

COMPREHENSIVE RESEARCH ON HB ESTIMATION BY SAHLI'S AND AUTOMATED METHODVarsha K¹, Ambika Vazhuthakat²¹Student, Department of Pathology, Government Medical College, Kozhikode, Kerala.²Assistant Professor, Department of Pathology, Government Medical College, Manjeri, Malappuram, Kerala.**ABSTRACT****BACKGROUND**

Deficiency of Haemoglobin leads to anaemia. There are different methods of Hb estimation. Of these Sahli's method is one of the most acceptable visual method while automated method is more sensitive and reliable method. This work is an attempt to compare the Sahli's method (manual method) and automated method of haemoglobin estimation and thereby to check reliability of Sahli's method.

MATERIALS AND METHODS

This was a case control study and was carried out in government medical college, Kozhikode. A total of 30 cases in March 2016 were taken for evaluation.

RESULTS

The results of all the 30 samples by Sahli's and automated method were entered in a table and analysis done by descriptive statistics.

CONCLUSION

Haemoglobin concentration determined by Sahli's method is as reliable as automated method and in a developing country like India Sahli's method can be used as a common method for the estimation of haemoglobin.

KEYWORDS

Hb-Haemoglobin, EDTA -Ethylene Diamine Tetra Acetic Acid, PHC-Public Health Centre.

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BACKGROUND

Haemoglobin an important component in human blood and deficiency of this leads to anaemia. Anaemic condition in women and children across the world is a serious concern and the severity is more in Indian population. Hence estimating the haemoglobin level of patient's blood is a preliminary and important investigation.¹

The world health organization has defined the lower limit of normal for haemoglobin concentration at sea level as 12.0 gm/dl in females and 13.0 gm/dl in males.² In general practice Hb estimation is a routine and frequently performed valuable primary sensitive screening test and it gives reliable prediction on the need to do full blood count.³

Haemoglobin content may be estimated by measurement of its colour, O₂ carrying capacity, specific gravity or iron content. The methods commonly used in many government PHC's are colour or light intensity matching techniques that measure inert pigments in blood with different degree of efficiency.⁴

Sahli's method is one of the most acceptable visual method, used since olden days.⁵ It is relatively inexpensive, simple to use, does not require electricity and require only small sample of blood. In developing country like India Sahli's Hb estimation is most common method used.⁶ Automated method is more sensitive and reliable method for estimation of haemoglobin but the facility is not available in many laboratories. This work is an attempt to compare the Sahli's method (manual method) and automated method of haemoglobin estimation and thereby to check reliability of Sahli's method.

Aims of study

1. To compare the value of haemoglobin in Sahli's (manual) method and automated method of haemoglobin estimation.
2. To check the reliability of Sahli's method of haemoglobin estimation.

MATERIALS AND METHODS

This study which is a case control study was done in pathology department, government medical college, Calicut in March 2016. Blood samples from 30 patients coming for blood R/E was collected in EDTA bottle and taken for analysis.

Inclusion Criteria- Samples of patients coming for R/E and without haematological malignancy were taken.

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Exclusion Criteria- Clotted samples, samples without proper label were excluded.

Collection of Specimens done by venous method. Blood was collected from the cubital fossa under strict aseptic precautions. Collected blood mixed with EDTA and haemoglobin content of the blood is estimated by both automated and Sahli's method.

Sahli's acid haematin method was introduced by Hermann Sahli who invented 'Sahli's haemoglobinometer', an instrument used for colorimetric determination of blood haemoglobin content. The principle of Sahli's acid haematin method is that Haemoglobin is converted to acid haematin by the action of HCl, which gives brown colour. Since brown is more easily matched by the human eye than red (the colour of haemoglobin), this method for testing haemoglobin is one of the most acceptable visual methods. Also, visual methods (especially Sahli's) are convenient and cost of estimation is less, they are usually practiced in haematology laboratories in clinical medicine and for performing practicals for the students in medical field. The acid haematin solution is further diluted until its colour matches exactly with that of the permanent standard of the comparator block. The haemoglobin values read directly from the calibration tube and expressed as gm%.

