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HAEMOGLOBIN ESTIMATION BY NON CYANIDE--- SLS METHOD

Pathology

KEYWORDS:

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.The international standard of estimation by the cyanide method (HiCN). This method is known to be hazardous to the environment and harmful to the laboratory personnel as it contains KCN

Aims and Objectives: 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 haemoglobincyanide method, for laboratory investigations.

Materials and Methods: 1000 samples were tested for haemoglobin were done by the cyanmeth method and Sodium Lauryl Sulphate method (SLS) Was compared using Pearson's correlation coefficient.

Results: The haemoglobin concentrations which were estimated by the Sodium Lauryl Sulphate method were similar to those which were obtained by the conventional cyanmethaemoglobin method

Conclusion: The SLS 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-carring chromoprotein in the red blood cells of, human. 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 Sodium lauryl sulphate (SLS) method 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 cyanmethhaemoglobin method, this cyanide free methods can be insisted to be used in teaching institutions, blood bank camps and 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 one month in the RIMS, Ranchi, with a sample size of 1000 randomly selected patients. Haemoglobin was estimated by the cyanmethhaemoglobin method and the Sodium Lauryl Sulphate method.

Procedure:

TheCyanmethhaemoglobin 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 as blank at 540nm.Actual haemoglobin percentage in gm% was calculated by the formula(OD OF TEST/OD OF STD) x conc. Of STD.

The Sodium Lauryl Sulphate (SLS) Method: HEMOSAFE from the Tulip group Coral containing SLS - Reagent - 1000ml.

Hemosafe SLS – Standard (60mg/dl) – 10ml: The total conversion to the SLS-Haemoglobin was rapid and SLS converted haemoglobin fully to methaemoglobin.

Procedure: 0.02ml of the test sample was added to 5.0ml of sodium lauryl sulphate. 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 the SLS solution. The value of the sample was determined by the formula(OD OF TEST/OD OF STD) x conc. Of STD. 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 sodium lauryl sulphate solution. Depending on the optical density values, the results were compared with the standard curve. The stability of the sodium lauryl sulphate reagent at room temperature was assessed by preparing solutions with haemoglobin 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 sodium lauryl reagent and Drabkin's solution.

These samples were used for estimating the stability of the sodium lauryl sulphate reagent and the HbF and have not been included in the current data.

RESULTS

The haemoglobin which was estimated, ranged from 4.2gm% to 18.4gm% and the patients who were included in this study were newborns to 78years old. The optical density was measured at 540nm for all the 2 methods. The reaction completion time for the haemoglobincyanide method was 10 minutes, whereas for the sodium lauryl sulphate method 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 NaN3 which cause environmental pollution. the above non-cyanide method was compared for repeatability, reagent stability, and accuracy in correlation with the cyanmethhaemoglobin method and the SLS reagent gave excellent results.

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 and calibrator. However, its use may create a problem, as the disposal of large volumes of reagent which contains cyanide may constitute a potential bio-toxic hazard. As an alternative, the non-toxic sodium lauryl sulphate method 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, teaching hospitals and in diagnostic laboratories.

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