



## Evaluation of three hemoglobin estimation methods for screening blood donors: An experience in a south Indian Hospital

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### Abstract

**Background & Aims:** Hemoglobin (Hb) estimation is one of the mandatory tests should be carried out on all blood donors before accepting for blood donation to prevent collecting blood samples from anemic donors. Presently various methods are available for pre-donation screening of donor hemoglobin. Total Hb concentration is calculated from measured absorbance at multiple wavelengths. In this study we reviewed the performance of Compolab TS with automated SYSMEX XN 1000i hematology analyzer and with standard cyanmethemoglobin method.

**Materials & Methods:** This is a prospective study carried out in the department of Transfusion Medicine, Narayana Medical College and Hospital, India. A total of 466 blood donors were screened and evaluated for hemoglobin estimation by three different methods. Comparison and their test performances were evaluated by analysis of coefficients of variation (CV), linear regression, and mean differences, using SPSS Software version 20.0.

**Results:** There was no difference between the mean Hb determinations. The CVs for three methods Compolab TS, 0Coulter, and Cyanmethemoglobin methods were 0.71%, 0.44%, and 0.85%, respectively. Compolab TS and Coulter showed best agreement and the Cyanmethemoglobin showed lower determination rate compared to both the Coulter and Compolab TS tests. However, pair methods involving Cyanmethemoglobin seem to narrower limits of agreement ( $\pm 0.1636$  and  $\pm 0.2152$ ) than Compolab TS and Coulter combination ( $\pm 0.2730$ ).

**Conclusion:** Estimation of Hb by Compolab TS is rapid, simple and easy and showed good agreement with the standard methods.

**Keywords:** Compolab TS, Hb Estimation, Screening, Blood Donors

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## Introduction

Hemoglobin (Hb) estimation is one of the mandatory tests should be carried out on all blood donors before accepting for blood donation to prevent collecting blood from anemic donors. In India, Hb estimation is mandatory according to Drugs and Cosmetics Act 1940. According to this Act, the blood donors should have a minimum Hb of 12.5 g/dL and hematocrit of 38% to be accepted for blood donation (1). Presently various methods are available for pre-donation screening of donor hemoglobin. In India, simple and rapid tests like gravimetric copper sulfate method is using for Hb testing. Though a cheap and easy method, it does not provide an acceptable degree of accuracy (2,3). Another method is based on photometric detection of a stable complex after reaction with reagents in Drabkin's solution. Nowadays most of the blood banks move towards more rapid and simple methods for Hb estimation. Compolab TS (Fresenius Kabi, Diaspect Medical GmbH, Germany) is an easy method which is based on a broad spectrum measurement of Hb in whole blood. Reagent free cuvettes (by Diaspect Medical GmbH) are used in this instrument. The total Hb concentration is calculated from measured absorbance at multiple wavelengths. In this study we reviewed the performance of Compolab TS in comparison with coulter and standard cyanmethemoglobin method.

## Materials & Methods

This is a prospective study carried out in the Department of Transfusion Medicine in Narayana Medical College and Hospital, Nellore in South India after obtaining permission from the institute ethics committee. A total of 466 blood donors were screened and evaluated for hemoglobin estimation by three different methods namely Compolab TS (Fresenius Kabi Company), Drabkin's, and SYSMEX XN 1000i hematology analyzer. After obtaining a detailed health history and medical examination, 2 ml of blood was collected in a dipotassium ethylene diamine tetra acetic

acid (K2 EDTA) tube from each donor. The same sample was tested for hemoglobin by the above 3 methods within a short interval of 10-15 minutes in order to avoid changes in blood components resulted from delay in processing. In Compolab TS a drop of whole blood was placed in a reagent free cuvette and its absorbance was measured against multiple wavelengths. In cyanmethemoglobin method, 0.5 ml was mixed with 5 ml of Drabkin's reagent. Its absorbance was measured against 540 nm. In automated SYSMEX XN 1000i hematology analyzer, the whole blood sample's Hb is estimated by the same principle as that of cyanmethemoglobin method.

