CYANMETHEMOGLOBIN (HEMOGLOBIN-CYANIDE) METHOD FOR ESTIMATION OF HEMOGLOBIN

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This method is optional for estimation of hemoglobin and this method is recommended by International Committee for Standardization in hemotology. This is because in this method all type of hemoglobin are transformed to cyanmethemoglobin (except sulfhemoglobin), and a firm and trustworthy standard is available.

Principle

When Blood is mixed with a solution of potassium cyanide, potassium ferricyanide and Drabkin’s solution, the erythrocytes are lysed by producing evenly disturbed hemoglobin solution. Potassium ferricyanide transforms hemoglobin to methemoglobin, and methemoglobin combines with potassium cyanide to produce hemoglobin cyanide (cyanmethemoglobin). This way all types of hemoglobin present in blood are entirely transformed to a single compound cyanmethemoglobin. When the reaction is entire, absorbance of the solution is deliberate in a spectrophotometer at 540 nanometer. Hemoglobin cyanide has a wide absorbance peak at this wavelength. The absorbance is compared with that of the standard hemoglobin cyanide solution by using a formula to obtain the amount of hemoglobin.

Equipment

1. Spectrophotometer or photoelectric colorimeter
2. Pipette 5 ml
3. Sahli’s pipette

Reagents

1. Drabkin’s Solution
2. Cyanmethemoglobin standard solution with known hemoglobin value
Specimen

Blood obtained from skin puncture or EDTA-anticoagulated venous blood.

Procedure

1. Take 5 ml of Drabkin’s solution in a test tube and add 20 μl of blood. This way, we will get the dilution of 1:25. Now mix the mixture and allow to stand for atleast 5 minutes. This time is adequate for transformation of hemoglobin to hemiglobincyanide.

2. Pour the test sample to a cuvette and read the absorbance of the test sample in a spectrophotometer at 540 nanometer or in a photoelectric colorimeter using a yellow-green filter. Also read the absorbance of the standard solution. Absorbance must be read against Drabkin’s solution.

3. From the formula given below, the hemoglobin value is derived.

\[
\text{Hemoglobin in gm/dl} = \left[ \frac{\text{Absorbance of test sample} + \text{Absorbance of standard}}{\text{concentration of standard}} \right] \times \text{Dilution factor} / 100
\]

Preparation of table and graph: Result can be obtained quickly, if the table of graph is prepared which correspond absorbance with hemoglobin concentration. This is markedly acceptable when huge number of samples are daily processed on the same instrument.

For the preparation of a calibration graph, adulterate cyanmethemoglobin standards are commercially available. As another option, standard cyanmethemoglobin solution is diluted serially with Drabkin’s solution. Concentration of hemoglobin (horizontal axis) in each dilution is arranged against the absorbance (vertical axis) on a linear graph paper. A straight line connecting the points and passing through the origin is obtained. A table can be prepared relating absorbance to concentration of hemoglobin from the help of this graph.

Notes:

1. The hemiglobincyanide solution is stable so that delay in getting the reading of absorbance does not influence the result.

2. High TLC (total leukocyte count) (> 25,000/μl), abnormal plasma proteins (e.g. in Waldenström’s macroglobulinemia, multiple myeloma) or lipemic blood (hypertriglyceridermia), can cause the error in results.