



HEMOGLOBIN ESTIMATION

By

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Learning Objectives

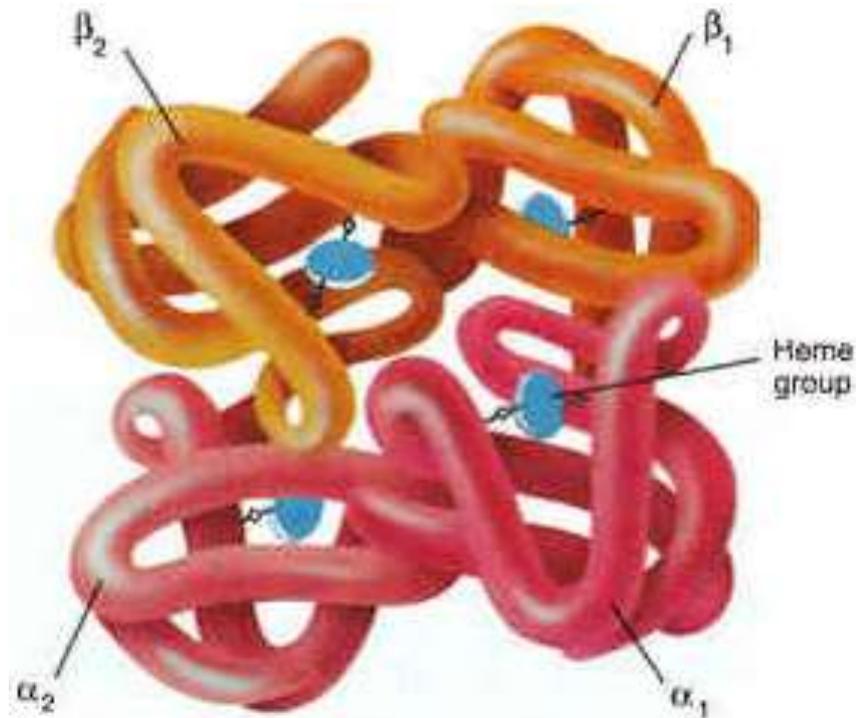
- By the end of this class, you should know about
- Basic Structure of Hemoglobin.
- Function of Hemoglobin.
- Various laboratory methods for estimation of Hemoglobin.
- Enumerate the advantages and disadvantages of each method.

Introduction

- Hemoglobin is the major constituent of the red cell cytoplasm, accounting for approximately 90% of the dry weight of the mature cell.
- It is comprised of **heme** and **globin**.

