

Hemoglobin Estimation by Invasive and Non-Invasive Methods: A Review

^[1]Nidhi R. Patel, ^[1]Arti N. Kabra, ^[2]Shreya Vora
^{[1],[1],[2]}Parul University, Waghodia.

Abstract:

Hemoglobin is an imperative element in the human blood, the deficiency of which may lead to Anemia. Thus identifying the level of Hemoglobin in patients' blood is a vital investigation. In order to evade such a condition, there is a requirement for a regular blood test to detect the presence of Anemia in the blood. There are multiple techniques with different applications for estimation of hemoglobin; however, it is broadly categorized into two types namely: Invasive method and Non-Invasive method. In an Invasive method, there is a requisite of patients' blood sample whereas in Non-invasive technique there is no need to draw blood from the patients'. This review article focuses on an assortment of methods for estimating Hemoglobin. Direct cyanmethemoglobin method has been the gold standard for Hemoglobin estimation but other methods like Color scale, Sahli's technique; Tallqvist, Hemocue, etc. are also available. The study, in addition, explores the frontier involving latest methods such as image processing techniques, pulse oximetry and occlusion spectroscopy which embraces the enhanced scope for the detection of Hemoglobin.

Keywords: Hemoglobin, Anemia, Invasive and Non-Invasive methods, Image Processing Techniques.

Abbreviations: Hemoglobin (Hb), WHO (World Health Organization), National Family Health Survey (NFHS), The Haemoglobin colour scale (HCS), The Cyanmethemoglobin method (HiCN), Fetal haemoglobin (HbF), arterial oxygen saturation (SaO₂), Methemoglobin (MetHb), Carboxyhemoglobin (COHb), Near Infrared (NIR), plethysmo-graphic (ppg)

INTRODUCTION

Hemoglobin is a significant respiratory protein in Red corpuscles have a molecular weight of approximately 64,500 and consists of four polypeptide chains linked together by the non-covalent bonds each with electroactive iron heme group ^[1]. The iron contained in hemoglobin is responsible for the red color of blood. ^[2]Hb is frequently measured by complete blood count method and is expressed in grams (gm) per deciliter (dl) of whole blood. The volumetric determination of Hb in red corpuscles in blood is defined as Hematocrit. ^[3]The normal ranges of Hb and hematocrit depend on the age, beginning in adolescence, the gender of the person which is mentioned in Table 1. ^[4]:

TABLE 1: Showing the normal Ranges of Hb and Hematocrit

Population	Hemoglobin	Hematocrit
Infants	10.5 - 19.5 g/dl	32 - 60%
Children (1-9)	11.0 - 14.0 g/dl	33 - 40%
Children (10-12)	11.5 - 15.0 g/dl	35 - 45%
Men (adults)	13.0 - 18.0 g/dl	40 - 50 %
Women (adults)	12.0 - 16.0 g/dl	36 - 44%
Pregnant Women	11.0 - 14.0 g/dl	33 - 42%

Anemia is one of the most serious global public health problem and world's second-leading disability which is generally defined as a reduced hemoglobin concentration in red corpuscles in the blood, which is associated with augmented perinatal mortality, increased child mortality, impaired immune competence and reduced performance at work. ^[5]In developing countries of Southeast Asia an alarming 600 million populace are suffering from iron deficiency anemia, mainly disturbing adolescent girls,

women of reproductive age and young children. ^[6]The situation is predominant amongst pregnant women in the area ranging from 13.4 % in Thailand to 87% in India. Approximately 74% of pregnant women in Bangladesh, 63% in Nepal, 58% in Sri Lanka and Myanmar, and 51% in Indonesia suffer from Anemia. ^[7]According to the National Family Health Survey (NFHS)-3rd in 2005- 2006, more than half of women in India 55% & 24% of men have Hemoglobin level lower than the cutoff. According to the studies made an increasing trend in prevalence of anemia from 49.7% (NFHS-2, 1998-1999) to 50.5% (NFHS-3, 2005-2006) has been reported. ^[8]There is wide range of clinical methods accepted by World Health Organization (WHO) to identify abnormal level of hemoglobin in the blood in the pathology laboratory. ^[9]

