**Hemoglobin Determination**

Hemoglobin (HB) consists of :

- heme → pigment that contain iron (ferrus).

- globin →protein consists of subunits.

•Its main function is carrying O₂ and CO₂.

•It’s formed in the bone marrow (during RBC’s formation).

₪ The hemoglobin in circulating blood isn’t only pure hemoglobin, how is that??? Let’s see…

•The hemoglobin in circulating blood is a mixture of:

1. Hemoglobin 2-Carbohemoglobin (HB + CO₂)

 3-Oxihemoglobin (HB + O₂)

 4-Minor amount of this pigment (مش مطلوبة بالتفصيل )

•Each gram of hemoglobin carry 1.34 ml of O₂.

•The O₂ ability to combine with blood depends on the concentration of hemoglobin **NOT** on the number of RBC’s, why? Because some RBC’s have hemoglobin more than others.

₪ Hemoglobin also serve as pH buffer in the plasma of blood.

\*The importance of **Hemoglobin Determination:**

1• Used to screen(تكشف عن وجود ) for anemia.

2• Identify the severity ( مدى شدة ) of anemia.

3• Assist in evaluating ( تقدير) the patient’s response to therapy.

**The principle of hemoglobin determination:**

•The used reagent is [Drabkin’s Solution]

•Drabkin’s solution contains:

1) **Potassium ferricyanide (important for us)**

2) **Potassium syanide (important for us)**

3) Potassium dehydroen phosphate (not important for us)

4) Distilled water (not important for us)

\*\*The (potassium ferricyanide) oxidize the ferrous (Fe²⁺) in hemoglobin to the ferric (Fe³⁺) to form [**Met**hemoglobin].

•(Potassium cyanide) provide **cyanide** ions.

\*\*These cyanide ions immediately react with the methemoglobin to form [**Cyan**methemoglobin (**HiCN**)].

•This cyanmethemoglobin(also called HiCN) is radioactive (مشعة)

\*\*The amount of HiCN can be measured according to its radioactivity by an instrument called (**SPECTROPHOTOMETER)** at a wave length of 540nm.

•The spectrophotometer compare its radioactivity with known hemoglobin standards (*like the negative (-ve) control in the biolab*).

•It compare it in order to determine the hemoglobin concentration of the sample.

•You can do it without comparing by using a table (جدول) of values to convert the radioactivity of the sample into the concentration of the hemoglobin in it.

₪ All forms of circulating hemoglobin are converted into cyanmethemoglobin except the sulfhemoglobin (not mentioned).

-It doesn’t really matter because the sulfhemoglobin is rarely present in significant (يمكن ملاحظتها) amounts.

•you can take specimens for the test using blood capillary, but anticoagulated blood (EDTA) by vein punctureis recommended.

•The best results are obtained with blood taken on the same day.

**The normal HB values are**:

- For adult male : 14-18 g/dl

- For adult female : 12-16 g/dl

- For newborn :17-19 g/dl

**Clinical significance of the test:**

**A-** Physiological variation:

•Physiologically; hemoglobin values are affected by:

1) Age: HB values in newborns are more than the values in adults.

2) Sex: HB values in males are more than the values in females.

3) Pregnancy :

•During pregnancy, body gains fluids.

→ Then the RBC’s concentration will become less **<**The hemoglobin is contained in RBC’s **<** It’s concentration will become less, too.

4) Altitude (الارتفاع):

•At high altitude, there is less O₂ partial pressure.

→ RBC’s count will become more **<** thenThe hemoglobin is contained in these RBC’s **<** Its concentration will become more, too.

•At lower altitudes the partial pressure of O₂ is higher.

→ The RBC’s count becomes more.

→ HB values become more too.

**B-** Pathological variation:

❶ Decreased level of HB:

1) Hemoglobin is a component in all RBC’s, so the conditions that make the RBCs’ count low will lead to low values of HB, and these pathological conditions are:

ᵒ Blood loss. ᵒ Bone marrow suppression.

ᵒ Abnormal types of HB ᵒ Hemoglobinopathies(HBخلل ال)

2)Some patients have normal RBC’s count but low HB level.

•This situation happens when RBC’s have less HB than normal, like in: ᵒ Iron deficiency anemias.

❷Increased levels of hemoglobin:

•Any condition that have higher number (count) of circulating red blood cells than normal.

•Examples of increased levels of hemoglobin are:

ᵒ polycythemia vera

Clinical significance of the test

**A-** Physiological variation: **B-** Pathological variation:

1. Age 1) Decreased level of HB
2. Sex 2) Increased levels of hemoglobin
3. Pregnancy
4. Altitude

☺ The bad times make the good times better ☺

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