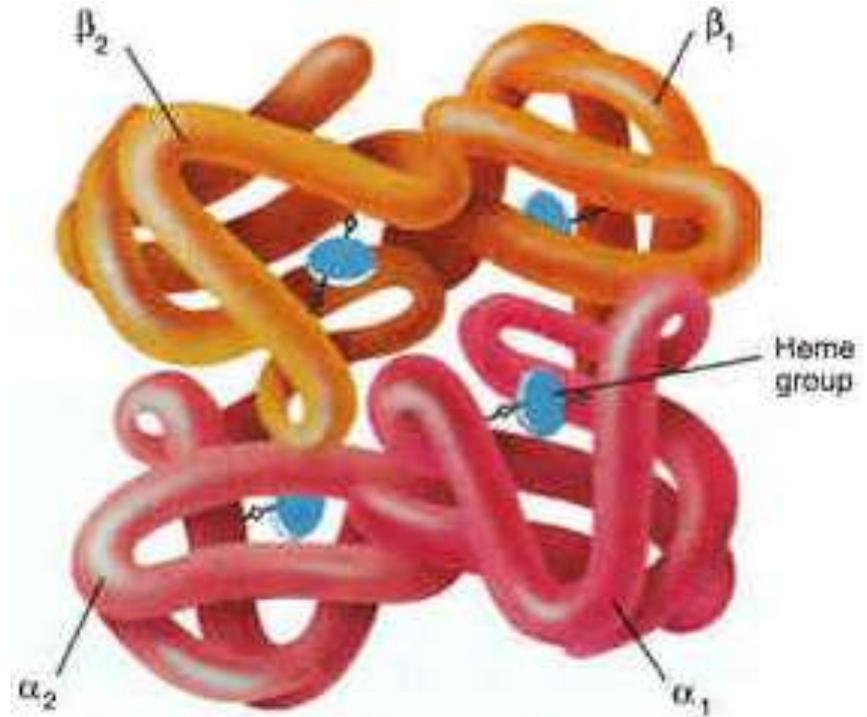
Structure of Hemoglobin

- Hemoglobin molecule is a tetramer consisting of two pairs of similar polypeptide chains called **globin chains**.
- To each of the four chains is attached heme which is a complex of iron in ferrous form and protoporphyrin.
- The major (96%) type of hemoglobin present in **adults** is called **HbA** and it has
 - ✓ 2 alpha globin chains and
 - ✓ 2 beta globin chains ($\alpha_2\beta_2$).



Structure of Hemoglobin

- The gene that codes for
- the formation of α globin chains is located on chromosome 16.
- The gene that codes for the formation of β globin chains is on chromosome 11.
- In adults, a minor amount of HbA2 ($\alpha_2\beta_2$) is also present and constitutes less than 3.5%.



Haemoglobin variants

Name	Structure	Present in
Adult Hb (HbA)	$\alpha_2\beta_2$	Adults
Fetal Hb (HbF)	$\alpha_2\gamma_2$	Fetal life
Hb Portland	$\zeta_2\gamma_2$	Embryonic life
Gower-1	$\zeta_2\varepsilon_2$..
Gower-2	$\alpha_2\varepsilon_2$..

During embryonic and fetal life, other different types of hemoglobins predominate.

- **Gower I, Gower II and Hb Portland** present in early embryonic life.



- After the 8th week of development, embryonic hemoglobins are replaced by **Fetal hemoglobin HbF ($\alpha_2\beta_2$)**
 - **This remains the predominant hemoglobin** until after birth and constitutes 50-90% of the total hemoglobin.
 - After birth, it's concentration decreases to less than 2% by 30 weeks of age.

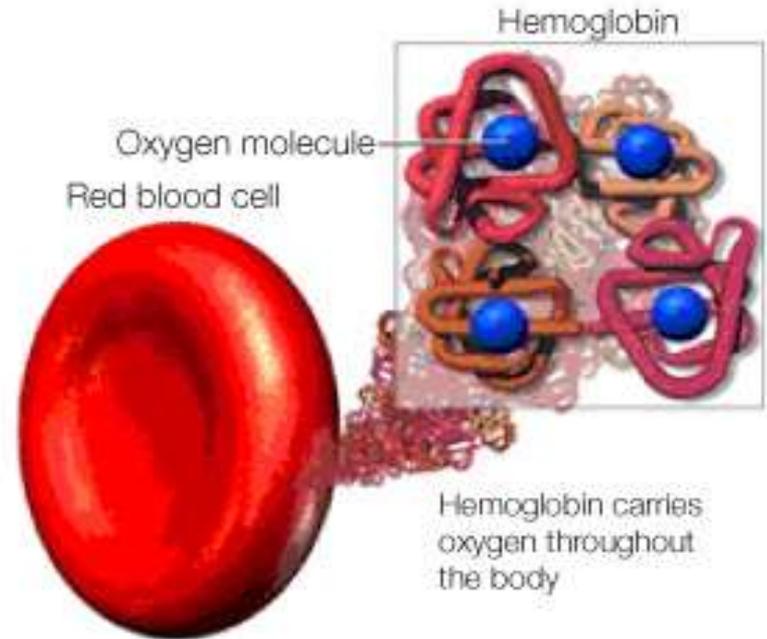


- HbA is then the predominant hemoglobin.
- HbA2
- **Abnormal - HbS, HbC, HbD, HbE**