Invasive method: A medical process pervades the body cavity i.e., it requires a puncture, an prick, a catheterization, etc. into the body can be termed as invasive technique. ^[10]Direct cyanmethemoglobin technique has been the gold standard designed for hemoglobin estimation and is economical but time consuming. A number of additional methods with their specific working principle are available such as hemoglobin color scale, Sahli technique, Tallqvist technique, copper-sulfate method and HemoCue. Every technique has a different functioning principle and its own advantages and disadvantages. Simple techniques to measure Hb exist but they are relatively expensive and require commercial reagents and good technical skills to interpret. ^[11]**Non invasive** methods are generally defined as the type of hemoglobin estimation methods that has been introduced with the aim of preventing ache to blood donor. Other than avoiding venipuncture, this technique in addition minimizes the risk of infection for health care personnel, eliminates the production of biohazardous squander, reduces

the need for trained workers, cuts down on consumables and is sampling error proof.^[12]

METHODS:

Hemoglobin estimation has conventionally relied on the services of a well-resourced clinical laboratory. A number of methods with their specific working principle, advantages and disadvantages are present.

INVASIVE TECHNIQUE

Haldane technique designed for hemoglobin (Hb) estimation is the oldest method, mainly based on carbon monoxide (CO) carrying ability of blood and comparison by using a standard. Nevertheless, commercially accessible standards are faded quickly & unreliable.^[11] **The Haemoglobin colour scale (HCS)** is an easy, rapid and inexpensive method which has been developed where there is no laboratory for estimating the concentration of hemoglobin by means of a finger prick. The process relies on comparing the color of a plunge of blood absorbed onto a clean 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.^[13] **Dare method** uses a diminutive glass chamber that is entirely filled with blood by capillary deed. The chamber is illuminated via a battery- lit bulb and the color of blood is matched with a standard following by viewing all the way through an eyepiece.^[14] **The Tallqvist technique** is a type of filter paper process which does not require lysis or blood dilution. The development of method took place in 1995. A plunge of blood is obtained by puncturing either the earlobe or fingertip with a sterilized lancet. Wipe up the primary drop of blood. Subsequently collect next drop onto the filter paper and remain until the blood spot has almost dried. The concentration of Hb is interpolated by comparing the color of the blood with a set of color standards on paper.^[15] **The Sahli's technique** is commonly defined as a process based on the theory of converting Hb to acid hematin and then visually matching its color adjacent to a solid glass standard. The haemometer is a supreme measuring system by graduation with g/dl reading.. The key requirements designed for this method are Sahli blood pipette and Sahli hemoglobinometer. The measuring graduated tube is filled up to the base graduation line with hydrochloric acid. A capacity of 20 µl of blood is blown into the tube and water is added until the solution's color matches the colour of the test rods. The result can be read by gentle daylight, 3 min after adding up the 20 µl of blood to hydrochloric acid. The amount of dilute acid added will be determined by the hemoglobin level of the blood samples. The readings are based on the judgment of human eye.^[16]

Hemocue is yet another method extensively used for as a point-of-care device for Hb estimation in health facilities. It is also known as portable hemoglobinometer which is highly sensitive and specific in nature.^[17] The portable hemoglobinometer mainly requires only a small sample of capillary/venous blood, is relatively inexpensive and simple

to use. Thus giving immediate, digitally displayed results.^[18] This technique requires the lysis (breaking down of a cell) of the blood frequently by viral, enzymic, or osmotic mechanisms that compromise its integrity. HemoCue primarily uses a disposable cuvette that is treated with chemicals that rip apart the red blood cell wall and coalesce with the hemoglobin to figure a compound that can be deliberate photometrically. The absorbance is measured at two wavelengths (570 nm and 880 nm) in order to compensate for turbidity in the sample. The end result is displayed in digital form on the face of the instrument.^[11] Reference Range for Adult male's 130 – 170 g/L

Adult females 120 – 150 g/L Infants, after neonatal period 110 – 140 g/L Children, two years to teenage: gradual increase to adult normal.^[19]

