semester, just chill and have fun...

We will discuss four topics:

- 1- Blood groups
- 2- Clotting time
- 3- Bleeding time
- 4- Fragility test

### **Blood groups:**

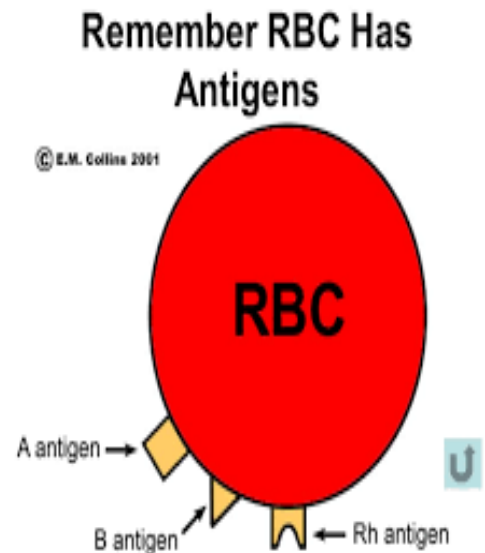
We have four blood groups (A, B, AB, and O).

Blood groups are classified according to the antigen found on the RBCs' surface.

Antigen: is a protein (glycoprotein) on the surface of RBCs and it appears before birth

| Blood Group | Antigen (Ag) | Antibody (Ab)  |
|-------------|--------------|----------------|
| A           | A            | anti-B         |
| B           | B            | anti-A         |
| AB          | A,B          | ---            |
| O           | ---          | anti-A, anti-B |

We also have the Rh antigen (also called the "D" antigen) found on RBCs. People who have this antigen are called Rh+ (D+), and the ones who don't have it are called Rh- (D-).



- ❖ In our body, the situation is as follows: a person with an "A" blood group will have the "A" antigen and the "B" antibody.
- ❖ The reaction (agglutination) occurs when an antigen and its corresponding antibody meet: which means (antigen A and anti-A), (antigen B, anti-B). That is exactly what we did in the lab, the agglutination granules appear like sand granules, and so we can see it.

## How to know the blood group of a patient?

- ❖ Simply, we bring a clean slide, we add three blood drops (separately), and we add a drop of antibodies over each drop of blood: (each of the three drops of blood will have a specific type of antibody added to it), we mix the drops well, and the result will be noticed within seconds.
- ❖ The agglutination sites represent the patient's blood type.

The importance of this procedure is illustrated in:

### 1- Blood transfusion:

- ❖ AB: universal recipient
  - ❖ O: universal donor
- In emergency cases, we give O- to the patient

### 2- Pregnancy:

If the mother's blood is D- and her fetus is D+ (some blood may pass through the placenta causing the blood of the mother to recognize the D antigen and produce antibodies that are going to attack the next baby's blood and destroy the RBCs leading to the baby's death. we call this condition Hydrops Fetalis. This problem doesn't affect the first baby, instead it effects the second one. In case the baby managed to survive and was born, he will suffer from severe anemia that causes death immediately after birth. That is why we give those mothers an antibody injection (anti-D) 24 or 72 hours before the delivery.

### 3- Paternity test:

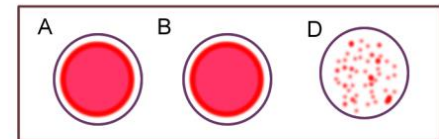
It is not a specific way to be used, but it can help a little bit. For example: could the parents of a person with an O blood group both have A blood groups? Yes, since they could be recessive.

Note: we use a white background under the used slide in order to see the agglutination clearly.

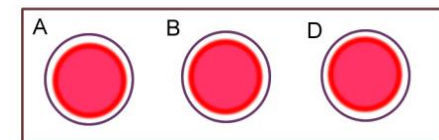


| Anti-A | Anti-B | Anti-D | Blood type      |
|--------|--------|--------|-----------------|
|        |        |        | A <sup>+</sup>  |
|        |        |        | B <sup>+</sup>  |
|        |        |        | AB <sup>+</sup> |
|        |        |        | O <sup>-</sup>  |

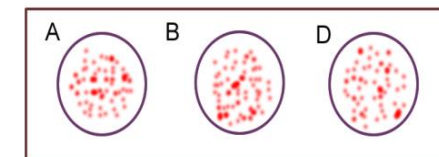
### Blood Groups



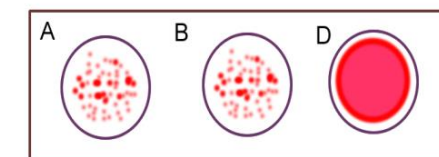
O<sup>+</sup>



O<sup>-</sup>



AB<sup>+</sup>



AB<sup>-</sup>

### Notes:

In the lab, usually the anti-A we use is **blue**, and the anti-B is **yellow**, while the anti-D is **transparent**, but it is preferable to write down the name of the used antibody in order not to get confused.