Function of Hemoglobin

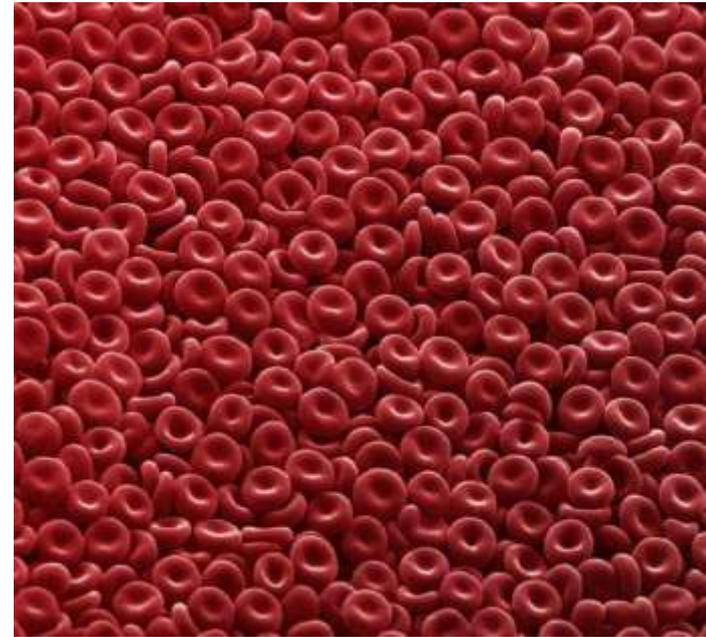
- **Heme has the ability to bind oxygen reversibly and carry it to tissues.**
- It also facilitates the exchange of carbon dioxide between the lungs and tissues.

Thus, hemoglobin functions as the primary medium of exchange of oxygen and carbon dioxide.



Normal Haemoglobin concentration in humans

- Men - 150 ± 20 g/l
- Women(non pregnant)- 135 ± 15 g/l
- Pregnant women –
 - ✓ 1st trimester 124–135 g/l
 - ✓ 2nd trimester 110–117 g/l
 - ✓ 3rd trimester 106–109 g/l
- Birth- 180 ± 40 g/l



Physiological variations in HB concentrations

- Elderly-renal insufficiency, inflammation, testosterone deficiency, diminished erythropoiesis, myelodysplasia **reduce** Hb concentration
- Exercise - **increases** Hb concentration
- Posture - lying to sitting **increases** Hb concentration
- Altitude - **increases** Hb concentration
- Smoking - **increases** Hb concentration

Blood can be collected from 3 different sources:

- ❖ Capillary blood.
- ❖ Venous blood.
- ❖ Arterial blood.

Tube cap color	Additive	Function of Additive	Common laboratory tests
Light-blue 	3.2% Sodium citrate	Prevents blood from clotting by binding calcium	Coagulation
Red or gold (mottled or "tiger" top used with some tubes is not shown) 	Serum tube with or without clot activator or gel	Clot activator promotes blood clotting with glass or silica particles. Gel separates serum from cells.	Chemistry, serology, immunology
Green 	Sodium or lithium heparin with or without gel	Prevents clotting by inhibiting thrombin and thromboplastin	Stat and routine chemistry
Lavender or pink 	Potassium EDTA	Prevents clotting by binding calcium	Hematology and blood bank
Gray 	Sodium fluoride, and sodium or potassium oxalate	Fluoride inhibits glycolysis, and oxalate prevents clotting by precipitating calcium.	Glucose (especially when testing will be delayed), blood alcohol, lactic acid

Indications for Hb estimation

- Determine presence and severity of anemia
- Screening for polycythemia
- Response to specific therapy in anemia
- Estimation of red cell indices
- Selection of blood donors

Colorimetric methods

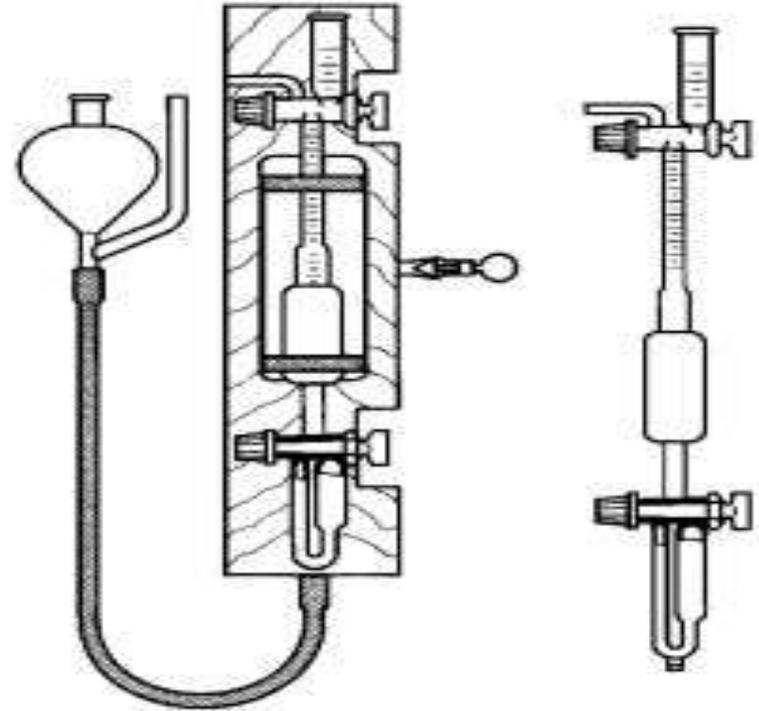
- Colour comparison between standard and test sample by
 - ❖ Visual methods
 - Sahlis acid hematin,
 - Tallqvist hemoglobin chart,
 - WHO hemoglobin Color scale,
 - Oxyhemoglobin Method
 - Specific gravity method
 - ❖ Photoelectric methods
 - ❖ Cyanhemoglobin method
 - ❖ Oxyhemoglobin Method
 - ❖ Alkaline Hematin Method

