



EVALUATION OF THE COLORIMETRIC CYANMETHEMOGLOBIN METHOD AND THE AUTOMATICAL ANALYZER FOR HEMOGLOBIN ESTIMATION



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Article Type: Research Article

Article Citation: Diana CA, Krishnan V, Sree P, Manikandan V. Evaluation of the colorimetric cyanmethemoglobin method and the automatic analyser for hemoglobin estimation. SALT J Sci Res Healthc. 2021 February 20; 1(1): 17-27.

Received Date: January 29, 2021

Accepted Date: February 19, 2021

Published Date: February 20, 2021

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ABSTRACT

Hemoglobin estimation by cyanmethemoglobin method was carried out in the present study. The intensity of the color was measured by colorimeter and autoanalyser. The samples were collected from St Isabel's Hospital, Chennai and estimation of hemoglobin was done in autoanalyser. The remaining sample was transported to Loyola Health Centre, Chennai and then estimation of hemoglobin was performed with colorimeter. The readings obtained from colorimeter and autoanalyser processed statistically and was compared. Results indicates the insignificant variation between the autoanalyser and colorimeter for estimation of Hemoglobin respectively. Autoanalyser detects more anemic cases than colorimetric method. However, in resource poor condition where laboratory cannot afford expensive autoanalyser, colorimeter can be used as a suitable alternative.

Keywords: Colorimeter, Autoanalyser, Hemoglobin.

1.0 INTRODUCTION

Hemoglobin is a conjugated protein present in red blood cells. Haemoglobin consists of two components – haem (iron-protoporphyrin) and globin (amino acid chains). After the normal life span of the red blood cells is over (120 days), the red blood cells are destroyed by the reticuloendothelial cells and the components of haemoglobin undergo metabolic degradation. The iron component of haem is recycled and used up again in hemoglobin synthesis. The globin breaks down into its constituent's amino acids which are also recycled. The only component which cannot be recycled is protoporphyrin and it leads to the formation of bilirubin.

1.1 SYNTHESIS

Hemoglobin is synthesized in a complex series of steps. The heme part is synthesized in a series of steps

in the mitochondria and the cytosol of immature red blood cells, while the globin protein parts are synthesized by ribosomes in the cytosol. Production of Hb continues in the cell throughout its early development from the proerythroblast to the reticulocyte in the bone marrow. At this point, the nucleus is lost in mammalian red blood cells, but not in birds and many other species. Even after the loss of the nucleus in mammals, residual ribosomal RNA allows further synthesis of Hb until the reticulocyte loss its RNA soon after entering the vasculature.

1.2 STRUCTURE

In humans, the hemoglobin molecule is an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-protein heme group. Each protein chain arranges in to set of alpha- helix structural segments connected together in a globin fold arrange-

ment, so called because this arrangement is the same folding modified used in other.

In adult humans, the most common hemoglobin type is a tetramer called hemoglobin A. consisting of two α and two β subunits non-covalently bound, each made of 141 and 146 amino acid residues, respectively. This is denoted as $\alpha\beta$. The subunits are structurally similar and about the same size. Each subunit has a molecular weight of the tetramer of about 68,000 daltons. Hemoglobin A is the most intensively studied of the hemoglobin molecules. The four polypeptide chain are bound to each other by salt bridges, hydrogen bonds, and hydrophobic interactions. There are two kinds of contacts between the α and β chains: $\alpha 1\beta 1$ and $\alpha 2\beta 2$.

Variations of Hemoglobin

The variations of hemoglobin occur due to structural differences in the globin protein. These differences are genetically controlled. The normal hemoglobin components are hemoglobin A (HbA), hemoglobin A2 (HbA2), and fetal hemoglobin (HbF). HbA constitutes most of the hemoglobin of a normal adult while HbA2 constitutes a much smaller amount. HbF is present during the first 4 to 6 months of life and not normally present in adults. Hemoglobin S and hemoglobin C are the most commonly occurring abnormal hemoglobins. Others (D, E, H, etc.) are found in rare occurrences associated with several types of anemia. The various types of hemoglobin are separated by electrophoresis.

1.3 FUNCTION

The role of hemoglobin in the blood was elucidated by Physiologist **Claude Bernard**. Hemoglobin transports oxygen from the lungs to the rest of the body where it releases the oxygen for cell use. It also has variety of other role of gas transport and effect-modulation.

