

Estimation of Hemoglobin

▶ hemoglobin is the pigment of red blood cells , it is important in the carriage of oxygen . ▶ the amount of hemoglobin is measured in g/100 ml. ▶ different species has different hemoglobin values. ▶ hemoglobin pigments are colored so that intensity of the color in solution is directly proportional to the amount of pigments. ▶ colorimetric estimation is useful for estimation of hemoglobin which is a mixture of :

- a. Reduced hemoglobin .
- b. Oxyhemoglobin .
- c. Methemoglobin (small amount).
- d. Sulphemoglobin .
- e. Carboxyhemoglobin .

-The best colorimetric estimation method of Hb. is Sahli method .

Animal	Horse	Cow	Sheep	Dog	chicken	human
g\dl	12.5	12	11	13.5	6.5-9	13.4 - 15.5

Sahli method It depends upon converting hemoglobin into acid hematin in presence of Hcl. The intensity of color is measured by comparing it with standard color with the use of the eyes . Hemometer is consist of :

1. Comparator block containing color standard .
2. Capillary pipette (especial pipette) mark to 20 . Or 0.2 ml .
3. Graduated tube (has scale represent the percentage of hemoglobin in the blood.

In some graduated tube has 2 scales one as percentage and other as gram / 100 ml .

Procedure

1. Fill the graduate tube to the mark 10 with 0.1N Hcl .
2. Suck the blood by using Hb pipette up to the mark 20.

3. Insert the tip of pipette in the graduated tube and gently blow out the blood .
4. Mix the blood and Hcl by stirring with a glass rod and let the tube stand in comparator block for 10 minutes until forming of acid hematin (brown color) .
5. Drop distilled water drop by drop to the acid hematin solution with stirring by glass rod after each drop until the color matches the standard color .
6. Read the scale on the graduate tube to obtain the % Hb or g/1 dl. Each 100 % contain 14.4 g/1dl. Hb.

For example if Hb percentage is 65% it mean

$$\begin{array}{r} 100 \quad 14.4 \\ 65 \quad \quad x \end{array}$$

$$-X = 14.4 * 65 / 100 = 9.36 \text{ g / dl}$$