The Cyanmethemoglobin method or modified Drabkin's method (HiCN) is used for the measurement of hemoglobin and has been in wide applicability worldwide; its pros are available as steady and internationally acknowledged cite standard calibrator.^[20] The cyanmethemoglobin method entirety is on the explanation of modification of hemoglobin to cyanmethemoglobin by the summation of potassium cyanide and ferricyanide whose absorbance is calculated at 540 nm in a photoelectric colorimeter against a standard solution.^[11] When Blood is mixed with a solution of Drabkin's solution (potassium cyanide, potassium ferricyanide and potassium dihydrogen phosphate)^[21], the erythrocytes are lysed by producing evenly disturbed hemoglobin solution. Potassium ferricyanide change hemoglobin to methemoglobin, and methemoglobin mix with potassium cyanide to produce hemoglobin cyanide (cyanmethemoglobin). And then all forms of hemoglobin present in blood are completely changed to a single conjugate cyanmethemoglobin. When the reaction gets completed, 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.^[22]

$$c(g/l) = \frac{A_{HiCN}^{540} \times M \times F}{\epsilon_{HiCN}^{540} \times 1 \times 1000}$$

Where,

$c(g/l)$ = concentration of hemoglobin gram per liter of blood

A_{HiCN}^{540} = absorbance of the solution at $\lambda = 540$ nm;

M = relative molecular mass of haemoglobin, derived from 64 458/4;

F = dilution factor used (for example, 251);

ϵ_{HiCN}^{540} = molar absorptivity = 11.0;

l = light path in cm; and

1000 = conversion factor mg to g.⁽²³⁾

Alkaline hematin method measure total pigment with a reasonable degree of accuracy and yet it is simple enough. to be employed in a laboratory where many determinations are performed daily.^[24] This procedure consist of a solution of crystalline haemin in sodium hydroxide.^[25] In the alkaline hematin D-575 (AHD-575) method of haemoglobin estimation, blood is diluted using an alkaline solvent sodium hydroxide comprising of a non-ionic detergent Triton X100. All haemoglobin derivatives are converted into

a steady end-product, alkaline hematin D-575, whose absorption maximum is at $X = 575 \text{ nm}$ ^(26,27) Alkaline hematin methods in which blood is diluted using alkaline solutions without detergent have been described; however, in these methods, certain forms of haemoglobin are resistant to alkali denaturation, notably fetal haemoglobin (HbF).⁽²⁴⁾

EXPECTED RESULTS can be calculated by the following formula

The absorbances obtained were converted into haemoglobin concentrations (g/dl) using the following relationship:

Haemoglobin concentration of test sample = $A_t / A_s \times$ concentration of standard

Where,

A_t = absorbance of the test sample and

A_s = absorbance of the standard.⁽²⁶⁾

NON- INVASIVE METHOD

Pulse oximetry is a form of Spectrophotometric method which is ubiquitously used for monitoring oxygenation in critical care setting. Pulse oximetry measures oxygen saturation by illuminating the skin and measuring changes in light absorption of oxygenated and deoxygenated blood using two light emitting diodes that absorb light at two different wavelengths: 660 nm (red) and 940nm (infrared). At the defined wavelengths the ratio of absorbance is calculated and calibrated against direct measurements of arterial oxygen saturation (SaO_2) to establish the pulse oximeter's measure of arterial saturation (SpO_2).^[28] The light passing as of the LED through the finger is calculated by a photo detector positioned contradictory to LED. The

PHOTOPLETHYSMOGRAPHY (IMAGE PROCESSING)

Saif Al Zahir and Han Donker disclose the non invasive method to estimate hemoglobin level in the blood which utilizes the approach to estimate hemoglobin using colour analysis.^[33] The proposed method of haemoglobin detection is to get signal image scanning using photoplethysmo-graphic technique, when extremity is placed near proximity to Near Infrared(NIR).^[34] The model fetches the color information from the image of the fingertip under the standard condition. The method in use creates two images for each person one without applying pressure on the fingertip in the assistance of rubber band which directs the deposition of blood at the fingertip.^[13]