Statistical Analysis: The mean, range, standard deviation (SD) and coefficient of variation (CV) were calculated for each method separately. Following this initial assessment of central tendency, the test methods were paired with each other as the following three combinations: Compolab TS/cyanmethemoglobin methods, Compolab TS/cell counter methods, and cell counter/cyanmethemoglobin methods. The coefficient of correlation (r) was calculated for each pair of methods by using linear regression analysis. After tabulating these 'r' values, Bland and Altman approach was used finally. By this method, the mean of difference in estimating Hb for each of the above pairs of methods was calculated and its statistical significance was assessed by paired t-test. P value < 0.001 is considered as significant. Statistics analyzed using SPSS Software version 20.0.

## Results

In the initial step we assessed the mean, range, and standard deviation for all the three methods. Among these, the lowest mean of 14.95 g/dl was noted for cell counter along with the least range value of 5.10 (12.5 g/dL – 18.0 g/dL). The CV for the Compolab TS, Cell Counter, and Cyanmethemoglobin methods were 0.08%, 0.07%, and 0.08%, respectively as shown in [Table 1](#).

**Table 1:** Measurements of central tendency and variation for the three methods of Hb determination

Method	N	Range	Mean	SD	Median
Compolab TS	466	7.50	15.17	1.19	15.2
Cell Counter	466	5.50	14.95	1.08	15.0
Cyanmethemoglobin	466	7.40	15.09	1.28	15.0

The correlation coefficient (r) was assessed for pairs of these methods by performing the linear regression analysis. Among the 3 pairs, the Compolab TS and cell counter showed the highest correlation of

0.89. The correlation between the Compolab TS and Cyanmethemoglobin method is 0.77. The Cyanmethemoglobin method and cell counter showed the least correlation of 0.74 ([Table 2](#)).

**Table 2:** Linear Regression Analysis between pairs of the three methods used for Hb determination (n=466)

Reference Method	Testing Method	Coefficient		
		Correlation	Slope	Y-Intercept
Compolab TS	Cell Counter	0.891	0.979	0.542
Compolab TS	Cyanmethemoglobin	0.770	0.721	4.30
Cyanmethemoglobin	Cell Counter	0.741	0.87	2.08

In order to evaluate the agreement between the three different methods of Hb estimation, Bland and

Altman methods were used. The results of using Bland and Altman approach for the three pairs of tests in our study are shown in [Table 3](#).

**Table 3:** Use of the Bland and Altman approach for determining the agreement between pairs of the three methods used for Hb measurement

Reference Method	Testing Method	Coefficient		
		Mean of the Difference	Limits of Agreement	P value for The difference
Compolab TS	Cell Counter	0.2238	± 0.2730	< 0.0001
Compolab TS	Cyanmethemoglobin	0.0873	± 0.1636	0.025
Cell Counter	Cyanmethemoglobin	-0.1365	± 0.2152	0.001

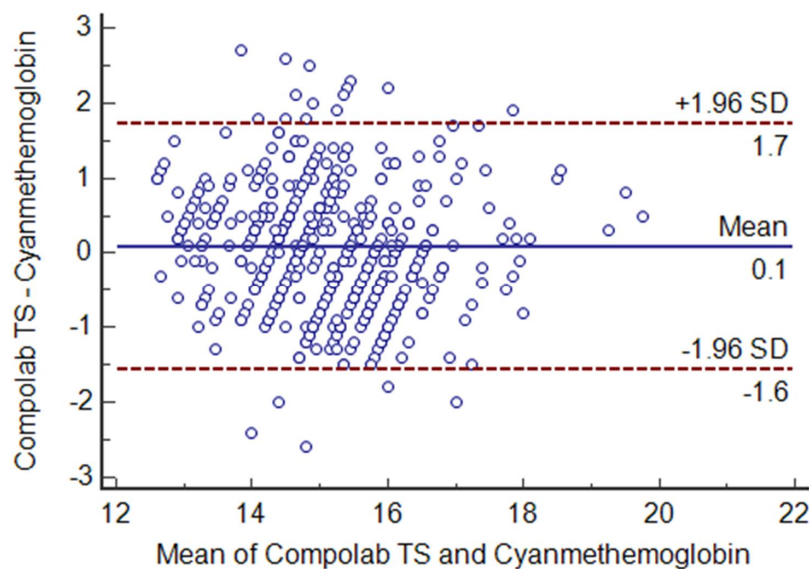
It must be noted that the means of differences were statistically zero for the pairs of tests having Cyanmethemoglobin method i.e., Compolab TS/ Cyanmethemoglobin method and cell counter/ Cyanmethemoglobin method were in good agreement with each other as their means of difference is not statistically significant. The test pair of Compolab

TS/Cell counter has a statistically significant means of difference ( $p < 0.0001$ ).