Compounds of Hemoglobin

Oxyhemoglobin: Oxygen combines loosely with iron (ferrous state) in hemoglobin. The loosely attached oxygen diffuses into the tissues for oxidative processes. The hemoglobin then binds carbon dioxide and exists as reduced hemoglobin.

Carboxyhemoglobin: Hemoglobin combines with carbon monoxide to form carboxyhemoglobin. Carbon monoxide has an affinity 200 times greater for hemoglobin than oxygen does. Hemoglobin in this

combination is incapable of oxygen transport.

Methamoglobin: This compound is formed when the ferrous state of the heme is oxidized to the ferric state.

This compound is incapable of oxygen transport.

Sulfhemoglobin: This compound results from the combination of inorganic sulfides and hemoglobin. This compound is incapable of oxygen transport. This is an irreversible reaction.

Cyanmethemoglobin: This compound results when methemoglobin combines with the cyanide radical. This compound is used in hemoglobinometry.

1.4 CLINICAL IMPORTANCE

1.4.1 Low Haemoglobin

A decrease in hemoglobin concentrations in blood below normal values is a sign of anemia (Table 1.3). The Haemoglobin concentration is lower in adult women as compared to adult males. Haemoglobin values further drop during pregnancy due to haemodilution. Children also have values lower than those seen in adult males. Anaemia, defined as a reduced haemoglobin concentration, is associated with increased perinatal mortality, increased child morbidity and mortality, impaired mental development, impaired immune competence, increased susceptibility to lead poisoning, and decreased performance at work (**Dallman, 1990; Basta et al., 1979; Pollit E et al., 1989; Soewondo S et al., 1989**). To identify the most likely cause of anaemia, such as low iron intake or infection, distribution curves of the haemoglobin concentration of different groups within a population can be compared.

As absence of iron decreases heme synthesis, red blood in iron deficiency anemia are hypochromic (lacking the red hemoglobin pigment) and microcytic (smaller than normal). Macrocytic (increase in cell size) can also be seen, In hemolysis (accelerated breakdown of red blood cells), associated jaundice is caused by the hemoglobin metabolite bilirubin, and the circulating hemoglobin can cause renal failure.

There are many reasons for anemia some of the most common causes are: 1. Loss of blood (traumatic, injury, surgery bleeding, colon cancer or stomach ulcer), 2. Nutritional deficiency (iron, vitamin B₁₂, folate), 3. Bone marrow problems (replacement of bone marrow), 4. Suppression by chemotherapy drugs by

cancer., 5. Kidney failure and abnormal haemoglobin (sickle cell anemia).

Anemia can be classified in a variety of ways, based on the morphology of RBCs, underlying etiology, mechanisms, and discernible clinical spectra.

Types of Anaemia: Drug induced immune haemolytic anaemia, Haemolytic anaemia due to G6PD deficiency, Idiopathic aplastic anaemia, Idiopathic autoimmune haemolytic anaemia, Immune haemolytic anaemia, Iron deficiency anaemia, Paroxysmal cold haemoglobinuria.

1.4.2 High Haemoglobin Elevated levels of hemoglobin are associated with increased numbers or sizes of red blood cells, called polycythemia. This elevation may be caused by congenital heart disease, pulmonary fibrosis, too much erythropoietin, or polycythemia vera. Defects in hemoglobin called haemoglobinopathies are congenital diseases (sickle cell anemia). Such defects originate from the abnormalities in the chemical structures of globin and its amino acid sequence. The haemoglobinopathies– thalassemias and abnormal haemoglobins – constitute a major burden of genetic diseases in India (**Sujata Sinha et al., 2004**).

Higher than normal haemoglobin levels can be seen in people living at high attitudes and in people who smoker, dehydration produces falsely high haemoglobin which disappears when proper fluid balance is restored. Some other infrequent causes are: Advanced lung disease (for eg emphysema), Certain tumours, A disorder of the bone marrow known as polycythemia rubra vera and abuse of the drug erythropoietin by athletes for blood dropping purposes, Congenital heart disease, Cor pulmonale, Pulmonary fibrosis, excess production of RBC in the bone marrow, Several other conditions.