Sysmex Kx-21 Analyser was used to do automated analysis of haemoglobin. The Sysmex Kx-21, a new class of automated haematology analyser, is distinguished by its space saving design, operational simplicity and small size requirement. Automated method works on the principle of impedance. To analyse haemoglobin by automated methods, the Cyanmethemoglobin method or ox haemoglobin method was used as main stream. Cyanmethemoglobin method was recommended as the international standard method in 1966 by ICSH (International Committee for Standardization in Haematology). This method however, is so low in haemoglobin conversion rate that it cannot be said as an appropriate method in the automated process since in automated machines multi-sample processing has to be done. In addition, this method uses the reagent containing cyanide compound which is a poisonous substance and waste disposal is real problem; thus, it can hardly be called

an environment favourable method. In many hospitals, this method cannot be said to be suitable for a fully automated instrument where they have to handle a large no of samples and thereby waste material.

Non-cyanide haemoglobin analysis method rapidly converts blood haemoglobin and contains no poisonous substance, making it suitable for automated method. Being capable of analysing methaemoglobin, this method accurately analyses blood sample which contain methaemoglobin.

Procedure- Blood collected in EDTA bottle, mixed well and fed to the automated haematological analyser in such a way that, after mixing the bottle containing blood, it is placed below the aspiration needle of the automated analyser and press the button for aspiration. The required volume of the blood was aspirated by the machine, and analysed. The result was monitored in the screen and a print out of the result taken.

General Methodology of the Analyser- After aspiration of the blood, it is diluted (1:500 dilution for haemoglobin determination). The diluted sample is directed to two sides of the instrument. Particles on the red cell side that are larger than 36 fl are counted as red cells. Measurement of haemoglobin done after red cell lysis.

RESULTS

The values of Haemoglobin concentration obtained by Automated and Sahli's (manual) methods in 30 samples were taken. The results of all the 30 samples by Sahli's and automated method were entered in a table. From this it was found that in automated method maximum value obtained was 14.1 gm% and minimum value 6.2 gm% which was seen only in one case and it was rechecked. The Sahli's method showed a maximum of 15.4 gm% and minimum of 6.4 gm%. This minimum value was seen in two cases and the test was repeated twice.

Statistical analysis showed a mean of 10.95 in automated method of haemoglobin estimation and that of Sahli's method was 11.14 (Table 1). Standard deviations on analysis were 2.62 and 2.46 respectively.

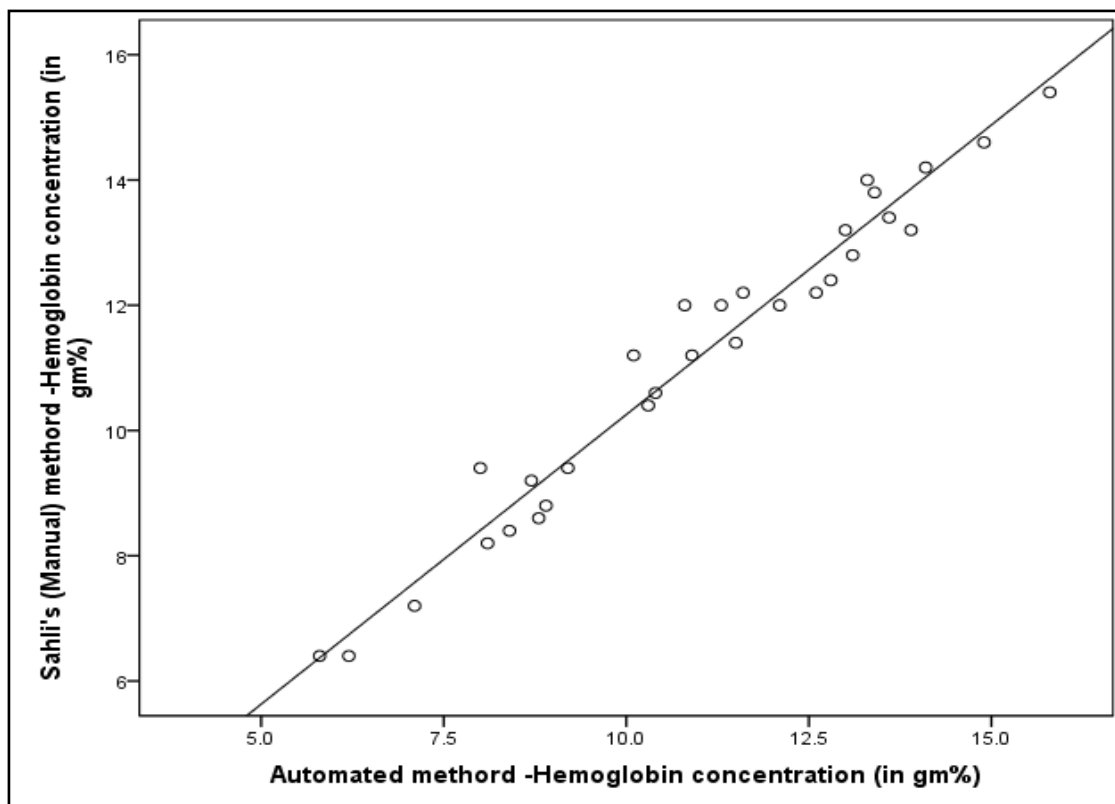
	Minimum	Maximum	Mean	Std. Deviation
Automated Method haemoglobin concentration (gm%)	5.8	15.8	10.957	2.6202
Sahli's (Manual) Method Haemoglobin Concentration (Gm%)	6	15	11.14	2.466
Average methods	6.10	15.60	11.0483	2.53212
Difference methods	-1.40	.70	-.1833	.50040