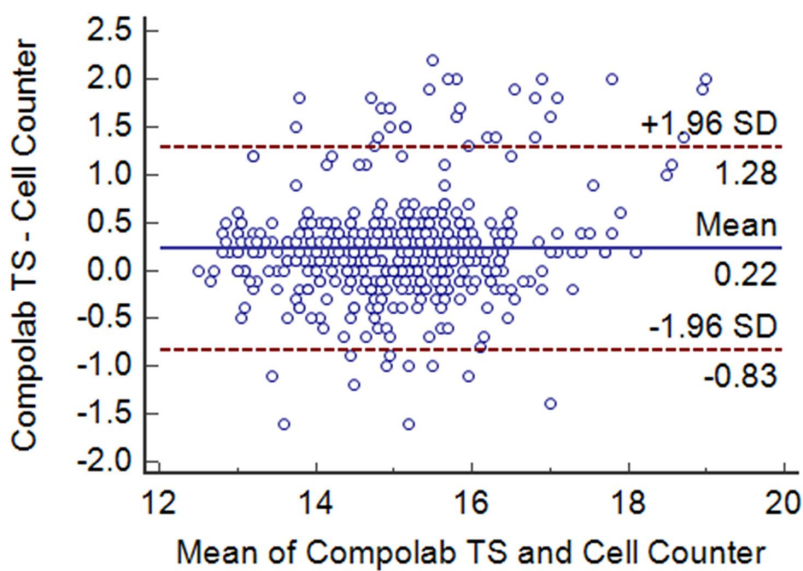
The pair of tests Compolab TS/ Cyanmethemoglobin method has the lowest limit of agreement i.e., 0.16 g/dL compared to Compolab TS/Cell counter and cell counter/ Cyanmethemoglobin methods (0.27 g/dL and 0.22 g/dL, respectively). These limits of agreement also reflect the dispersion of Hb

estimation data around the mean of difference for the

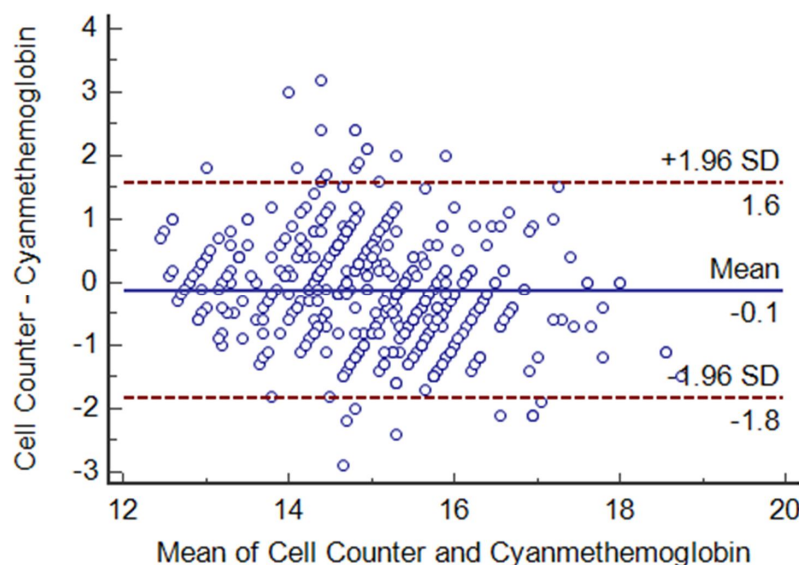
various pairs of tests as shown in Figure 1, 2, and 3.



**Fig. 1.** scatter plot showing the dispersion of values around the mean for Compolab TS and Cyanmethemoglobin method



**Fig. 2.** scatter plot showing the dispersion of values around the mean for Compolab TS and Cell Counter method



**Fig. 3.** scatter plot showing the dispersion of values around the mean for Cyanmethemoglobin method and cell counter method

The most important parameter in deciding the need for blood transfusion in a massive blood loss patient is blood Hb concentration (4). One of the routine screening tests that are performed prior to blood donation on an individual intending to donate blood is estimation of blood Hb. This single most important step helps in protecting the health of both the recipient and the donor. By performing this test, one ensures that the recipients receive the blood that meets his/her requirement and the donor also will not be affected by anemia by losing blood. In India, as per the Drugs and Cosmetics Act 1940, a donor is accepted to donate blood only when he/she has at least a Hb of 12.5 g/dL (5).