1.5 LABORATORY DIAGNOSIS

The hemoglobin concentration is directly proportional to the oxygen-combining capacity of blood. Therefore, the measurement of the hemoglobin concentration in the blood is important as a screening test for diseases associated with anemia and for following the response of these diseases to treatment. Various methods are available for hemoglobin estimation for example:

Sahli or Acid Haematin Method: Haemoglobin (Hb) is converted to acid haematin by addition of 0.1 N Hy-

drochloric acid and the resulting brown colour is compared with standard brown glass reference blocks.

Alkali Haematin Method: Haemoglobin, methaemoglobin, carboxyhaemoglobin and sulphaemoglobin are converted to alkaline haematin by addition of sodium hydroxide - a strong alkali. It forms a true solution and the brown colour can be read against comparable standards or in a colorimeter. Foetal haemoglobin and Hb- Barts are alkali-resistant, but can be converted by heating in a boiling water bath for 4 min. or by collecting the blood first in to acid and then adding alkali (acid alkali method).

Haldanes Carboxyhaemoglobin Method: Haemoglobin is converted to carboxyhaemoglobin (which is bright red in colour), by exposing it to carbon monoxide. It is a relatively accurate method but carbon monoxide is dangerous.

Oxyhaemoglobin Method: Haemoglobin is converted into oxyhaemoglobin by mixing blood with a dilute solution of sodium carbonate or ammonium hydroxide. The intensity of the colour obtained is measured colorimetrically. It is a fast and accurate method, but traces of copper can give errors.

Cyanmethaemoglobin Method: Hb, Methaemoglobin, Carboxyhaemoglobin but not sulphaemoglobin are converted to cyanmethaemoglobin when blood is diluted in a solution containing potassium cyanide and potassium ferricyanide. The absorbance of the solution is then measured in a photoelectric colorimeter at a wavelength of 540 nm or with a yellow-green filter. Abnormal plasma proteins or a high leukocyte count may give erroneously high Hb content due to turbidity. The latter can be avoided by centrifuging the diluted sample.

DHT meter (HMB.010; Developing Health Technology, Ipswich, UK): This is based on the oxyhaemoglobin method and has a direct readout of the haemoglobin estimation.

HemoCue (HemoCue AB-haemoglobin photometer; Angelholm, Sweden): The HemoCue® haemoglobinometer measures haemoglobin concentration using light absorption technology. The Hb measurement performed by HemoCue is based on a method described by Vanzetti. The sodium desoxycholate present in the cuvette hemolyzes red cells, and the free Hb is converted into methemoglobin by sodium nitrite.

Then, methemoglobin is converted into azide-methemoglobin by sodium azide. Transmitted light absorbance is measured at 565 and 880 nm to compensate for any turbidity in the sample (**Vanzetti, 1966**). The haemoglobin concentration is displayed in less than 45 seconds (**Nicholls, 1990**).

Some advantages of this equipment are simplicity and speed in measuring Hb, requiring just a single drop of blood collected in a special cuvette. It is claimed by the manufacturer that the HemoCue is suitable for Hb estimation using capillary, venous and arterial blood samples. Previous evaluations of the HemoCue have revealed conflicting results, with some studies presenting a good precision and accuracy of this equipment, and others emphasizing the need of a better methodological control (**Chen et al., 1992; Cogswell et al., 1998; Conway et al., 1998**).

Haemoglobin colour scale (Copack GmbH; Oststeinbek, Germany): A drop of undiluted blood is placed on specific chromatography paper and matched against a range of colours representing different haemoglobin values in 20 g/liter increments.

The Lovibond comparator: (ECHO International Health Services Limited, Coulsden, Surrey, UK): This is the predominant method used in Malawian district hospitals and assessments were carried out using the equipment already in routine use. It is based on the oxyhaemoglobin method and involves matching the colour of the diluted blood sample with colours on a disc equivalent to known haemoglobin values in 20 g/litre increments (**Woodliff et al., 1966**).

A simple, rapid and cheap method for estimating haemoglobin concentration with a finger prick blood sample, has been developed for use in resource-poor settings where there is no laboratory. The method relies on comparing the colour of a drop of blood absorbed onto a filter paper with standard colours on a laminated card, varying from pink to dark red. These colours correspond to haemoglobin levels of 4, 6, 8, 10, 12, and 14 g/dl. Intermediate shades can be identified, allowing haemoglobin levels to be judged to 1 g/dl. This is the only method currently available that is affordable for widespread primary care use by the most impoverished countries (**Julia & Imelda, 2005**).

