

Research Article

Evaluation of hemoglobin estimation with non-cyanide alkaline haematin D- 575 method

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ABSTRACT

Background: Anemia is serious cause for concern in the world as it impacts on psychological and physical development, behavior and work performance. There are various methods recommended for estimation of hemoglobin for detection of anemia. Each method has its advantages and disadvantages. Aims and objectives of the present study were to compare conventional Hemoglobinocynide (HiCN) method containing potassium ferricyanide and potassium or sodium cyanide with non- cyanide alkaline haematin Method for hemoglobin estimation.

Methods: The prospective study was conducted to evaluate the performance of two methods - HiCN method with Drabkin's reagent and non- cyanide alkaline haematin method with AHD 575 reagent, for hemoglobin determination utilizing 201 blood samples. The data statistically analyzed by using coefficient of variation (CV), linear regression and mean differences.

Results: A good correlation was observed for hemoglobin estimation between the HiCN method and non-cyanide alkaline haematin Method with AHD 575 reagent. The correlation coefficient of $r = 0.9998$ was statistically significant.

Conclusions: It was concluded that both methods are accurate and precise, however the toxic and biohazardous effects of potassium ferricyanide and sodium cyanide in HiCN method can be prevented by using alkaline haematin method with AHD 575 reagent.

Keywords: Hemoglobin, Hemoglobinocynide, Alkaline haematin

INTRODUCTION

Hemoglobin (Hb) estimation is the primary method for anemia screening. Hb being most commonly advised test in laboratory, needs a most suitable and economical method of estimation. The various methods have been recommended for estimation of Hb for assessment of anemia like acid haematin method, Hemoglobinocynide (HiCN) method and automated cell counter method. The most widely used HiCN method contains potassium ferricyanide and potassium or sodium cyanide which are hazardous to environment and occasionally to laboratory technicians also.^{1,2} The present study was conducted to analyze the precision, accuracy, suitability, cost effectiveness and feasibility of HiCN method and non-

cyanide alkali haematin method with AHD 575 reagent for the estimation of hemoglobin.

METHODS

A tertiary care hospital based study was conducted. Fresh blood samples from 201 adult cases were collected with the vacutainer system, containing EDTA-K₃ (Becton-Dickinson) to a total volume of 4.5 ml was included in the study. Samples with hemolysis were excluded from the study. Sample was thoroughly mixed and hemoglobin estimation was done by HiCN method with Drabkin's reagent and non- cyanide alkaline haematin method with AHD 575 reagent. Commercial controls were run with each batch.

HiCN method

0.02 ml of well mixed blood sample was taken in 5 ml of Drabkin's reagent (Biolab Diagnostics) and incubated at room temperature for 5 minutes. Absorbance was taken at 546 nm (530-550nm) wavelength.²

Alkaline haematin method (AHD method)

0.02 ml of well mixed blood sample was taken in 3 ml of Hemoglobin AHD (AHD 575) reagent (Biorapid Diagnostics) and incubated at room temperature for 5 minutes. Absorbance was taken at 578 nm wavelength.²

Hemoglobin AHD (AHD 575) reagent (Biorapid Diagnostics) is commercially available, ready to use, cyanide free reagent with high storage stability of upto 45 degrees Celsius and for more than three years.

The 201 samples were performed by both the methods within an interval of 10-20 minutes to avoid variation during processing and measurement. For the reproducibility analysis of the Hb measurements, we used a single sample that was evaluated 15 times by each method.

Statistical analysis was done for test performances and their comparisons by using coefficient of variation (CV), linear regression and mean differences.

RESULTS

The reproducibility of both the methods was evaluated by measuring the Hb level 15 times from a single blood sample and determining the coefficient of variation (CV) for each assay. The CV for the Drabkin's method and AHD method was 0.43% and 0.49% respectively (Table 1).

Table 1: Determination of the coefficient of variation for the two methods of hemoglobin measurement.

Method	Range	Mean	SD	CV
Drabkin's Method	10.4-10.6	10.506	0.045	0.43%
AHD Method	10.4-10.6	10.486	0.051	0.49%

SD= Standard Deviation, CV= Coefficient of Variation

The assessment for the measurements of central tendency (mean and median) and variation (range and standard deviation) for the 201 Hb determinations from each method is as shown in Table 2. There was no any statistical difference for these parameters. The Drabkin's method showed the mean (13.01 g/dl) and median (13.0 g/dl) when compared to the AHD method (mean 13.0 g/dl and median 13.1 g/dl, respectively).