The hospitals and blood banks have been using many invasive and non-invasive methods of Hb estimation for screening the donors prior to blood donation (6). For any method to be considered ideal for screening Hb, besides from being inexpensive and portable, it should also be friendly for both donor and recipient by providing accurate results. The more accurate the results of Hb estimation are, the lesser the chances of deferral of a donor due to falsely low Hb

levels, which also aids in protecting the donors having a low Hb (7).

Drabkin's method that is considered as gold standard method for Hb estimation uses cyanide. In this method when the blood mixes with the Drabkin's reagent, the potassium cyanide and ferricyanide that are present in the reagent converts all forms of Hb in the blood to cyanmethemoglobin. Hb is estimated by measuring the absorbance of cyanmethemoglobin at 540 nm against a standard solution in a spectrometer. Apart from cyanmethemoglobin, hemicyanide (HiCN) is also formed (8). Though this method measures all forms of Hb in a blood sample, in order to evade the toxic effects of cyanide on environment, non-cyanide methods of Hb estimation are the need of the hour. A study comparing the non-cyanide and cyanide methods of Hb estimation had been conducted in Mumbai. In addition to being cyanide free, the results showed a very good correlation between these two groups of tests. The non-cyanide tests were also cost effective in comparison with the cyanide tests (9).

The automated hematology cell counters make use of Coulter principle and perform Hb estimation.

Nowadays, cell counters use non-cyanide reagents for this purpose. Although these automated hematology analyzers results have a high reliability and accuracy, they are expensive and not easily portable. It is not always feasible to use these cell counters for Hb estimation, especially in developing countries (10).

In our study, we compared Compolab TS, a very simple, easy, and portable method of Hb estimation with the gold standard cyanmethemoglobin method as well as with accurate cell counter method. A cuvette containing a drop of whole blood is placed in the Compolab TS and Hb estimation is done. The Compolab TS has the highest range and means of Hb estimation, i.e., 7.5 g/dL for range and 15.17 g/dL for mean Hb concentration. The CV of Compolab TS was 0.08%, which was same as that of Cyanmethemoglobin method. On performing linear regression analysis, the correlation coefficient was highest for the Compolab TS and cell counter combination followed by Compolab TS and Cyanmethemoglobin methods i.e., were 0.89 and 0.77, respectively. The least coefficient of correlation was 0.74 and is observed between cell counter and cyanmethemoglobin methods. This shows that the cyanmethemoglobin method which is considered as gold standard is more in agreement with the Compolab TS than the cell counter.

In our study, the Bland and Altman approach was used to assess the means of differences in Hb estimation and its statistical significance between the pairs of tests. According to this method, if two paired methods are in agreement with each other, their means of differences will be statistically zero. This method has the advantage of establishing the limit of agreement as well as the graphical visualization of the dispersion of the various differences of Hb levels (11). Current study observed that the means of differences was not statistically significant for the pairs of tests using cyanmethemoglobin method as one of the methods in the pair. Among the combinations, also the pair of Compolab TS and cyanmethemoglobin had the least mean of difference of 0.09 compared to that of cell counter and cyanmethemoglobin methods pair, i.e., 0.14. On evaluating the p-value, they were found to be

statistically significant. We observed that Compolab TS method is more in agreement with the gold standard cyanmethemoglobin method than the automated hematology analyzer.

The limit of agreement for the difference in values of Hb was least between the Compolab TS and cyanmethemoglobin methods, i.e., 0.16 g/dL. The difference was 0.22 g/dL for cell counter and cyanmethemoglobin methods whereas it is highest i.e., 0.27 g/dL for Compolab TS and automated hematology analyzer methods. Hence the Hb estimation by Compolab TS was statistically more in agreement with the gold standard method of Hb estimation i.e., cyanmethemoglobin method.

## Discussion

The advantages of using Compolab TS for Hb estimation are its simplicity, portability, reagent independency and its agreement with the cyanmethemoglobin method, which is considered as the gold standard method of Hb estimation. Being a reagent independent method, it poses no toxic effects to the environment.

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## Conflict of interest

The authors have no conflict of interest in this study.

## Ethical Statement

This study carried out in the Department of Transfusion Medicine in Narayana Medical College and Hospital, Nellore in South India after obtaining permission from the institute ethics committee.

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