The block diagram of the system for the measurement of haemoglobin, from signal to image scanning using photoplethysmo-graphic (ppg), is shown in Figure 1. The clip-on sensor to be affixed on an extremity (say the finger) of a patient being tested concerns NIR on one side and a photo diode on the other side. NIR emit infrared rays which are captured by photonic detector. The signal from the device is recorded with Sigview software and then the recorded signal was processed using MatLab coding. Then signal to image conversion was done finally. A regressive logical thinking will be taken for the received values to determine the haemoglobin amount. In order to determine the haemoglobin content, variance and mean values are compared and output was plotted in PPG graph.^[34]

software determines the disparity between absorption during diastole and systole at both wavelengths.^[29] In general pulsatile signal's amplitude is approximately 1% of the d.c. level. The measurement of methemoglobin (MetHb), Carboxyhemoglobin (COHb) & total Hemoglobin can be done non-invasively using multi-wavelength pulse oximetry technique that follows theory of "The new Rainbow Technology" developed by Masimo Corp.^[29]

Occlusion spectroscopy

A peculiar apparatus named NBM200 by Orsense Ltd., Nez Ziona, Israel has been designed and assembled for in vivo occlusion spectroscopy (Fig.1). It comprise of three major units: the pressurizing assembly (occluder), the measuring unit, and the control unit.^[30] This new method is used to estimate Haemoglobin levels has been developed, using the principle of occlusion spectroscopy, which let off the donor finger stick and makes the process more easy,^[31] The system consist a ring-shaped re-usable sensor/probe, arranged for preference around the thumb, and a small portable monitor with a graphical display, comprise a microprocessor that compute the result and shows it on the screen. The probe is composed of eight diodes generating bands of light in the red (610 nm) and infrared (935 nm) fields, in addition to that a photocell receiving the light after it has passed through the patient's skin and circulation. At those wavelengths Hb absorbs light. The photocell records the light penetrating the thumb and converts the intensity of the light spectrum into an electronic signal which is transmitted to the device to be processed and the result displayed. A measuring is taken on five consecutive occlusion cycles.^[32]

DISCUSSION

For investigating various anemic or other blood related cases hemoglobin estimation plays a vital role. In rural areas for hemoglobin levels a method should be used which is less expensive and most reliable. Different invasive and non-invasive methods were analyzed and was interpreted that Hemocue and cyanmethemoglobin methods are widely used for invasive methods.^[11] Previously it was believed that Hemoglobin Color Scale has a sensitivity and specificity of 95% and 99.5% respectively.^[35] But a recent study in 2014 by WHO considered it to be less reliable method.^[36] Sahli's method is still routinely used in hospital settings but has inbuilt disadvantages mentioned in below table.^[37] Hemocue is yet another method for hemoglobin estimation whose sensitivity and specificity is 94.1% and 95.2%.^[38] Cyanmethemoglobin is referred to as gold standard invasive method and in India approximately 70% of laboratories still use direct cyanmethemoglobin method (HiCN) for hemoglobin estimation especially in rural areas.^[20] The non invasive methods such as pulse oximetry, occlusion spectroscopy and photoplethysmography have come in to picture for hemoglobin estimation recently which overcomes the pain faced by patients during tests. The study for the non invasive is yet to go through many challenges which includes the expenses initially where these methods cannot be utilized for rural areas. The advantages and disadvantages has been shown in the Table 2.

