Methods of Hemoglobin Estimation

Hemoglobin: is the most important pigment of the blood imparting red color to it, the **main function of Hb** is oxygen carrying from lungs to the body tissues and the Co2 transport in the blood.

The **object** of hemoglobin (Hb) estimating to: **1-determine the oxygen carrying capacity of the blood 2-detecting diseases which cause a deficiency of excess of Hb**, **3-studying changes in Hb concentration before or after operations and blood transfusion.**

Generally, Hb estimation relies on a comparison of colors. Depend upon matching the color produced by the test sample with the color produced by a standard sample of know Hb concentration.

Various methods for estimation of hemoglobin in the laboratory:

1-Methods based on development of color, which include:

a-Sahli method

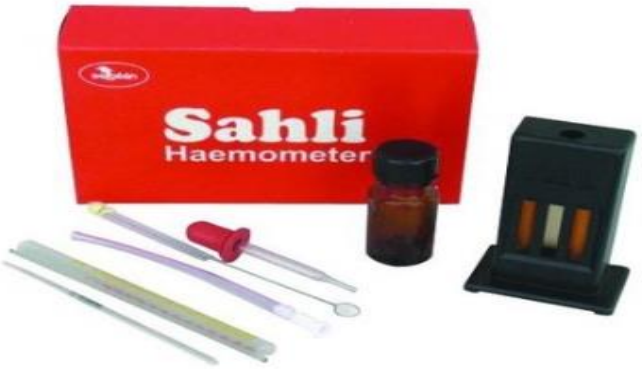
b-Drabkin (Cyanmethemoglobin) method

2- Automated Hematology Analyzer.

3- Measurement of oxygen combining capacity by blood gases analyzer (BGAs).

A-Sahli or acid hematin method

Blood is mixed with N/10 HCl resulting in the conversion of Hb to acid hematin which is brown in color. The solution is diluted till it's color matches with the brown colored glass of the comparator box. The concentration of Hb is read directly.



Equipment uses: **Hemocytometer** which consists of: **1-**Comparator box which has brown colored glass on either side **2-**Hb pipette which is marked upto 20mm3(0.02ml blood). **3-**Tube with markings of Hb on one side. 4-glass rod. 5-dropper **Reagents uses:** N/10 HCl, Distilled water.

Sample: Venous blood collected in EDTA

Procedure

1-Add N/10 HCl into the tube up to mark 2g%

2- Mix the EDTA sample by gentle inversion and fill the pipette with 0.02ml blood. Wipe the external surface of the pipette to remove any excess blood.

3-Add the blood into the tube containing HCl. Wash out the contents of the pipette by drawing in and blowing out the acid two to three times. Mix the blood with the acid thoroughly.

4-Allow to stand undisturbed for 10min.

5-Place the hemoglobinometer tube in the comparator and add distilled water to the solution drop by drop stirring with the glass rod till it's color matches with that of the comparator glass. While matching the color, the glass rod must be removed from the solution and held vertically in the tube.

6-Remove the stirrer and take the reading directly by noting the height of the diluted acid hematin and express in g%.

Advantage of Sahli: 1-Easy to perform **2-**Quick **3-**Inexpensive **4-**Can be used as a bedside procedure **5-**Does not require technical expertise

Disadvantage of Sahli: **1-**Less accurate **2-**All hemoglobins (oxyhemoglobin, sulphemoglobin) are not converted to acid hematin and hence the value of Hb obtained is less than the actual value. **3-**The color of acid hematin develops slowly. **4-**Individual variation in matching of color is seen. **5-**If the matching point is passed, the whole procedure has to be repeated. **6-**Color of glass in the comparator box tends to fade with time.

B- Drabkn's or Cyanmethemoglobin method

Procedure:

This is a type of colorimetric method, the principle of this method depend on mixing of blood with a solution containing potassium ferricyanide and potassium cyanide, the potassium ferricyanide oxidizes iron to form methemoglobin. The potassium cyanide then combines with methemoglobin to form cyanmethemoglobin, which is a stable color pigment read photometrically at a wave length of **540nm.**

Advantage of Drabkin method:

1-Visual error is not there as no color matching is required. **2-**Cyanmethemoglobin solution is stable and it's color does not fade with time so readings may not be taken immediately.

3-A reliable and stable reference standard is available from World Health Organization for direct comparison. **4-** All forms of Hb except sulphemoglobin are converted to cyanmethemoglobin.

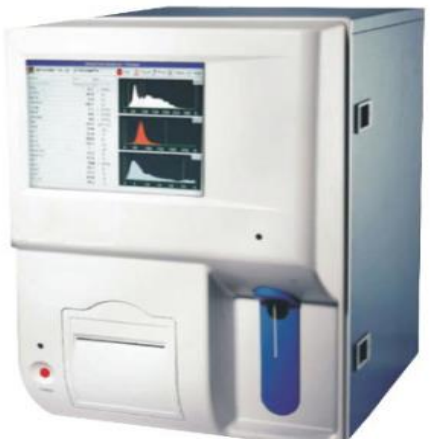
Disadvantage of Drabkin method:

1- Required prolonging time for reaction complete 2-Potassium cyanide is a poisonous substance.