Haemoglobin Colour Scale (HCS) is simple and inexpensive device for providing a reliable indication of the presence and severity of anaemia would be of considerable value in situations where laboratory based haemoglobinometry is not readily available. The simplest method is to match the colour of a drop of blood on absorbent paper against a colour scale; however, previous devices of this type have been too unreliable to serve their intended purpose (**Ingram & Lewis, 2000**).

1.5.1 HEMOBLOGINOMETRY

There are four basic ways to measure the hemoglobin concentration: (1) Measurement of the oxygen-combining capacity of blood (gasometric), (2) Measurement of the iron content (chemical method), (3) Colorimetric measurement of specific gravity (gravimetric method), (4) The cyanmethemoglobin method is the method of choice and is recommended by the Technical Subcommittee on Hemoglobinometry of the International Committee for Standardization in Hematology (ICSH).

1.5.2 Cyanmethemoglobin Method

Three advantages of the cyanmethemoglobin method are: 1. Measures all forms of hemoglobin except sulfhemoglobin; 2. Can be easily standardized 3. Cyanmethemoglobin reagent (also called Drabkin's solution) is very stable.

Principle: Blood is diluted with a dilute solution of potassium ferricyanide and potassium cyanide at a slightly alkaline pH. The ferricyanide converts the hemoglobin to methemoglobin. The cyanide then reacts with the methemoglobin to form the stable cyanmethemoglobin. The color intensity is measured in a spectrophotometer at a wavelength of 540 nm. The optical density is proportional to the concentration of hemoglobin.

1.5.3 COLORIMETER

These methods are based on the conversion of haemoglobin to haemiglobincyanide. A colorimeter, an instrument used in colorimetry refers to one of several related devices in scientific field, the word generally refers to the device that measure the absorbance of particular wavelength of light by a specific solution. This is mostly used to determine the concentration of a known solute in a given solution by the application of the Beer Lambert law, which states that the concentra-

tion of a solute is proportional to the absorbance.

1.5.4 AUTOMATED HAEMATOLOGY ANALYSER

An automated analyzer is a medical laboratory instrument designed to measure different chemicals and other characteristics in a number of biological samples quickly, with minimal human assistance. This can involve placing test tubes of sample in to racks, which can be moved along a track, or inserting tubes in to circular carousels that rotate to make the sample available.

Haematology analyser are used to perform complete blood counts, erythrocytes sedimentation rates (ESRs), or coagulation tests. Automated cell counters sample the blood, and quantify, classify, and describe cell populations using both electrical and optical techniques, electrical analysis involves passing a dilute solution of the blood through an aperture across which an electrical current is flowing. The passage of cells through the current changes the impedance between the terminals. Haemoglobin measurement is the most commonly performed laboratory test worldwide and is an essential component of any health system. In sub-Saharan Africa, haemoglobin estimations are used in district hospitals for individual patient management, to guide transfusion practice, and in the management of antiretroviral therapy. Surveys of haemoglobin concentrations are also used as tools to provide public health data, such as nutritional status, and to monitor malaria interventions. Despite the wide range of manual methods available for measuring haemoglobin in developing countries, no single technique has emerged as the most appropriate for this setting.

2.0 REVIEW OF LITERATURE

Neville demonstrated a much lower correlation between the HemoCue® and laboratory haemoglobin measurements when nurse practitioners were using the device in a clinical setting compared with results obtained by trained laboratory personnel in the laboratory (Neville, 1987).

(Chen *et al.*, 1992) found the device to be very accurate in an intensive care setting when using arterial or venous samples, but revealed a greater variance in capillary samples. They concluded that photometer values from capillary samples were not accurate enough to be used to guide therapeutic decisions.

Comparative study of haemoglobin estimated by drabkin's and sahli's methods. As photometer 4010 has the capability to do all the chemical analysis, separate photometer for Hb is not required in small laboratories (Balasubramaniam & Malathi, 1992).

Despite its advantages, the high recurrent costs will probably be a major disincentive for governments of poor countries to implement HemoCue at subdistrict levels where the burden of anaemia (Hudson *et al.*, 1994).

