



Research Article

Evaluation of Hemoglobin by Using Sahli's Method and Automated Analyser, A Comparative Study in Paediatric Age Group in A Tertiary Care Hospital of Assam

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ABSTRACT

Background and aim: Anemia is the most common hematologic abnormality identified in infants and children. The present study was conducted to evaluate the hemoglobin values in pediatric age group by Sahli's acid hematin method, cyanmethemoglobin method and automated hematology analyser and to compare and contrast sahli's acid hematin method and automated analyser with respect to cyanmethemoglobin method for hemoglobin estimation and to evaluate the diagnostic accuracy of both.

Material and Methods: This is a cross-sectional study. Blood sample was collected in two EDTA vials from 100 outdoor patients in the age group 1 year to 12 years attending CCL Pathology. The hemoglobin values of the collected blood samples was analysed by Sahli's Acid Hematin, cyanmethemoglobin method in the Department of Physiology and Automated Hematology Analyser (Elite 580, ERBA manheim) in the Department of Pathology. The methods were compared and analysed for diagnostic accuracy through chi-square tests and ANOVA. A value of <0.05 was regarded as statistically significant.

Results: In our study, all three methods exhibit a similar level of repeatability, with standard deviations around 1.94–1.95. This indicates that all methods are consistent in their measurements with minimal variability. Sahli's Method has a narrower prediction range (3.55–11.32), which might suggest lower reliability for measurements across the full spectrum of possible values. Cyanmethemoglobin and Automated hemoglobin show wider and more similar prediction ranges, indicating better capability to accommodate a broader range of hemoglobin values.

Conclusion: In our study, all three methods exhibit a similar level of repeatability which indicates that all methods are consistent in their measurements with minimal variability. The Automated hemoglobin and Cyanmethemoglobin methods are likely more suitable for applications requiring higher reliability and coverage of a wide range of hemoglobin values. The Sahli's Method, while consistent, might be less reliable for extreme or high values due to its narrower range.

Keywords: Hemoglobin values, Sahli's acid hematin method, cyanmethemoglobin method and automated hematology analyser.

INTRODUCTION

Hemoglobin levels are one of the most accurate measures of anaemia and are frequently used to identify anaemic people and assess how well therapies are working. [1] Anemia is the most common hematologic abnormality identified in infants and children. Approximately a quarter of the world's population suffers from anemia, almost 2 billion people, with almost

half of children <5 years of age affected in 2016. Anemia is associated with increased morbidity and mortality in children, particularly children of preschool age. [2]

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.** Hemocue method and Cell counter are still costly and require good equipment, quality control, laboratory setup and trained personnel for proper functioning. But in determining the treatment protocol when other RBC indices and complete blood picture are required, automated cell counter is more useful Hemoglobin concentration is routinely measured using Automated Hematology Analyzers (AHAs).

Although these are very accurate and reliable, they are expensive, and problems of samples' transport to the laboratory may delay treatment.[3] In clinical measurement, comparison of a new measurement technique with an established one is often needed to see whether they agree sufficiently for the new to replace the old.

The present study was conducted to evaluate the hemoglobin values in pediatric age group by sahlis acid hematin method, cyanmethemoglobin method and automated hematology analyser and to compare and contrast sahlis acid hematin method and automated analyser with respect to cyanmethemoglobin method for hemoglobin estimation and to evaluate the diagnostic accuracy of both.

MATERIALS AND METHOD

2 ml of blood sample was collected in two EDTA vials from 100 outdoor patients in the age group 1 year to 12 years attending CCL Pathology. The hemoglobin values of the collected blood samples was analysed by Sahlis Acid Hematin, cyanmethemoglobin method in the Department of Physiology and Automated Hematology Analyser (Elite 580, ERBA manheim) in the Department of Pathology. All indoor patients and those who were below 1 year and above 12 years were excluded from the study. Written Consent was taken from all the participants' parents. The study was conducted after obtaining ethical clearance from the Institutional Ethical Committee.

The method of choice for hemoglobin determination is the cyanmethemoglobin method which is a type of colorimetric method. The principle of this method is that when blood is mixed with a solution containing potassium ferricyanide and potassium cyanide, the potassium ferricyanide oxidizes iron to form methemoglobin. The potassium cyanide then combines with methemoglobin to form cyanmethemoglobin, which is a stable color pigment read photometrically at a wave length of 540nm.

In Sahlis Haemoglobinometer (acid haematin method) method blood is mixed with N/10 HCL, resulting in the conversion of hemoglobin to acid hematin, which is brown in colour. The solution is diluted till its colour matches with the brown coloured glass of the comparator box. The concentration of hemoglobin is then read directly.

