



## COMPARATIVE STUDY OF HAEMOGLOBIN ESTIMATION BY USING SYSMEX XN550-L SERIES (5 PART) AUTOMATED ANALYZER AND CONVENTIONAL MANUAL TECHNIQUE IN A TERTIARY CARE HOSPITAL IN RURAL HARYANA

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### ABSTRACT

To compare the hemoglobin estimation by various methods (Sahli's acid hematin method, cyanmethemoglobin method and non-cyanide method of automated cell counter). This study included 100 cases of blood sample collected into ethylenediamine tetra-acetic acid (EDTA) from randomly selective patients for the analysis of hemoglobin by Manual and automated methods over a period of one year in the department of pathology at maharishi markandeshwar institute of medical sciences and research. The study cohort comprised mainly of females consisting of 55% while 45 % were males. Mean Haemoglobin obtained by Sahli's method, cyanmethemoglobin method and automated cell counter method was 13.0 gm/dl, 12.2 gm/dl and 12.5 gm/dl respectively. It was observed that 56% of patients had normal hemoglobin, whereas mild degree of anemia in 28{28%} cases, moderate degree of anemia in 14{14%} cases and severe degree of anemia in 02{02%} cases. There was significant difference between the Sahli's method and Cyanmethemoglobin method (P value = 0.0271) but there was no significant difference found between the cyanmethemoglobin method and automated cell counter method (P value = 0.4056). The present study showed there was no significant correlation between the cyanmethemoglobin method and automated cell counter method. There was a statistically significant difference between the Sahli's and cyanmethemoglobin method, there was a correlation. Sahli's method is inexpensive and easily available; therefore, it is beneficial in remote areas and resource constraint settings. Hence to conclude, the non-cyanide methods in automated cell counter can therefore easily replace cyanmethemoglobin method and moreover they do not have a bio-toxic hazard.

**KEYWORDS :** Hemoglobin, Sahli's Method, Cyanmethemoglobin method

### INTRODUCTION

Haemoglobin is an iron containing metalloprotein in RBCs (1). It is responsible for transportation of oxygen, carbon dioxide and protons between lungs and tissues. Haemoglobin below normal range for the age and sex of the person leads to 'Anemia'. It is caused by nutritional deficiencies commonly, also by acute and chronic inflammation, parasitic infestations and inherited or acquired disorders. Cyanmethemoglobin is gold standard method whereas, Sahli's method of haemoglobin estimation is relatively inexpensive, simple to use, does not require electricity & requires only small sample of blood. In developing country like India most common method used for haemoglobin estimation is the Sahli's method.(2) Photometric determination of haemoglobin cyanide (HiCN) is recommended as the reference method for haemoglobinometry in human blood by International Committee for Standardization in Hematology (ICSH standard 1995).(3) All other methods used in routine measurement are needed to be adjusted to obtain comparability with the cyanhaemoglobin method.(4) For laboratory practices it is needed to compare hemoglobin estimation by Sahli's acid haematin method and cyanide-free sodium lauryl sulphate method of the automated cell counter with the gold standard Drabkin's cyanmethemoglobin method.(5) Automated cell counter method uses sodium lauryl

sulphate for hemoglobin detection and is based on absorption photometric principle.(6) The present study was conducted to compare various methods of haemoglobin estimation and calculate the coefficient of variation.

### MATERIAL AND METHOD

This study was undertaken in the hematology section of department of pathology in M M Institute of medical science and research, Mullana, Ambala and included 100 cases of blood sample collected into ethylenediamine tetra-acetic acid (EDTA) from randomly selective patients for the analysis of hemoglobin by Manual and automated methods over a period of one year. Ethical clearance was obtained from hospital ethical committee. Hemoglobin levels were estimated and compared by three methods Sahli's, Drabkin's and automated method. The data was analyzed by using chi square test for statistical variations if any.(P value of <0.05 was considered statistically significant).

### RESULT

The present study was carried out on 100 randomly selective patients for comparison of hemoglobin by various manual methods and automated methods. Majority of cases were female (55%) while remaining were male (45%). Age wise data shows majority of the patients to be in 3<sup>rd</sup> decade followed by 4<sup>th</sup> and 5<sup>th</sup> decades respectively (Table I).