(Koul *et al.*, 1995) presents the estimation of two important biochemical parameters –haemoglobin and glucose, quantitatively by a single chemical cell. The chemical cell has been so designed to be made of layers of cellulose matrix containing dry reagents separated with a semipermeable membrane. The membrane acts as a barrier to red cells and allows plasma to filter through it. This method provides a single step analysis of both parameters by reflectance photometry having an integrated sphere attachment.

In an ideal situation, transfusion would be based only on rapidly assessed laboratory measurements of haemoglobin concentration. In reality, however, it can take 30-45 min to get the results of a stat sample of blood analyzed for a haemoglobin concentration returned from the laboratory. In many intraoperative situations, decisions have to be made much sooner. In a controlled laboratory setting, the HemoCue® is both reliable and valid, demonstrating its consistency in repeated sample measurements (Melanie *et al.*, 1996).

According to Cogswell *et al.*, 1998, the collection of capillary samples for the diagnosis of anemia and iron deficiency anemia is not appropriate, rather than an inadequacy of the HemoCue. It is important to point out that the measurements in capillary blood samples have been reported to be significantly higher than the corresponding values in venous samples (Daae *et al.*, 1998).

Elevated Hb levels can be observed in polycythemic patients and congenital cyanotic heart diseases. Estimation of blood Hb concentration is as important as an initial step in the detection of anaemia or erythrocytosis (Dessparis *et al.*, 1999). Further studies are therefore needed to assess the benefits and disadvantages of using the HCS in specif-

ic patient populations in high prevalence of rural areas. The aim of a device such as the HCS should be to improve identifications of anaemic individuals, and not to replace clinical examination, so it is important to investigate the most effective way of combining this approaches **(Van et al., 1999)**.

The study of the haemoglobin colour scale is simple, rapid and cheap method for estimating haemoglobin concentration with a finger prick blood sample, has been developed for use in resource – poor settings where there is no laboratory. The method relies on comparing the colour of a drop of blood absorbed on to a filter paper with standard colours on a laminated card **(Kumar A et al., 1999)**.

The method of choice for evaluating anaemia in remote areas would be the HemoCue method to assess venous blood; our second choice would be the HemoCue assessment of capillary blood; our third choice would be to the direct cyanmethaemoglobin method of assessing capillary blood. These choices were made using the comparisons of the sensitivity and specificity of the different methods **(Mayang Sari et al., 2001)**.

Anaemia is a major health problem, in poor countries most of the cases are diagnosed clinically. HCS has been developed as an inexpensive, simple alternative for assessing anaemia **(Mohanram et al., 2002)**.

According to the study results, the HemoCue has a lower precision than the Cell Dyn 3000 for analyses of both blood samples. Similarly, some studies have also found a high variation between paired Hb measurements obtained by the HemoCue when compared to a counter **(Adriana et al., 2004)**.

The HCS may improve anaemia diagnosis where there is no laboratory, but there is a need for policy relevant diagnostic research which is pragmatic, implementation-focused and assesses clinical outcomes. This requires a different approach and research skill-mix from efficacy studies **(Julia and Imelda, 2005)**.

Anemic conditions in women and children across the world are a serious cause for concern. Since, human interpretation errors are likely to creep in during the subjective processes involved with this method an artificial neural network (ANN) approach for the estimation of haemoglobin count in human blood has been evaluated. The ANN used color-coded values of

the samples as input and the Hb value, as obtained with the cyanmethaemoglobin method, as desired output **(Ranganathan & Gunasekaran, 2006)**.

Kafil Aktar et al., 2008 suggested some better alternatives of haemoglobin estimation in blood donors in the Hemocue photometer method. Measurement of plasma haemoglobin is useful in variety of clinical conditions. In the present study we have developed a kinetic method to estimate plasma haemoglobin by using o-tolidine. This method is sensitive, rapid, economical, simple and less influenced by interfering substances. It measures plasma haemoglobin in the range of 6 to 400mg/L in less minutes and can be easily automated **(Madhur & Anjan, 2009)**.

Automated cell counter costs Rs. 50 per sample for hemoglobin estimation, while cyanmethemoglobin method performed manually costs less. Cost of Medonic Cell Counter is about Rs. 0.65 million, while cost of photometer 4010 is about Rs. 0.22 million. As photometer 4010 has the capability to do all the chemical analysis, separate photometer for Hb is not required in small laboratories. Automated estimation is feasible, but should be carried out, when the whole blood picture and red cell indices are required **(Balasubramaniam & Malathi, 1992)**.

