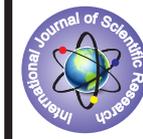


## HAEMOGLOBIN ESTIMATION BY NON CYANIDE--- MODIFIED ALKALINE HAEMATIN METHOD



### Pathology

**KEYWORDS:** haemoglobin, non cyanide, SLS method

**Dr R K Shrivastava**

MBBS, MD (path) Professor. Dept. of pathology RIMS ranchi.

**Dr Satyabrata Patra**

MBBS, MD(path) Tutor, Dept. of pathology, RIMS, ranchi

**Dr. Camellia Chanda**

MBBS, PGDMCH, Tutor Dept. of anatomy RIMS, Ranchi.

**Dr R K Singh**

MBBS, MD (path)Asst. Professor. Dept. of pathology RIMS ranchi

### ABSTRACT

Haemoglobin is an oxygen binding chromo protein in the red blood corpuscles. International standard of haemoglobin estimation is cyanide method (HiCN). This method is known to be hazardous to the environment and harmful for the laboratory personnel as it contains KCN

**Aims and Objectives:** To compare and study the haemoglobin conc. which were estimated by cyanide and non-cyanide methods. To estimate the advantages of the cyanide free methods over the cyanide methods. To assess whether these methods were better as compared to the haemoglobin cyanide method, for laboratory investigations.

**Materials and Methods:** 1000 samples were tested for haemoglobin were done by the cyanmeth haemoglobin method and modified alkaline haematin method which uses cetrimide were compared using Pearson's correlation coefficient.

**Results:** By the modified alkali denaturation method, haemoglobin concentration, were similar, to those, which were obtained by the conventional cyanmethaemoglobin method

**Conclusion:** The modified alkali denaturation method method was as accurate as the cyanmethaemoglobin method. It was economical. The toxic effects of cyanide can be prevented by using this method commercially.

### INTRODUCTION

Haemoglobin is an iron containing, oxygen-carrying chromoprotein in the red blood cells of human being. The haemoglobin in the blood carries oxygen from the lungs to the rest of the body.

Haemoglobin is estimated by the cyanmethhaemoglobin method (HiCN) in labs. is a stable method and as it has an internationally accepted reference standard and calibrator, but the potassium cyanide which is used in the cyanmethhaemoglobin method is toxic, making the management of the safe disposal of this reagent difficult. Automated cell counters now use Sodium lauryl sulphate (SLS) instead of toxic reagents.

Non-cyanide methods, the modified alkaline haematin method (MAH) which uses sodium tetraborate and cetrimide have been chosen to evaluate haemoglobin with respect to the cost, standard results, stability and safety in comparison to the cyanmethhaemoglobin method. If the haemoglobin values which are obtained by these methods are comparable to those which are derived from the standard cyanmeth haemoglobin method, this cyanide free methods can be insisted to be used in teaching institutions, blood bank camps and in laboratories, to avoid biohazards to the their staff and the environment.

### MATERIALS AND METHODS

This study was comparative and a hospital based study. It was conducted for the duration of one month in the RIMS, Ranchi, with a sample size of 1000 randomly selected patients. Haemoglobin was estimated by the cyanmeth haemoglobin method and the modified alkali denaturation method.

#### Procedure:

The Cyanmethhaemoglobin method-The reagent was Drabkin, 0.02ml of whole blood was mixed with 5 ml of drabkin solution. After 10mts OD was recorded with drabkin solution with reagent blank at 540nm. Actual haemoglobin percentage in gm% was calculated by the formula  $(OD \text{ OF TEST} / OD \text{ OF STD}) * \text{conc. Of STD}$ .

#### The Modified Alkaline Hematin Method (MAH)

Borate working solution is a solution of Sodium tetra borate (borax) – 1.9gm/lit, Sodium hydroxide – 2gm/l, cetrimide 0.5% (CETAVLON) was used as lysing agent. It is a quaternary ammonium compound which is a crystalline powder with a disinfectant activity.

Principle: Cetrimide acts by lysing the erythrocytes and precipitating haemoglobin which conjugates with boronic acid to form a red precipitate which is read at 540nm.

Procedure: 0.02ml of the test sample was added to 3.0ml of the borate working solution. The diluted sample was allowed to stand for 5 minutes, it was then transferred to a cuvette and the optical density was determined at 540nm against a blank of borate working solution.

A standard curve was plotted for the different haemoglobin values which were obtained by using Drabkin's solution and its standard, against the optical density which was obtained from the borate working solution. Depending on the optical density values, the results were compared with the standard curve. The stability of the modified alkaline haematin reagent at room temperature was assessed by preparing solutions of alkaline haematin and cyanmethaemoglobin from the samples of whole blood, and comparing the absorbance at different time intervals over a 24 hours period. The time which was taken for complete haemolysis was calculated by noting the absorbance values every minute and by centrifuging the samples to look for the cell buttons. There was no cell button formation from the third minute onwards. The ability to denature foetal haemoglobin (HbF) was assessed by comparing the haemoglobin values of 20 samples of cord blood by using the modified alkaline haematin reagent and Drabkin's solution.