Gasometric Method

- Oxygen carrying capacity measured by Van Slyke apparatus
- Based on formula, 1 gm of Hb carries **1.34 ml of oxygen**

It does not measure
carboxyhemoglobin

- sulfhemoglobin
- methemoglobin.
- Time-consuming and expensive.
- Result is 2 percent less than other methods.



Chemical method

- Iron content of hemoglobin is first estimated.
- Indirectly Hb is derived - 100 grams of hemoglobin contain 374 grams of iron.
- Time-consuming method.
- This method is used to calibrate all other methods of Hb estimation.

Specific Gravity method

- Rough estimate is made from specific gravity of blood
- Copper sulfate technique.
- Used in mass screening like selection of donors.

Specific Gravity Method

- Rapid and simple
- Commonly used in blood donor selection
- A drop of blood is allowed to fall in copper sulphate solution of specific gravity of 1.053 from a height of 1 cm
- Specific gravity is equivalent to 12.5 grams/dl
- Drop gets covered with copper proteinate
- If drop sinks, specific gravity is higher than copper sulfate

Sahli's Acid Hematin Method

Principle -

- Blood is mixed with an acid solution so that Hb is converted to **brown colored acid hematin**
- Diluted with water till brown colour matches that of brown glass standard
- Hb value is read directly from the scale

Sahli's Acid Hematin Method

- Equipments-
- Sahli hemoglobinometer
- Sahli pipette(marked at 20 microlite or 0.02 ml)
- Stirrer
- Dropping pipette
- Reagents
- N/10 hydrochloric acid
- Distilled water

Sahli's Hemoglobinometer



Sahli's Acid Hematin Method

- Place N/10 HCl into Hb tube upto 2 grams.
- Blood sample in Sahli's Hb pipette upto 20 micro litre.
- Add blood sample to acid solution.
- Mix with a stirrer.
- Allow to stand for 10 minutes.
- Add distilled water drop by drop till the colour of the solution matches to brown glass standard.
- Take the reading of the lower meniscus from the graduated tube in grams.



Sahli's Acid Hematin Method

Advantages

- Easy to perform
- Quick
- Inexpensive
- Can be used as a bedside procedure
- Does not require technical expertise

Sahli's Acid Hematin Method

Disadvantages

- For maximum colour, longer time is required
- Perfect matching with brown glass standard is not possible
- Carboxyhemoglobin, methemoglobin and sulfhemoglobin are not converted to acid hematin
- Developed of colour is slow and acid hematin is not stable
- Source of light will influence the comparison of colours

Cyanmethemoglobin Method

- Most accurate method for estimation of Hb.
- Recommended by International Committee for Standardisation in hematology because : -
 - ✓ All forms of Hb are converted to cyanmethemoglobin (except sulfhemoglobin)
 - ✓ Stable and reliable standard is available.