TABLE 2: ADVANTAGES AND DISADVANTAGES OF VARIOUS METHODS

METHODS	ADVANTAGES	DISADVANTAGES
INVASIVE METHODS		
1. Hemoglobin Colour Scale	Portable, No requisite of electricity & Inexpensive	Inter-observer variability
2. Sahli's method	Easy & Economical	Imprecise, Inter-observer unpredictability, Exercise of manual pipetting prone to inaccuracy, Color produced is unstable & No international standard
3. Hemocue method	Quick- immediate result, Convenient, Accurate, Non toxic, Battery Operated, Reliable & Easy to employ in poor settings where skills as well as resources are inadequate	Use disposable cuvette which makes it expensive
4. Tallquist method	Inexpensive, Rapid and Simple, Portable, Electricity not required & No reagents required	Results influenced by lighting, Temperature, Humidity & Size and thickness of blood spot
5. CYANMETHEMOGLOBIN METHOD	error due to subjective visual matching is avoided as spectrophotometer is used, provides accurate and precise results, precise and reliable & all hemoglobin can be estimated (except sulfhemoglobin).	Potassium cyanide is photosensitive and toxic, expensive, Turbidity can deviate the Estimate
6. alkaline hematin method AHD-575	AHD-triton reagent is not toxic or photosensitive; Reaction is very fast in non-cyanide methods & cheap.	method does not work when non-ionic reagents replaced by cationic reagents
NON INVASIVE METHODS		
7. Pulse oximetry method	No need to withdraw blood from patient body & Quick and easy to measure the reading	Affected by light interference & Error can occur in oxygen saturation reading
8. NBM-200	Donor Comfort, No Pain, No Blood, Easy & Safe to Use, Infection Free, Hands Free, Immediate Results batteries required No Disposables	Expensive, Screening Should Occur With Meticulous Attention

CONCLUSION

The study is done to provide the information regarding various Hemoglobin estimation techniques and rising advances in it. Also data generated for large number of anemic patients' in most of the developing countries for determining Hemoglobin level and to overcome

Hemoglobin complications. Future research can be done on developing more rapid and advance economic method for hemoglobin estimation.

REFERENCES:

1. Parveen.S et al. Preparation of Stable and Highly Active Hemoglobin by Using Antiglycosylspecific Hemoglobin Polyclonal Antibodies, April 2013.
2. Pranav Kumar, Usha mina: Biotechnology and a problem approach, 2014.
3. Celia Henry Arnaud, Paper device measures hematocrit Inexpensive red blood cell assay could be used in limited-resource settings, September 19, 2016.
4. Anemia Detection Methods in Low-Resource Settings: A Manual for Health Workers, December 1997.
5. Mayang Sari, Saskia de Pee et al. Estimating the prevalence of Anemia: a comparison of three methods, 2011.
6. The global prevalence of anaemia in world health organisation, 2011.
7. Kawaljit Kaur, Anaemia 'a silent killer' among women in India: Present scenario, Scholar Research Library, 2014.
8. Bharati S, Pal M, Som S, Bharati P. Temporal Trend of Anemia Among Reproductive-Aged Women in India. Asia Pac J Public Health. 2012.
9. http://www.path.org/publications/files/TS_anemia_guide_health_workers.pdf
10. <http://community.boredofstudies.org/422/psychology/164474/whats-difference-between-invasive-non-invasive-brain-research-methods.html>
11. Tanushree Srivastava, Himanshu Negandhi, Sutapa B Neogi, Jyoti Sharma and Renu Saxena Methods for Hemoglobin Estimation: A Review of "What Works", November 3, 2014.