These samples were used for estimating the stability of the modified alkaline haematin reagent and the HbF and have not been included in the current data.

### RESULTS

The haemoglobin which was estimated, ranged from 3.8gm% to 17.6gm% and the patients who were included in this study were newborns to 78years old. The optical density was measured at 540nm for all the two methods. The reaction completion time for the haemoglobincyanide method and the sodium lauryl sulphate method was 10mts but for modified alkali denaturation test it was less than 3 minutes.

### DISCUSSION

Haemoglobin estimation is one of the most common tests which are done at the bedsides of patients, at primary health care centres, in antenatal clinics; during the follow up of management of anaemic patients and during the screening for anaemia among blood donors. A good number of methods is available for haemoglobin estimation, with the colorimetric method being the most popular one. The manual HiCN method was phased out as a routine method and it gradually became a reference method. Other factors like legislation which affects the transportation of the reagents and the control of substances such as cyanides which are hazardous to health, have led to the development of

methods which do not employ cyanide for the determination of haemoglobin. In our country, approximately 70% of the laboratories still use the manual

HiCN method for Hb estimation in the rural areas. The cyanmethemoglobin method has been accepted as a standard method for Hb estimation. This method uses a stable standard solution which helps in deriving uniform and consistent results. But this solution contains potassium cyanide which in high quantities, makes its safe disposal difficult.

Sodium lauryl sulfate converts haemoglobin into methaemoglobin in the order of oxyhaemoglobin, haemochrome and methaemoglobin by its oxidative activity. Therefore, unlike other methods, this method does not need oxidative reagents and it does not generate toxic wastes such as KCN and NaN<sub>3</sub> which cause environmental pollution.

The alkaline haematin method (AHD-575) has been evaluated extensively by some authors in other countries and east Africa but in all cases it is proprietary confidential hand held machine, we tried it with commercially available CETAVLON (Cetrimide 0.5%, it is easily available and economical, this can be used in peripheral centers in the alkaline boronate method.

Both the above non-cyanide methods were compared for repeatability, reagent stability, and accuracy in correlation with the cyanmethaemoglobin method and both the reagents gave excellent results. The quantity of the reagent which was used by the MODIFIED ALKALI DENATURATION METHOD was low (3ml) as compared to that which was used by the other two methods (5ml). Stability of SLS haemoglobin is shorter.

Both sodium lauryl sulfate and cetrimide have been used as detergents in shampoo and soap industries extensively. Sodium tetra borate is a well-known food additive and it has been thought to cause gastric symptoms while it was used in high quantities. No other definite complications have been reported.

In both the non cyanide methods, the absorbance was read at 540nm on a colorimeter.

The stability of the modified alkaline haematin reagent promises a standard preparation for this method exclusively. The present study was done on 1000 samples and the alkaline borate method was modified by using CETAVLON as a lysing agent, which is easily available at primary health centres. The correlation between HiCN and the MAH was excellent. Reagent which contains cyanide may constitute a potential bio-toxic hazard. As an alternative, the non-toxic sodium lauryl sulphate and the modified alkaline haematin methods have been used. The results are reproducible as for HiCN for measuring haemoglobin at all concentrations. Hence, these methods can be employed in primary health care centres. **CONCLUSION**-The cyanmethemoglobin (HiCN) method for measuring haemoglobin is used extensively worldwide; its advantage is the ready availability of a stable and internationally accepted reference standard/calibrator. However, its use may create a problem, as the disposal of large volumes of teaching hospitals and diagnostic laboratories.

#### REFERENCES-

- [1] Bull BS, ICSH Expert Panel on cytometry, ICSH recommendations for "surrogate reference" method for packed cell volume. *Br J Prev Soc Med.* 1966 Oct;20(4):172-75.
- [2] Shah VB, Shah BS, Puranik GV. Evaluation of noncyanide methods for hemoglobin estimation. *Indian Journal of Pathology and Microbiology* 2011;(54): 764-68.
- [3] Lewis SM, Garwey B, Manning R, Sharp SA, Wardle J. Lauryl sulphate haemoglobin: a non-hazardous substance for HiCN in haemoglobinometry. *Clin Lab Haematology* 1991; 13(3):279-90.
- [4] Zander R, Lang W, Wolf HU. Alkaline haematin D-575: a new tool for the determination of hemoglobin as an alternative to the cyanmethemoglobin method. *Clin Chim Acta.* 1984; 136(1):83-93.
- [5] Theodorsen L. An automated cyanide - free method for haemoglobin determination on Technicon. *Scand J Clin Lab Invest* 1990; 50:643-48.
- [6] Bhaskaran P. Validation of hemoglobin estimation by using Hemocue. *Indian J. Paediatr* 2003;70(1)25-28.