In the Drabkin's Hb estimation method, Hb is oxidised to methemoglobin by potassium ferricyanide, which reacts with cyanide ions of potassium cyanide to form cyanmethemoglobin. The Hb is estimated with the help of cyanmethemoglobin curve. The advantages of this method are i) error due to subjective visual matching is avoided as spectrophotometer is used and hence reading is precise and reliable, ii) measures all forms of Hb except sulphaemoglobin, iii) single step procedure using single reagent, iv) cyanmethemoglobin formed produces broad absorbent band at 530nm and v) good stable Hb standards are available **(Waqar Azim et al., 2002)**.

2.1 AIM & OBJECTIVE OF THE PRESENT STUDY In the light of earlier literature, it was felt essential to evaluate the estimation methods of hemoglobin. Hence the present attempt was made: To adopt cyanmethemoglobin method for hemoglobin estimation. To compare the results obtained Colorimetric and Autoanalyser measurement. To assess the hemoglobin estimation with 354 samples in order to deter-

mine the sensitivity and accuracy of Colorimetric method (CM) with Autoanalyser method (AM).

3.0 MATERIALS AND METHOD

To identify a method for measuring haemoglobin that was simple, accurate, fast, and cheap and required minimal training and supervision that could be used in district hospital laboratories the present study was planned. Our study assessed the effectiveness and managerial aspects, of autoanalyser and colorimeter for measuring haemoglobin by cyanmethemoglobin in routine practice in a typical Loyola College Health Centre, Chennai against criteria predetermined by St Isabals Hospital, Chennai. The present study was conducted in Loyola Health Center, Loyola College, Chennai 600 034 (Table 3.1). The study group included 355 patients. Blood specimens were collected in 5ml Ethylenediaminetetra-acetic acid (EDTA) tubes from 354 outpatients and inpatients of St. Isabals Hospital, Chennai (Table 3.2) then transported to Loyola Health Centre, Chennai.

3.1 MATERIALS REQUIRED: Test tube, Syringes, Tourniquet, Micropipettes, Test tube stand, Eppendorf tube, Colorimeter, Automated analyzer, Cotton, Surgical spirit.

3.1.1 REAGENT REQUIRED: Drabkins Reagent (Cyanmethaemoglobin solution), EDTA, Cyanmethaemoglobin Standard, All commercially available.

3.2 COLLECTION OF SAMPLE

All things required for blood collection was assembled. The container was labeled with patient's identification number. The tourniquet was tied just above the elbow and the puncture area was selected. The skin was disinfected with 70% alcohol and the site of vein puncture was rubbed thoroughly. The needle was firmly and steadily injected to the center of the vein of a depth of 1.5 cm. After collecting required amount of blood the tourniquet was released. The swab of cotton wool was placed over the hidden point of the needle and it was gently withdrawn. The needle was removed from the syringe and the blood was expelled in to the test tube.

3.3 HAEMOGLOBIN ESTIMATION (CYANMET-HAEMOGLOBIN METHOD)

Label the test tube as blank (B), test (T), standard(S). Pipette 5ml and 2.5 ml of cyanmethaemoglobin re-

agent in to the marked test tubes. Correct volume is not important in case of the blank (B). Hence, while setting the automated dispenser, use the blank for removing air bubbles. Mix the blood specimen by gentle inversion or swirling. Draw the 0.02ml of blood sample in micropipettes and mix with the 5ml solution marked as test (T). Wipe the outside of the pipette with a wet gauze while holding the pipette in the horizontal position. If the outside of the pipette is not properly cleaned, the result may show a falsely high value. After cleaning the pipette, check that the blood is still up to the mark and there are no air bubbles. Mix the contents and incubate the test tube at room temperature for five minutes. Turn on the colorimeter and allow it to warm up for 10 minutes; set the wavelength selector to 540nm or use a suitable filter in this range. zero the instrument without cuvette using the first control knob. Transfer both, the solutions, blank (B) and test (T), to two matched cuvettes. The matched cuvettes should give same absorbance reading when filled with water. Insert the cuvette with the test solution (T) in to the socket of the photoelectric colorimeter and record the absorbance reading. Do not touch any adjustment knob while taking the absorbance reading of test solution.