Cyanmethemoglobin Method

Principle

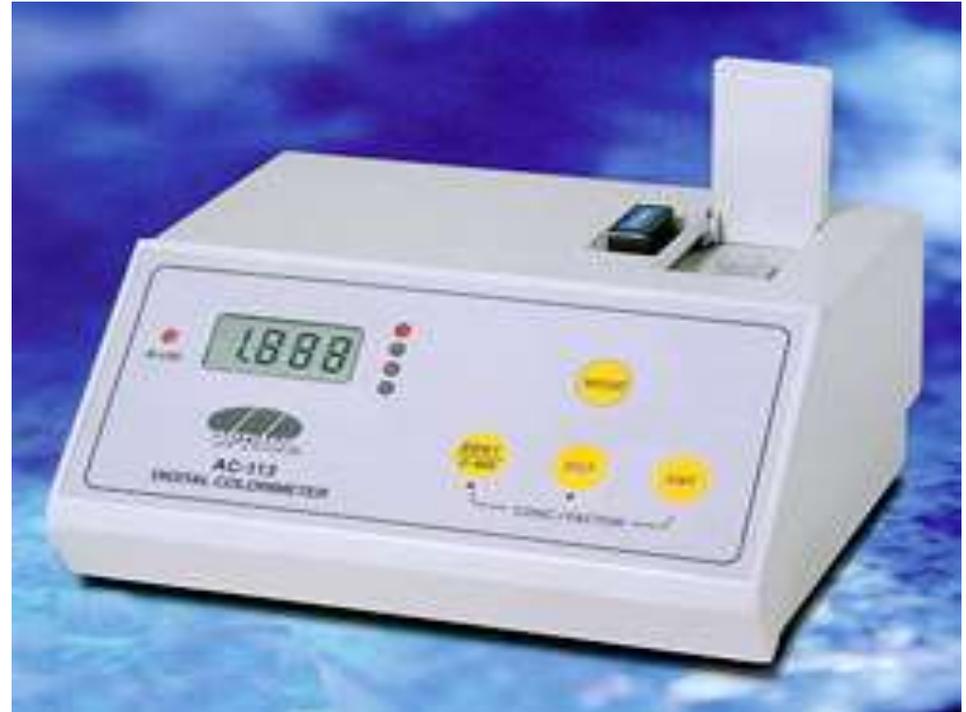
- Blood is mixed with Drabkins solution.

Drabkins solution –pH 7.0 -7.4

- ❖ Potassium ferricyanide
 - ❖ Potassium cyanide
 - ❖ Potassium dihydrogen phosphate
 - ❖ Non-ionic detergent
 - ❖ Distilled water
-
- Erythrocytes are lysed producing an evenly distributed Hb solution.
 - Potassium ferricyanide converts Hb to methemoglobin.
 - Methemoglobin combines with potassium cyanide to form cyanmethemoglobin.
 - All Hbs present in blood are converted to this form.
 - Absorbance is measured in spectrophotometer at 540 nm
 - To obtain amount of unknown Hb sample, its absorbance is compared with the standard cyanmethemoglobin solution

Cyanmethemoglobin Method - Equipment

- Photoelectric colorimeter or spectrophotometer
- Sahlis pipette at 20 micro litre
- Pipette 5 ml



Cyanmethemoglobin Method

- Take 5 ml of Drabkins solution and to it add 20 microlitres of blood
- Stopper the tube, mix by inverting several times
- Allow to stand for 5 minutes
- Transfer the sample to cuvette
- Read the absorbance in the spectrophotometer at 540 nm
- Also take the absorbance of the standard solution

Cyanmethemoglobin Method

- Hemoglobin is derived from the formula below

$$\text{Hemoglobin in gm/dl} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times$$

$$\text{Concentration of standard} \times \frac{\text{Dilution factor}}{100}$$

Cyanmethemoglobin Method

- A graph can be plotted when a large number of samples are processed
- Hb concentration on horizontal axis and absorbance on vertical axis

Note

- Hypertriglyceremia, leucocytosis, plasma cell dyscrasias cause erroneous results
- Cyanmethemoglobin solution is stable
- Any delay will not affect the result

Cyanmethemoglobin Method

Advantages

- All forms of Hb except sulphhemoglobin are converted to hemiglobincyanide/cyanmethemoglobin (HiCN).
- Visual error is not there as no color matching is required.
- Cyanmethemoglobin solution is stable and its color does not fade with time so readings may not be taken immediately.
- Absorbance may be measured soon after dilution.
- A reliable and stable reference standard is available from World Health Organisation for direct comparison

Cyanmethemoglobin Method

Disadvantages

- Diluted blood has to stand for a period of time to ensure complete
- conversion of Hb.
- Potassium cyanide is a poisonous substance and that is why **Drabkin's**
- **solution must never be pipetted by mouth.**
- The rate of conversion of blood containing carboxyhemoglobin is slowed
- considerably. Prolonging the reaction time to 30min can overcome this
- problem.
- Abnormal plasma proteins cause turbidity when blood is diluted with
- Drabkin's solution.
- A high leucocyte count also causes turbidity on dilution of blood.
Centrifuging the diluted blood can help overcome the turbidity.