Anti-D reaction takes seconds longer than the anti-A and anti-B to appear.

## Clotting time: (normally 4-10 minutes)

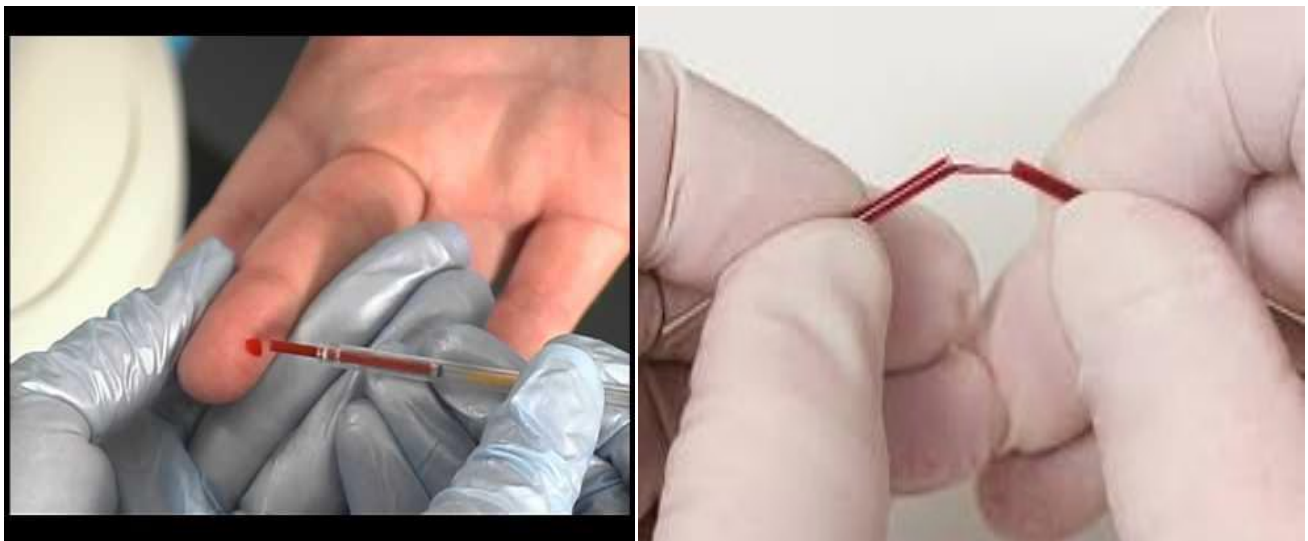
In this test, we are looking at the function of clotting factors;

- 1- We fill a capillary tube (the one tagged by blue that indicates it is free of anticoagulants) by blood.
  - Recall that we have two types of capillary tubes, one with a **red tip** (meaning that tube has anticoagulants and is used in PCV test), and one with a **blue tip** (it is free of anticoagulants and is used in this test).
- 2- After 30 seconds we start to break the capillary looking for the fibrin thread to appear, we break every 30 second until we find the fine thread.
- 3- We record the time.



One can continue doing this for up to 10 minutes, that's why we'll fill up to 10 or 15 capillary tubes. If the thread didn't appear during the 10 minutes then the patient has a problem in the clotting factors.

In blood, we have prothrombin that upon activation will give thrombin; thrombin along with the presence of clotting factors will convert fibrinogen (soluble) into fibrin (insoluble) that appears as a thread.



## **Bleeding time: (normally 1-3 minutes)**

Here we are looking at the function of the platelets.

- 1- We use a special lancet with a measured depth (like the one used by diabetic patients) to pierce the patient's fingertip.
- 2- Using a filter paper, we gently press on the patient's finger until the bleeding stops and we record the time.

Note: we cannot use any blade, because it might hurt the patient severely and it is not accurate as well.