3.3.1 CALCULATION: Hemoglobin (g/dl) = $\left[\frac{\text{Optical Density of Test}}{\text{Optical Density of Standard}} \right] \times 15$

3.4 HAEMATOLOGY ANALYSER (NON CYANIDE HB METHOD)

3.4.1 PROCEDURE

1. 2 ml of sample is collected in anticoagulated vacutainer.
2. The tuber is placed in the sample holder and pressed RUN button.
3. Sysmax hematology analyser provides 19 parameter test results including hemoglobin.

3.5 PRECAUTIONS

1. The reagent is poisonous, handle is carefully. To avoid using cyanide, lauryl sulfate has been proposed as a nonhazardous substitute, as lauryl sulfate has similar properties to HiCN. Drabkin's reagent contains 50mg of KCN per liter. Its toxic effects can only be seen if quantity in the range 600-1000ml is swallowed.
2. Mix anticoagulated blood by swirling properly

before pipetting. Adjust carefully the blood column in to the graduation mark and use dry cotton to wipe excess blood on the pipette.

3. Do not discard the drabkin's reagent in the sink. Poisonous cyanide gas is released if the sink has an acidic solution. Flush the sink with water and then discard the drabkin's reagent and to continue to flush water for some time.
4. Turbidity may develop due to HbS or HbC or due to lipemic blood. In that cases, if it is due to abnormal haemoglobin, add 0.1g of potassium carbonate, centrifuge and read absorbance of the supernatant solution. In the case of a lipemic blood specimen, use 0.02ml of serum or plasma, mix with 5ml of drabkin's reagent and it as a blank. Read test reading against this blank.
5. Deterioration of the reagent causes erroneously low results. Hence occasionally the reagent should be tested with blood specimen of known high Hb. Similarly, reagent from new batch should be tested with 2 to 3 blood specimen of known Hb concentration, belonging to low, moderate and high Hb values.

4.0 RESULTS

Results obtained for 354 different patients by auto-analyzer method (AM) yielded almost similar values as that of colorimetric method (CM), with a marginal variation. Data obtained by both the methods were subjected to statistical analysis by calculating the mean (M), standard deviation (SD), standard error (SE), Pearson's correlation, Paired T test, One-way ANOVA and Altman Bland test. Results reveal that, the calculated M were 11.42 and 11.84, SD were 2.2 and 5.6, SE were 0.11 and 0.30. The p value was less than 0.1560 which indicates the insignificant variation between the AM and CM for estimation of Hb respectively.

Out of 354 patients 215 were male and 139 were female, when estimated hemoglobin level with Colorimeter out of 215 males 80 were normal, 9 were high and 126 showed low values. Out of 139 females 28 were normal, 5 were high and 104 showed low values. However, when hemoglobin level was estimated with Autoanalyser, out of 215 males 28 were normal, 0 were high and 111 showed low value. Out of 139 fe-

males 73 were normal, 5 were high and 138 showed low values.

5.0 DISCUSSION

High cost and lack of facilities generally prevent the use of sophisticated equipment for haemoglobin (Hb) screening in the clinical situation in rural areas (**Politzer et al., 1988**). A tertiary care medical unit of public as well as private sector needs an economically suitable methodology. Automated cell counter costs Rs. 50 per sample for hemoglobin estimation, while cyanmethemoglobin method performed manually costs less. Cost of Medonic Cell Counter is about Rs. 0.65 million, while cost of photometer 4010 is about Rs. 0.22 million. As photometer 4010 has the capability to do all the chemical analysis, separate photometer for Hb is not required in small laboratories. Automated estimation is feasible, but should be carried out, when the whole blood picture and red cell indices are required (**Balasubramaniam & Malathi, 1992**).

In the Drabkin's Hb estimation method, Hb is oxidised to methemoglobin by potassium ferricyanide, which reacts with cyanide ions of potassium cyanide to form cyanmethemoglobin. The Hb is estimated with the help of cyanmethemoglobin curve. The advantages of this method are i) error due to subjective visual matching is avoided as spectrophotometer is used and hence reading is precise and reliable, ii) measures all forms of Hb except sulphaemoglobin, iii) single step procedure using single reagent, iv) cyanmethemoglobin formed produces broad absorbent band at 530nm and v) good stable Hb standards are available (**Waqar Azim et al., 2002**).