Table 2: Measurement of central tendency and variation for methods of hemoglobin determination.

Method	n	Range	Mean	Median	SD
Drabkin's Method	201	7.4-18.5	13.01	13	3.12
AHD Method	201	7.5-18.6	13.00	13.1	3.11

SD= Standard Deviation

The coefficient of correlation of the linear regression for analysis of Hb determination for Drabkin's method and AHD method was $r=0.9998$ which indicates that these two parameters have excellent correlation coefficient (Table 3).

Table 3: Linear regression analysis for methods of hemoglobin determination.

Reference method	Testing method	Correlation (r)	Slope	Y-intercept
Drabkin's Method	AHD Method	0.9998	1.002	-0.028

DISCUSSION

Hb estimation is an essential test carried out in the laboratories for the diagnosis and management of anaemia. The standard method for measuring hemoglobin (Hb) in human blood is the well-recognized HiCN method as recommended by the World Health Organization (WHO). There are a number of methods like Cell Counter Method, Sahli's Method, Gasometric method are available that give very approximate results as compared to HiCN method.^{2,3}

HiCN method employs Drabkin's reagent. When blood is mixed with Drabkin's reagent, the hemoglobin present in whole blood first converted to the methemoglobin (unstable) by the action of potassium ferricyanide and this methemoglobin is further converted to stable cyanmethemoglobin by the action of potassium or sodium cyanide. The resultant color complex formed is directly proportional to the hemoglobin concentration, which is directly measured spectrophotometrically at 540nm. However, the Drabkin's reagents contain potassium ferricyanide and potassium or sodium cyanide which is very toxic, biohazardous and photosensitive. Also, the disposal of large volumes of this reagent which contains cyanide constitutes a potential biohazard.¹⁻³

In view of disadvantages of HiCN method, the various alkaline haematin methods were analyzed for Hb estimation. One of the alkaline haematin methods was lauryl sulfate haemoglobin colorimetric method however the pigment formed was stable for only 4 hours at room temperature, and hence the method is not ideal for manual use in daily practice of laboratories. Also, no standards were available for this method.⁴

One of the other alkaline haematin methods is AHD-575 method. The AHD-575 reagent is non-toxic and is not photosensitive. In the alkaline haematin D-575 (AHD-575) method of haemoglobin estimation, small volume of blood is mixed with an alkaline solution containing a non-ionic detergent. All haemoglobin derivatives are converted into a stable end-product, alkaline haematin D-575, whose absorption maximum is at $\lambda = 575$ nm and measurement can be taken on colorimeter.^{2,3,5,6}

The concentration of Triton X-100 can be varied over the range 25-50 g/l and that of sodium hydroxide over the range 0.01-0.1 mmol/l without altering the test results. Thus the AHD reagent can be prepared in laboratories. Chlorohaemin is a stable compound that can be obtained in pure form and primary standards for the AHD-575 method can be prepared from it. The standards are stable for 8 months at 4-8 degree Celsius. Also, the total cost of the AHD-575 method significantly lower the reagents used in HiCN method.⁵ Now days, AHD-575 'ready to use' reagents are commercially available and have storage stability of upto 45 degree Celsius and for more than three years.

The present study showed that AHD method using Hemoglobin AHD (AHD 575) reagent showed comparable results with the HiCN method for Hb estimation which was statistically proved with various tests like coefficient of variation (CV), standard deviation (SD) and correlation coefficient. Similar findings were also observed by various authors in the past. Zander R et al., Moharram NMM et al. and Parikh S et al. observed that Alkaline haematin D-575 reagent is a precise new tool for Hb estimation as an alternative to HiCN method.^{2,3,5}

Thus, the AHD method using Hemoglobin AHD (AHD 575) reagent method therefore offers the possibility of safe and quality hemoglobin estimation at a reduced cost.

CONCLUSION

The AHD method using AHD-575 reagent is cheap, non toxic, non biohazardous, stable and gives rapid, accurate

and precise comparable Hb estimation results with internationally accepted reference HiCN method. Also, this method overcomes constraints of HiCN method and hence clearly warrants its introduction into healthcare facilities and laboratories for Hb estimation.

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Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee

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