Table 1. Descriptive Statistics

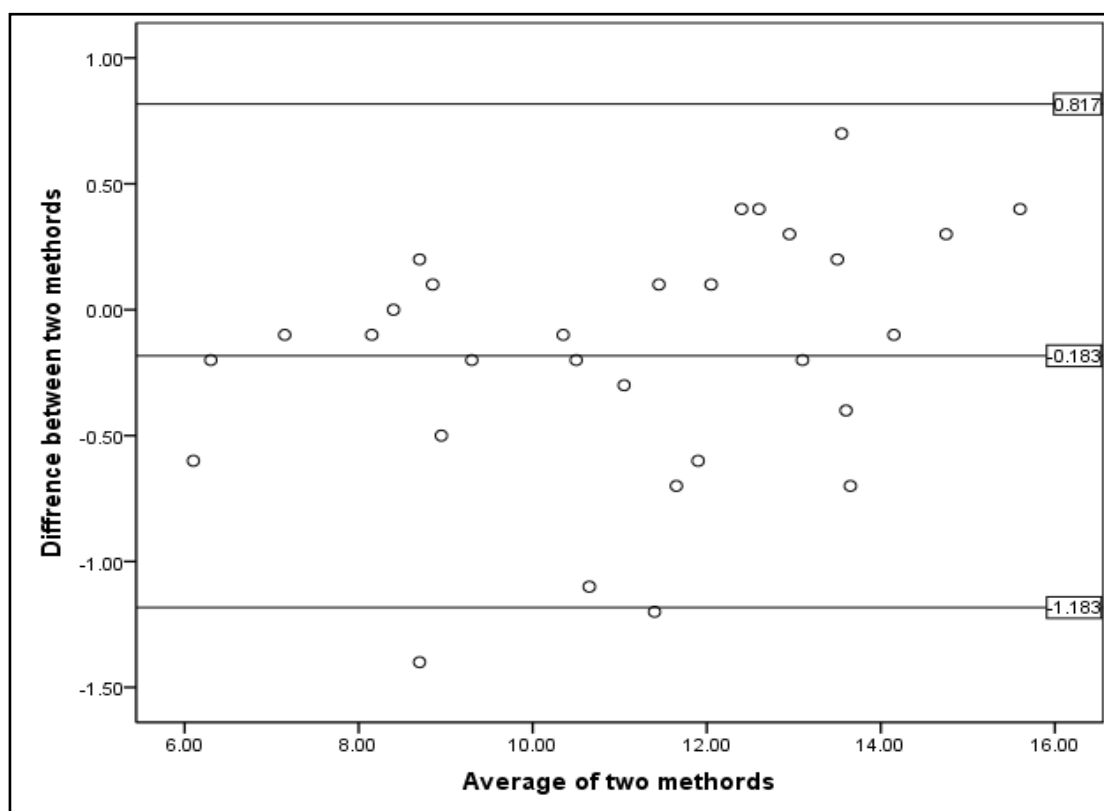
		Sahli's (Manual) Method Haemoglobin Concentration (gm%)	Difference Methods
Automated Method Haemoglobin Concentration (gm%)	Pearson Correlation	.982**	
	Sig. (2-tailed)	.000	
Average methods	Pearson Correlation		.309
	Sig. (2-tailed)		.097

**. Correlation is significant at the 0.01 level (2-tailed).

Table 2. Correlations



Graph 1



Graph 2

DISCUSSION

The values of haemoglobin concentration obtained by automated and Sahli’s methods in 30 samples were taken and the values were analysed statistically.

