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Cyanmethemoglobin Method

Hemoglobin

Hemoglobin, the main component of the red blood cell, functions in the transportation of oxygen and CO₂. Hemoglobin consists of 1 molecule of globin and 4 molecules of heme (each containing 1 molecule of iron in the ferrous state). Globin consists of 2 pairs of polypeptide chains. In the hemoglobin molecule, each polypeptide chain is associated with 1 heme group; each heme group can combine with 1 molecule of oxygen or CO₂.

Hemoglobin carries oxygen from places of high oxygen pressure (lungs) to places of low oxygen pressure (tissues), where it readily releases the oxygen. Hemoglobin also returns CO₂ from the tissues to the lungs.

Methods

Methods for hemoglobinometry can be grouped into 4 main classes



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depending on the basic technique employed with variants within each class:

1. Colorimetric Methods
2. Gasometric Methods
3. Specific Gravity Methods
4. Chemical Methods

The method of choice for hemoglobin determination is the cyanmethemoglobin method (This is a type of colorimetric method).

The principle of this method is that when blood is mixed 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.

Three advantages of the cyanmethemoglobin method are:



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1. measures all forms of hemoglobin except sulfhemoglobin
2. can be easily standardized
3. cyanmethemoglobin reagent (also called Drabkin's solution) is very stable

Normals: women 12 - 16 g/100 ml blood (g/dl) (g%)
 men 14 - 18 "
 newborn 14 - 20 "



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**Cyanmethemoglobin Method for Determining Hemoglobin
Concentration**

. Procedure:

1. Label a series of tubes as follows:

BLANK

Lo STD

Norm STD

Hi STD

Norm CONTROL

Hi CONTROL

Patient (PT)

2. Pipette 5 ml of Cyanmethemoglobin reagent into each tube.

Add 20 μ l of the appropriate sample into each tube. Do not add



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anything other than the Cyanmethemoglobin reagent to the reagent BLANK.

3. Allow tubes to stand for 10 minutes.
4. Read Absorbance (A) in the spectrophotometer at 540 nm, zeroing the spectrophotometer with the BLANK solution.
5. Plot Absorbance vs. Hemoglobin Concentration in grams % on linear graph paper.



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