12. Rajendra Chaudhary, Anju Dubey, Techniques used for the screening of hemoglobin levels in blood donors: current insights and future directions. 3 July, 2017.
13. Julia Critchley Imelda Bates Haemoglobin colour scale for anemia diagnosis where there is no laboratory: a systematic review *International Journal of Epidemiology*, Volume 34, Issue 6, Pages 1425–1434, 1 December 2005.
14. Stone JE, Simmons WK, Jutsum PJ, Gurney JM. An evaluation of methods of screening for Anaemia. *Bull World Health Organ*, 1984.
15. Elwood PC, Jacobs A. Haemoglobin estimation: a comparison of different techniques. *Br Med J*, 1966.
16. Paolo Barduagni, Ahmed Saad Ahmed, Filippo Curtale, Mohamed Raafat, Layla Soliman, Performance of Sahli and colour scale methods in diagnosing Anaemia among school children in low prevalence areas, June 27, 2003.
17. Hayedeh J. Shahshahani, Nahid Meraat, et al., Evaluation of the validity of a rapid method for measuring high and low haemoglobin levels in whole blood donors, July 2013.
18. Bernard Nkrumah, Samuel Blay Nguah, et al. Hemoglobin estimation by the HemoCue® portable hemoglobin photometer in a resource poor, 21 April, 2011.
19. <http://www.appn.net.au/Data/Sites/1/appn/02implementation/technicalresources/haematology/hemocuehb201methodandsamplecollection.pdf>
20. Shah VB, Shah BS, Puranik GV. Evaluation of non cyanide methods for hemoglobin estimation. *Indian J Pathol Microbiol.*, 2011.
21. Professor Dr. Riaz Ahmad Bhutta, Dr. Naheed Afroz Syed, Dr. Asad Ahmad, Dr. Shehpar Khan, M.D. Haemoglobin - Part 4 - Haemoglobin Solution (Drabkin's Solution) Preparation.
22. Dayyal Dg. Cyanmethemoglobin (hemoglobin-cyanide) method for estimation of hemoglobin, *Cell Biology*, 2016.
23. Recommendations for reference method for haemoglobinometry in human blood and specifications for international haemoglobin cyanide standard, *International Council for Standardisation in Haematology: Expert Panel on Haemoglobinometry*, 1995.
24. J. W. CLEGG, M.R.C.S., L.R.C.P., D.C.P. Lond. E. J. KING, M.A., Ph.D., Estimation of haemoglobin by the alkaline haematin method, *British Medical Journal*, 1942.
25. Dr. J. W. Clegg, Alkaline Haematin Method for Haemoglobin Determination, *BRITISH MEDICAL JOURNAL*, 1947.
26. O.E. Lema,¹ J.Y. Carter,² P.A. Arube,³ C.G. Munafu,³ M.W. Wangai,³ & P.H. Rees⁴, Evaluation of the alkaline haematin D-575 method for haemoglobin estimation in east Africa, *WHO Bulletin OMS*. Vol 72, 1994.
27. Zander R, Lang W, Wolf HU. Alkaline haematin D-575, a new tool for the determination of haemoglobin as an alternative to the cyanhaemoglobin method. I. Description of the method. *Clinica Chimica Acta*, 1984.
28. Amal Jubran: Pulse oximetry, July 16, 2015
29. Jared Stephen Ruckman B.S., Kettering University, a comparative study of total hemoglobin measurement technology: noninvasive pulse co-oximetry and conventional methods, 2008.
30. Llya Finea, Boris Fikhtea, Leonid D. Shvartsman', Occlusion spectroscopy as a new paradigm for noninvasive blood measurements, *Proceedings of SPIE - The International Society for Optical Engineering* · June 2001.
31. Márcio Pinto, et al. The new noninvasive occlusion spectroscopy hemoglobin measurement method: a reliable and easy anemia screening test for blood donors, *TRANSFUSION* Volume 53, April 2013.
32. Pasquale Paolo, Pagliaro, et al. A non-invasive strategy for haemoglobin screening of blood donors, *Blood Transfus*, 2014.
33. Vinit Kharkar, VRRatnaparkhe; Hemoglobin Estimation Methods: A Review of Clinical, Sensor & Image Processing Methods, *International Journal of Engineering and Technical Research* 2(1), February 2013.
34. A. Mohamed Abbas, et al., Haemoglobin Detection in Blood by Signal to Image Scanning using Photo-Plethysmo-Graphic-Technique (PPG), *Indian Journal of Science and Technology*, Vol 9(1), January 2016.
35. Haemoglobin color scale practical answer to a vital need Department of blood safety and Clinical Technology. World Health Organization, Geneva: WHO, 2001
36. Darshana LG, Uluwaduge DI. Validation of the WHO Hemoglobin Color Scale Method. *Anemia*. 2014.
37. Balasubramaniam P, Malathi A. Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. *J Postgrad Med*. 38:8-9, 1992.
38. Akhtar K, Sherwani RK, Rahman K, Hasan J, Shahid M. HemoCue photometer: a better alternative of hemoglobin estimation in blood donors *Indian J Hematol Blood Transfus.*; 24:170-172, 2008.