**Table I: Age Distribution of Patients in the study.**

AGE GROUP (YEARS)	NUMBER OF PATIENTS	PERCENTAGE (%)
0-10	06	6
11-20	06	6
21-30	28	28
31-40	17	17
41-50	16	16
51-60	15	15
61-70	12	12
TOTAL	100	100%

The anemia profiles in our study showed that in children (0-14 years), the normal homologized patients were seen in 62.5% of cases while mild, moderate and severe degree of anemia was seen in 25%, 12.5% and 0% of cases respectively. In adult females (>15 years), half of the patients had normal hemoglobin while mild degree of anemia was seen in 33%, moderate degree of anemia in 15% while remaining 2% had severe degree of anemia. In adult males (>15 years), the normal hemoglobin was seen in 70% of cases while mild, moderate and severe degree of anemia was seen in 17%, 10% and 3% respectively. (Table II)

**Table II: Distribution of patients according to severity of anemia.**

Haemoglobin	0-14 years of age (n=8)	15 years and above females (n=52)	15 years and above males (n=40)	Total patients (n=100)
Normal	05	26	28	56
Mild anemia	02	17	07	28
Moderate anemia	01	08	04	14
Severe anemia	0	01	01	02
Total	08	52	40	100

Standard deviation was calculated for the results and it came out as for cyanmethemoglobin method and automated cell counter to be  $13.0 \pm 2.54$  gm/dl,  $12.2 \pm 2.54$  gm/dl and  $12.5 \pm 2.55$  gm/dl respectively, which showed statistically significant correlation between Sahli's and Drabkin's method with p value 0.0271 but no significant correlation between Drabkin's and automated method. (Table III, Table IV)

**Table III: Mean  $\pm$  SD of haemoglobin results by Sahli's method and Drabkin's method**

PARAMETERS	SAHLI METHOD	DRABKIN METHOD	P value
Haemoglobin	$13.0 \pm 2.54$	$12.2 \pm 2.54$	0.0271

**Table IV: Mean  $\pm$  SD of haemoglobin results by automated method and Drabkin's method**

PARAMETERS	AUTOMATED METHOD	DRABKIN METHOD	P value
Haemoglobin	$12.5 \pm 2.5$	$12.2 \pm 2.5$	0.4056

## DISCUSSION

Out of total 100 randomly selected Patients majority of cases (28%) were between 20-30 years of age group. The male to female ratio was 9:11. The cyanmethaemoglobin method is the gold standard method but gradually it has been phased out as a routine method because the factors including the transportation of the reagents and the control of substances such as cyanides. Lewis study showed that some of the methods of haemoglobin estimation may have an error of  $\pm 20\%$  or more which when compounded with poor technique makes the method highly unreliable. (7) In our country, approximately 70% of the laboratories still use the manual HiCN method for Hb estimation in the rural areas. (8) In the present study, hemoglobin estimation by Sahli's method and cyanmethemoglobin method showed a statistically significant correlation. Similar findings were seen by Balasubramaniam P (9) but Agrawal V P (10) did not find any correlation.

For accuracy of Sahli's method the correction factor should be considered to ensure comparability of results with the reference method. In our study, Sahli's, cyanide method and automated method had a coefficient of variation of  $\pm 2.54$  g/dl,  $\pm 2.54$  and  $\pm 2.55$  respectively which was comparable to study done by Shah VB (11) who concluded that non-cyanide methods had a coefficient of variation 2.34%. In our study, Sahli's method had a higher value than cyanide method with mean difference of 0.8 gms/dl which was not in coherence with Patil P J (12) where sahli's method had lower values than Haemiglobincyanide method with mean difference of 1.1 gms/dl (95%CI: 0.92 to 1.26,  $p < 0.01$ ) in venous blood.

In present study, on comparing hemoglobin estimation by non-cyanide method in automated cell counter method to Drabkin's method there was no statistically significant difference. However, study done by Chandra D N (13) yielded significant findings. Our study also emphasized that the reagents used in non-cyanide methods are non-bio hazardous and do not affect the reliability of data determination. Similar to the study by Shah VB (11). Thus, non cyanide method can easily be incorporated in hemoglobinometers by using very minute quantities of reagents and test sample.

## CONCLUSION

The present study showed there was a statistically significant correlation between the sahli's method and cyanmethemoglobin method. The cyanmethemoglobin method is a gold standard method for hemoglobin estimation. (14) Hence the non-cyanide method in automated cell counter does not have a bio- toxic hazard. (15) However, there was no significant difference between the cyanmethemoglobin method and automated cell counter method as there was a good correlation. As Sahli's method is easily available in rural setup, this can be concluded that this method can be useful in remote areas and resource constraint settings.

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