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Original Research Article

# Comparative Study of Different Haemoglobin Estimation Methods

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**Conflict of interest: Nil**

**Abstract:**

**Aim:** The aim of the present study was to assess the different haemoglobin estimation methods.

**Methods:** This study was conducted on blood samples obtained in 2 ml blood in K3 EDTA vacutainer from various indoor wards and outdoor patient departments. 100 adult patients sent for Hb estimation from outpatient clinics and wards were included in the study.

**Results:** The repeatability standard deviations for Sahli's method, Drabkin's method, and the cell counter were found to be 0.68 g/dl, 0.42 g/dl, and 0.18 g/dl, respectively. Upon comparing Sahli's method with Drabkin's method and the cell counter, a statistical analysis revealed a p-value of less than 0.0001, indicating a significant difference between the two methods. Conversely, Drabkin's method was found to be comparable to the cell counter, as evidenced by a p-value greater than 0.05. The analysis yielded a mean difference of 0.455, which was found to be statistically significant with a p-value of less than 0.001. A statistically significant disparity was observed in the average measurements obtained from the colorimeter and the 5 part method ($p<0.001$), despite the presence of a significant correlation between these two techniques.

**Conclusion:** Sahli's method although cheap and easy, is inaccurate and has subjective bias. So it can be used for screening purpose, but not for diagnosis and follow up of anaemia, Haemoglobin measurement by Drabkin's method is very cost effective and it is as efficient as cell counter. It is especially useful in fund deprived areas and where only haemoglobin value is required. Cell counter although highly accurate and versatile, requires good equipment, quality control, laboratory setup and trained personnel. So it should be preferably used when complete blood count is required. The result showed a mean difference of 0.455 and with significant p-value of <0.001. A significant difference was found in the mean values of colorimeter and 5 part ($p<0.001$) despite a significant correlation between these methods.

**Keywords:** Anaemia, Automated haematology analyser, Haemoglobin estimation, Drabkin's cyanmetheamoglobin method, Sahli's method



## Introduction

Hemoglobin (Hb) is a porphyrin-iron protein compound that transports oxygen from the lungs to the body tissues where it is utilized for energy metabolism.[1-3] Hemoglobin estimation is of prime importance in medical investigations. The diagnosis of anemia is an important aspect in the practice of hematology.[4] The grading of anemia is based upon serum hemoglobin levels (Hb) as per the World

Health Organization (WHO) definitions.[5] Haemoglobin has multiple functions: transport of oxygen from the lungs to the tissues, to facilitate oxidative phosphorylation in the mitochondria, carriage of carbon dioxide from the tissues to the lungs as carbaminohaemoglobin, buffering of hydrogen ions formed in the erythrocyte from the conversion of carbon dioxide into bicarbonate, nitric oxide metabolism.[6]

Hemoglobin estimation, usually measured on venous blood or capillary blood and sometimes in clinical situations in arterial blood, is the most frequent laboratory investigation requested.[7] For accuracy and reliability of the measurement, both sample collection and analysis technique are critical.[8] Hemoglobin measured through different sources do show variability in values obtained. The reasons are several and include the instrument variability, type of blood samples and certain other factors.[9] Reference ranges for HGB concentration (according to WHO definition) are considered as 12–16mg/dL for women and 13–18mg/dL for men.[10] Different methods utilized for Hb estimation include acid haematin, photometric cyanmethemoglobin estimation and automated estimation with the help of counters.[11] The standard method for measuring hemoglobin (Hb) in human blood is the well-recognized HiCN method as recommended by the World Health Organization (WHO).[12]

Anaemia is a major public health problem in developing countries. One of the reliable indicators for assessing anaemia in population is the determination of haemoglobin concentration.[13] Direct cyanmethaemoglobin method[14] is the most widely used and recommended method by the International Committee for Standardization in Haematology for quantitative estimation of haemoglobin. It involves formation of a stable compound, cyanmethaemoglobin and is relatively a simple and cost-effective method.[15] This direct method involves estimation of haemoglobin in whole blood samples using spectrophotometer and hence requires laboratory facility which limits its applicability in large-scale surveys, as transportation of whole blood in vials from long distances to central laboratories for analysis may not be feasible. Thus, indirect cyanmethaemoglobin (filter paper) method which is based on the same principle but involves spotting of blood on filter paper is often used for the estimation of haemoglobin in population since it is simple and produces reliable results.[16]

The aim of the present study was to assess the different haemoglobin estimation methods.

## Materials and Methods

This study was conducted on blood samples obtained in 2 ml blood in K3 EDTA vacutainer from various indoor wards and outdoor patient Department of Pathology, Krishna Mohan Medical college and Hospital, Mathura, UP , India from January 2018 to December 2018. 100 adult patients sent for Hb estimation from outpatient clinics and wards were included in the study.