According to descriptive statistics, mean of automated method of haemoglobin estimation was 10.95 and that of

Sahli’s method 11.14. And their standard deviations were 2.62 and 2.46 respectively. The average of two methods were 11.04 and difference was -0.183 and standard deviations were 2.532 and 0.500 respectively.

A correlation table was made, and according to that table the correlation between the automated and Sahli’s method

was 0.982 and the difference of the two methods were 0.097. As per the correlation if the method is perfect or if the one method has complete correlation with the other, the correlation will be 1 and the difference will be zero. The values obtained in this study were almost close, and the closeness of the values indicate the method is almost reliable. This was described in a study by Atilola.⁷ and McCarthy.⁸ In 1948 another study was conducted by Rock et al; and he observed that manual method has many advantages over the automated methods and that is well evident in this study also especially regarding the cost effectiveness and simplicity.

From the values obtained two graphs were plotted.

One is scatter plot based on the correlation (Graph 1).

In this graph, haemoglobin concentration (in gm%) by automated method was plotted on X axis and haemoglobin concentration (in gm%) by Sahli's method on the Y axis. It gives a good impression that almost all values are clustered within a specific range.

The next graph is Bland - Altman plot(Graph 2), which have mainly 3 regions-

- a. Mean, that is -0.183 based on the descriptive statistics
- b. 95% reference range upper limit that is 0.817 and
- c. 95% reference range lower limit that is -1.183.

In this plot, almost all values lies within the reference range, only two values lie out of the range. From the above statistical studies, it was evident that automated haematology analyser (Sysmex KX-21) readings correlated well with manual methods. In this study it is also clear that Sahli's method is a reliable method and can be used to validate the automated methods. A similar picture was well explained in a study by Lantis et al.⁹

CONCLUSION

- Since a good correlation has been obtained in the statistical analysis, we finally conclude that, haemoglobin concentration determined by Sahli's method is as reliable as automatic method.
- In developing country like India Sahli's method is used as a common method for the estimation of haemoglobin.
- Since it is relatively inexpensive, simple to use, does not require electricity, and requires only small amount of

blood, it can be used as a reliable method for haemoglobin estimation especially in rural areas and laboratories.

REFERENCES

- [1] Kharkar VP, Ratnaparkhe VR. Hemoglobin estimation methods: a review of clinical, sensor and image processing methods. *International Journal of Engineering Research & Technology (IJERT)* 2013;2(1):1-7.
- [2] WHO. Haemoglobin concentration for the diagnosis of anaemia and assessment of severity. WHO 2011.
- [3] Lewis SM, Osei-Bimpong, Bradshaw A. Measurement of haemoglobin as a screening test in general practice. *Journal of Medical Screening* 2004;11(2):103-105.
- [4] Dacie JV, Lewis SM. *Practical haematology*. 7th edn. Edinburgh; New York: Churchill Livingstone 1991:37-41.
- [5] Wintrobe MM, Lec GR, Boggs DR, et al, eds. *Clinical haematology*. 7th edn. Philadelphia: Lea and Febiger 1975:114-115.
- [6] Zwart A, van Assendelft OW, Bull BS, et al. Recommendations for reference method for haemoglobinometry in human blood (ICSH std 1995) and specifications for international haemoglobinocyanide standard (4th edition). *J Clin Pathol* 1996;49(4):271-274.
- [7] Atilola LR, Kamensky LA. Routine differential leucocyte count. *Clinical Laboratory Medicine* 1996;15:289-291.
- [8] McCarthy JM, Capullari T, Spellacy WN. The correlation between automated hematology and manually read smears for the determination of nucleated red blood cells in umbilical cord blood. *J Matern Fetal Neonatal Med* 2005;17(3):199-201.
- [9] Lantis KL, Harris RJ, Davis G, et al. Elimination of instrument-driven reflex manual differential leukocyte counts. Optimization of manual blood smear review criteria in a high-volume automated hematology laboratory. *Am J ClinPathol* 2003;119(5):656-662.