2-Hematology Analyzer

An automated hematology or hemoglobin analyzer is commonly used for providing high quality to analyze a variety of red and white blood cells as well as hematocrit and **hemoglobin** levels from the blood sample.

Disadvantage: 1-highly cost 2-required stable climate conditions



different types of hematology analyzer

3-Measurement of oxygen combining capacity by blood gases analyzer (BGAs):

(Blood gases analyzer)

Typically used **arterial blood**, BGAs measure the combination of blood gas, pH, electrolytes, and metabolite parameters. Some laboratories may use BGAs for hemoglobin testing, but they are more commonly seen in **critical care units,** and **emergency rooms**. (Each red blood cell can hold approximately **270 million** hemoglobin molecules, each of which can bind 4 oxygen molecules).

Other methods of hemoglobin estimation:

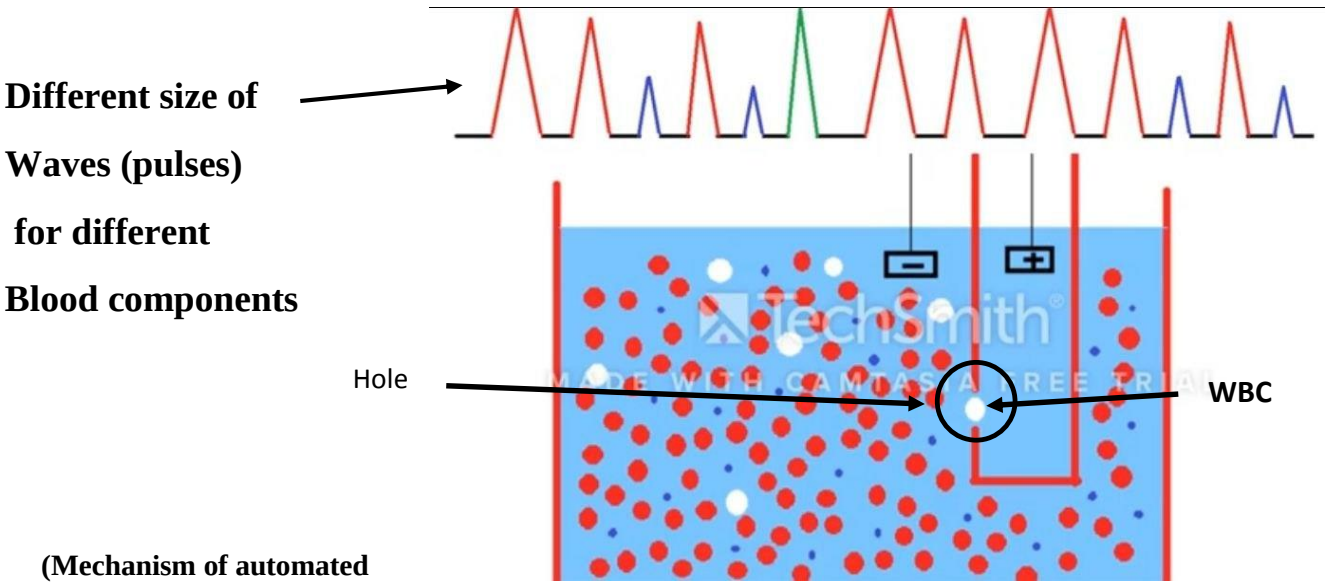
-Oxyhemoglobin method has semi principal of drabkin method.

-Alkaline hematin method has semi principal of sahli method but depend on alkaline medium

Note / each automated analyzer has specific principle for working that depend on manufactured company, (appendix with devise).

The description of simple form automated analyzer (**Coulter counter**), by automatic needle transport small size from blood sample to several different tubes in the devise, each of one tubes has diluent solution and 2 polar (- , +) in electric cycle, and all cells passes throw very small hole in tube, this hole in tube specific for cell size.

Each cell movement in hole generate impulse, and each cell has special size of impulse (pulses for all cell in blood sample), the devise calculated these pulses by special sensors after recognized then printed result on paper of devise or printer connected with it.



(Mechanism of automated hematology analyzer)

Normal values of Hemoglobin: male:13-18 g/dL female: 11.5-16.5 g/dL

References:

1- **Ranganathan, H., & Gunasekaran, N. (2006). Simple method for estimation of hemoglobin in human blood using color analysis. *IEEE transactions on Information Technology in Biomedicine*, *10*(4), 657-662.**
2- **Hoffman, R., Benz Jr, E. J., Silberstein, L. E., Heslop, H., Anastasi, J., & Weitz, J. (2013). *Hematology: basic principles and practice*. Elsevier Health Sciences.**
3- **DeNicola, D. B. (2011). Advances in hematology analyzers. *Topics in companion animal medicine*, *26*(2), 52-61.**