Samples were taken randomly and their Hb was measured by Sahli's Haemoglobinometer, Cyanmethemoglobin Method &7-part haematology analyzer.

· Hb estimation by Sahli' shaemoglobinometer (acid haematin method): Blood is mixed with N/10 HCL, resulting in the conversion of Hb to acid hematin, which was brown in colour. The solution was diluted till its colour matches with the brown coloured glass of the comparator box. The concentration of Hb was read directly.

Hb estimation by Cyanmethemoglobin Method: Blood is diluted in a solution containing potassium cyanide and alkaline potassium ferricyanide. The latter converts Hb to methaemoglobin which is converted to cyanmethemoglobin (HiCN) by

potassium cyanide. The absorbance of the solution is then measured in a spectrophotometer at a wavelength of 540 nm.

If p-value obtained from t-test is >0.05, it means that there is no significant difference between values obtained from both methods and both methods are comparable. While if p value is <0.05, it shows that there is significant difference between results of both methods and they are not comparable.

**Results**

**Table 1: Comparison of Sahli's method, Drabkin's method & Cell counter**

| | Sahli's method | Drabkin's method | Cell counter |
|---|---|---|---|
| Range of Hb values | 12.6-14.2 g/dl | 12.7-14 g/dl | 13-13.7 g/dl |
| Mean | 13.27 g/dl | 13.40 g/dl | 13.25 g/dl |
| Repeatability standard deviation | 0.68 g/dl | 0.42 g/dl | 0.18 g/dl |
| Method prediction range | 12.8-13.7 g/dl | 13.05-13.6 g/dl | 13.07-13.34 g/dl |

Repeatability standard deviations of Sahli's method, Drabkin's method and cell counter respectively were 0.68 g/dl, 0.42 g/dl and 0.18 g/dl. When comparing Sahli's method with Drabkin's method and cell counter, we found p value of <0.0001, suggesting significant difference between two methods whereas Drabkin's method was found to be comparable with cell counter with p value of >0.05.

**Table 2: Mean values of Hb obtained using colorimeter and 5 part**

| Method | Mean | SD | Mean Diff. | t-value | p-value |
|---|---|---|---|---|---|
| Colorimeter | 14.260 | 2.625 | 0.455 | 3.654 | <0.001 |
| 5 part | 11.725 | 2.314 | | | |

This showed a mean difference of 0.455 and with significant p-value of <0.001. A significant difference was found in the mean values of colorimeter and 5 part (p<0.001) despite a significant correlation between these methods.

**Discussion**

There are many methods available for hemoglobin (hb) estimation. In developing countries we are encountered with fund crunch and overcrowded hospitals, so we must design the laboratory method in a way that it should be fast, cost effective and as accurate and reliable as possible. Sahli's method, CuSo4 method and Drabkin's method are very cost effective. Mayang et al, in their study concluded that haemoglobin Concentration should be assessed with the direct cyanmethemoglobin method, the gold standard.[17] The photometer is easy to transport because it is small and light; it is battery operated and gives consistent results.[18 ]

Repeatability standard deviations of Sahli's method, Drabkin's method and cell counter respectively were 0.68 g/dl, 0.42 g/dl and 0.18 g/dl. When comparing Sahli's method with Drabkin's method and cell counter, we found p value of <0.0001, suggesting significant difference between two methods whereas Drabkin's method was found to be comparable with cell counter with p value of >0.05. The result showed a mean difference of 0.455 and with significant p-value of <0.001. A significant difference was found in the mean values of colorimeter and 5 part (p<0.001) despite a significant correlation between these methods. When compared to other studies, Prashant et al 2013[19] found that Sahli's method underestimated the hemoglobin by 1.12gm/dl in venous blood and p value <0.01 between Sahli's method and cyanmethemoglobin method. In a study by

P Balasubramanian & A Malathi[20], 1.13g/dl of difference was found between sahli's method and HiCN method. However a study done by MadhuraWasnik et al using 51 subjects did not find any significant difference between results obtained from Sahli's and HiCN methods (p= 0.954 i.e. >0.05).[21]

Study by Bezarra da silva et al comparing Sahli's method with cell counter did not find any significant difference between the two methods.[22] They found mean difference of 0.2267g/dl. An interesting study done by Dr. MP Brundha and S Priyadharshini, 2019 compared Sahli's two time average and three time average methods with automated cell counter. In this study they found Sahli's three-time average method to be most comparable with autoanalyzer with mean difference of 0.9g/dl.[23]

## Conclusion

Sahli's method although cheap and easy, is inaccurate and has subjective bias. So it can be used for screening purpose, but not for diagnosis and follow up of anaemia, Haemoglobin measurement by Drabkin's method is very cost effective and it is as efficient as cell counter. It is especially useful in fund deprived areas and where only haemoglobin value is required. Cell counter although highly accurate and versatile, requires good equipment, quality control, laboratory setup and trained personnel. So it should be preferably used when complete blood count is required.

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