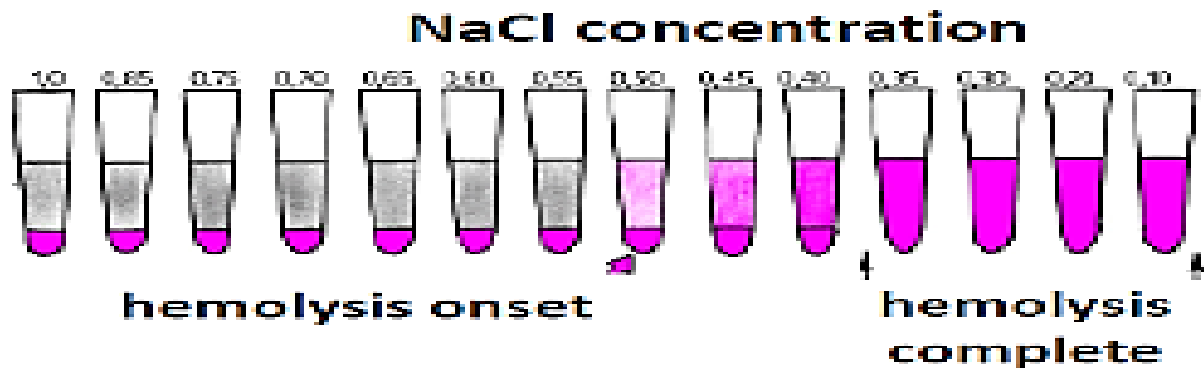
## **Fragility test: normally starts at (0.55- 0.45)%**

The intracellular fluid of erythrocytes is a solution of salts, glucose, protein and hemoglobin. A 0.9% NaCl solution is said to be isotonic: when blood cells reside in such a medium, the intracellular and extracellular fluids are in osmotic equilibrium across the cell membrane, and there is no net influx or efflux of water.

In case we put an RBC in a **hypotonic solution** (below 0.9%), then the water will move to the inside of the cell, causing it to swell and lyse (the whole contents will be released to the solution changing its color into red). While if we put the cell in a **hypertonic solution** (higher than 0.9%) then the blood will leave the cell causing it to shrink.

Fragility: measures the RBCs' resistance to hypotonic solutions (below 0.9%).

## Isotonic 0.9%



- 1- We add a blood drop to different tubes that contain different hypotonic solutions from 0.2% to 0.85%
- 2- Normally the cells will start to lyse at 0.55% or 0.45% and reach a complete lyses at 0.2% (the color changes gradually from clear to yellow to orange and finally red).
- 3- Let us assume that a patient's RBCs started to lyse at 0.2% then his cells are less fragile, higher resistance. It happens in cases of sickle cell anemia, iron deficiency anemia, thalassemia.
- 4- Let us assume that a patient's RBCs started to lyse at 0.7% then his cells are more fragile with lesser resistance. It happens in cases of spherocytosis where the cells are spherical (due to mutations in some proteins).

### Notes:

Putting blood in distilled water (hypotonic solution): the RBCs lyse and the tube is red.

Putting blood in an isotonic solution: the tube is normal (plasma is up and the RBCs are settling at the bottom of the tube); the solution is clear.

Fragility is concerned about surface area to volume ratio.

The end, best of luck.

Special thanks to the sweetest ♥♥ Aseel Abukishek ♥♥



# لك في هذا العالم حلم فانهض اليه

كان صوت المنبه، ذلك الأحمق الذي أوقف تدفق أحلامي وألغى استمرارية راحتي المؤقتة، فعلقت هناك في تلك الحالة اللاإنسانية، تلك التي تخلو من النوم والراحة وتخلو من الاستيقاظ والعمل، تلك المفرغة من المنطق ومن القواعد البشرية، تلك التي لا تشبه شيئاً إلا الرغبة بضم الوسادة والعودة إلى نوم أبدي. في تلك اللحظة استيقظت بضعة بقايا في داخلي لم أدرك ماهيتها ولكنها كانت كفيلة بإبعادي عن السكون للحظة، أدركت أن منبهي الأحمق لم يوقف أحلامي ولكنه أعلن بداية جديدة للسعي وراء الأحلام الحقيقية، تلك الأحلام المطلة على شرفة التحقق، تلك التي كانت حلمًا و أضحت هدفًا، تلك التي تنبض بحق وتعيدني إلى الصواب، أدركت أن المنبه لم يكن أحمقًا ولكن خمولي هو الأحمق، لأنني أستطيع ببساطة أن أعود إلى سبات لا أعلم نهايته ولكن الطريق السلس لم يكن يومًا خيارًا، لم أعتد على الطرق المرصوفة يومًا، لأنها تلك العقبات من كانت تعلمني بحق المحاولة تلو المحاولة لتتبع بالتحقق، لطالما كانت أهدافي تورقني فلم أسمح للصمت أن يسود الآن! النهوض في تلك اللحظة لم يكن خيارًا، كان لزامًا عليّ أن أنهض، كان لزامًا عليّ أن أبدأ يومي الآن، كان لزامًا عليّ أن اختار الطريق المحفوفة بالمكاره لأن الختام هو ما يجول في خاطري منذ زمن، والوقت قد اقترب حقًا من الخواتيم. بقلم: روان أبو داود