Automated Blood Count Method

- Modification of cyanehaemoglobin method
- Other chemicals-sodium lauryl sulphate,imidazole,sodium dodecyl sulphate
- Measurements are made at various wavelengths depending on final stable product

3 part Differential Analyzers

- MCV, MCHC, RDW, hematocrit and platelet parameters
- Two chambers-
 - Hb/WBC chamber
 - RBC/platelets chamber



5 part Differential Analyzers

- Classify cells as neutrophils, eosinophils, basophils, lymphocytes and monocytes
- These provide accurate platelet count, red cell parameters including various reticulocyte parameters, immature platelets



Tallqvist Hemoglobin Chart

- Series of lithographed colors said to correspond to Hb values ranging from 10 to 100 percent
- Blood obtained from finger puncture
- Placed on a piece of absorbent paper
- Colour is matched against the colour on the chart
- Corresponding reading taken
- Cheap and simple
- Error-20 to 50 percent

Direct matching methods

1-Tallquist method



WHO Hemoglobin Colour Scale

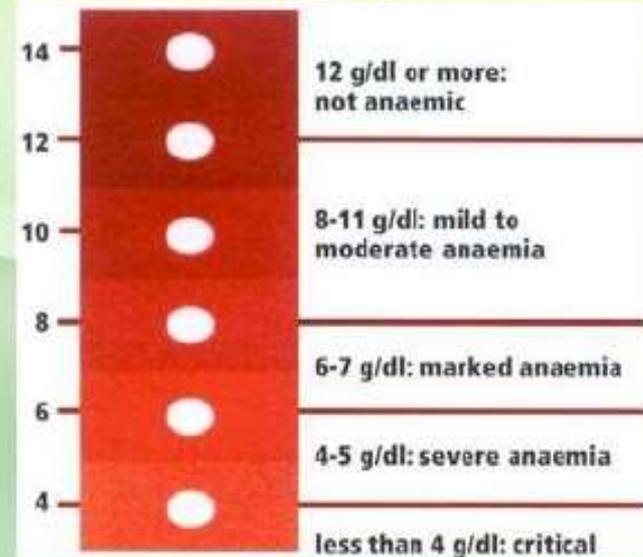
- Devised by Scott and Lewis
- Principle is similar to Tallqvist method
- Rapid, simple, inexpensive, reliable
- 1 gram/dl for diagnosis of anemia
- Printed set of colors corresponding to Hb values from 4-14 grams/dl
- Efficiency-greater than 90 percent in detecting anemia
- 86 percent-in classifying its grade

WHO Hemoglobin Colour Scale

- Useful for screening blood donors
- Screening women and children in health programmes
- Iron-therapy

WHO Haemoglobin Colour Scale:

This technique of estimating haemoglobin is based on comparing the colour of a drop of blood absorbed on a particular type of chromatography paper, against a printed scale of colour corresponding to different levels of haemoglobin ranging from 4-14 gram/dl.



Oxyhemoglobin Method

- Blood mixed with weak ammonia solution
- Absorbance compared with the standard
- Rapid and simple
- No stable solution is available

Summary

- Adult males- 150 ± 20 g/l.
- Adult females(non pregnant)- 135 ± 15 g/l.
- Various methods of Hb are-
 - Sahli's acid hematin.
 - Cyanhemoglobin Method.
 - Gasometric Methods.
 - Tallqvist Hemoglobin Chart.
 - WHO Hemoglobin Color Scale.
 - Oxyhemoglobin Method.
 - Oxyhemoglobin Method.
- Most commonly practiced is Sahlis acid hematin method.
- Principle - Hb converted to hematin on mixture with acid solution.
- Most accurate method for estimation of Hb is Cyanhemoglobin Method.

Thank

you