The gold standard for assessing haemoglobin concentration is the direct cyanmethaemoglobin method. However, this method requires that a laboratory with a spectrophotometer is available within a few hours' travelling time from where the blood is collected and, to avoid inter-laboratory variability, it is necessary that all measurements are conducted by the same laboratory (**Mayang Sari et al., 2001**).

Health managers in poorer countries do not have technical training in laboratory issues and have great difficulty in obtaining the evidence that will enable them to make rational decisions about purchasing technological services. They need to take account not only of

costs, but also simplicity, accuracy, speed, available manpower, and technical skills of their laboratory workforce and the health needs of the population. Although the World Health Organisation does provide guidance on the selection of laboratory tests, details of all the components needed to make an evidence based judgement, such as we have provided in our study, are lacking. One of the major roles that international organizations could play would be to provide detailed, independent evaluations of new laboratory methods, which include information about start up and recurrent costs, training duration and costs, complexity of the method, and the inputs needed to maintain calibrations and quality. This type of independent and comprehensive information would be invaluable to health purchasers in developing countries, who face conflicting pressures over equipment purchase from agencies such as bilateral donors, charities, and the commercial sector.

Cyanmethemoglobin is the most stable of the various hemoglobin pigments showing no evidence of deterioration after 6 years of storage in a refrigerator. The availability of prepared standards is a distinct advantage of this technique. All hemoglobin derivatives are converted to cyanmethemoglobin with the exception of sulfhemoglobin.

This method is highly accurate and is the most direct analysis available for total hemin or hemoglobin iron. Its disadvantage is the use of cyanide compounds, which, if handled carefully, should present little hazard. For accuracy in hemoglobin determinations, it is absolutely necessary that the spectrophotometer and Sahli pipets be accurately calibrated. Venous samples give more constant values than capillary samples. If the procedure is performed properly, the degree of accuracy is +2 to 3 percent.

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The present study shows that more among 354 collected sample majority suffer from anemia. Colorimeter identifies more anemic conditions than autoanalyser. When hemoglobin were estimated by colorimeter males were anemic than females, However when analyzed by autoanalyser females were anemic than the males.

Autoanalyser Method (AM) reduces the usage of reagent and blood sample compared to conventional Colorimetric Method (CM), however increases the test cost. In the present study CM reveals same results as AM with minute variations. These minute variations can be rectified by proper training to reduce pipetting error and using standard Drabkin's solution. Hence, it may be recommended that CM being cost effective can be used in primary level health centers for the estimation of Hb, where autoanalyzer facilities are not available.

The cost of CM is bearable by the poor people and will be helpful in diagnosing anemia and malnutrition. In future, evaluation of the suitability, cost effectiveness and feasibility of AM of Hb estimation is desirable.

6.0 CONCLUSION

Autoanalyser detects more anemic cases than colorimetric method. However, in resource poor condition where laboratory cannot afford expensive autoanalyser, colorimeter can be used as a suitable alternative. The cost of CM is bearable by the poor people and will be helpful in diagnosing anemia and malnutrition. In future, evaluation of the suitability, cost effectiveness and feasibility of AM of Hb estimation is desirable.

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ARTICLE TYPE: Research Article; **ORCID ID:** Open Researcher and Contributor Identifier (ORCID) ID of corresponding author: <https://orcid.org/0000-0002-4061-2840>; **ETHICAL:** Institutional Ethics Committee; **ACKNOWLEDGEMENT:** None; **FINANCIAL DISCLOSURE:** The authors declare that there was no financial aid received.; **CONFLICT OF INTEREST:** No conflict of interest associated with this review work.; **AUTHORS CONTRIBUTION:** Conceptualization: A.L.D., Methodology: V.K., Investigation: P.S., Result Analysis and Writing: V.M.; **CORRESPONDING AUTHOR AFFILIATIONS:** Vyshnav Manikandan. Masters in Human Clinical Embryology and assisted Conception, University of Dundee, Nethergate, Dundee DD1 4HN, Scotland.; **CORRESPONDING AUTHOR EMAIL:** 2472888@dundee.ac.uk; **ARTICLE CITATION:** Diana CA, Krishnan V, Sree P, Manikandan V. Evaluation of the colorimetric cyanmethemoglobin method and the automatical analyser for hemoglobin estimation. SALT J Sci Res Healthc. 2021 February 20; 1(1): 17-27.

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Originally published in the SALT Journal of Scientific Research in Healthcare (<https://saltjsrh.in/>), 20.02.2021.

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