In automated analyser (Elite 580, ERBA manheim) the hemoglobin diluents is delivered to the hemoglobin bath where it is mixed with certain amount of lyse which converts hemoglobin to hemoglobin complex that is measurable at 525 nm. The LED light passes through the sample and is measured by a optical sensor. The signal is then amplified and the voltage is then measured and compared with the blank reference reading. The hemoglobin is then calculated using the following equation and expressed in g/L

Hemoglobin=constant x Ln(Blank photocurrent/sample photocurrent)

The methods were compared and analysed for diagnostic accuracy.

RESULTS AND OBSERVATION

A total of 100 patients of both sexes were included in the study, with a mean age of 43.83 years. Hemoglobin values predicted by the automated cell counter were compared with those obtained using the cyanmethemoglobin method and Sahli's Acid Hematin method through chi-square tests and ANOVA (Analysis of Variance). The results were expressed as mean $\pm$ SD, with statistical significance defined as a p-value $\leq$ 0.05. The analysis was conducted using Microsoft Excel 2010 and SPSS Version 2.0 (IBM Statistics).

Closely related results were seen among automated cell counter (10.427 ± 1.94) and cyanmethemoglobin (10.32 ± 1.95) methods, while a huge difference was observed in estimation with Sahli's method (7.43 ± 1.94).

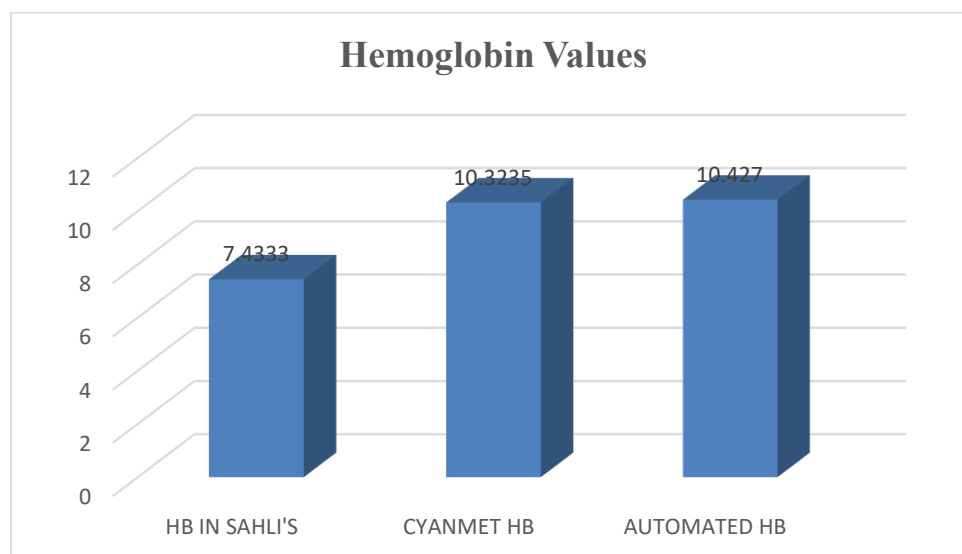


Figure 1-Graphical representation showing estimated Hemoglobin variation between automated cell counter, cyanmethemoglobin method and Sahli's acid-hematin method.

Table 1: Shows Analysis of Variance among the Hemoglobin value estimated via automated cell counter, cyanmethemoglobin method and Sahli's acid-hematin methods

ANALYSIS OF VARIANCE						
		Sum of Squares	df		F	P value
HB IN SAHLI'S	Between Groups	373.67582	99	3.774503	76.18	0.000
	Within Groups	0	0			
	Total	373.67582	99			
CYANMET HB	Between Groups	377.367275	99	3.811791		
	Within Groups	0	0			
	Total	377.367275	99			
AUTOMATED HB	Between Groups	374.7571	99	3.785425		
	Within Groups	0	0			
	Total	374.7571	99			

Interpretation: Since the p-value is extremely small (much less than 0.05), we reject the null hypothesis. This means there is a statistically significant difference in the mean hemoglobin levels across the three methods (Sahli's, Cyanmet, and Automated).

Table 2: Accuracy of Automated cell counter with respect to Gold Standard Cyanmethemoglobin method.

	Cyanmethemoglobin Method			
Automated Cell Counter				
		AnaemiaPresent	AnaemiaAbsent	Total
	AnaemiaPresent	38	0	38
	AnaemiaAbsent	1	61	62
	Total	39	61	100

Accuracy = 99%

Table 3: Accuracy of Sahli's Method with respect to Gold Standard Cyanmethemoglobin method.

Table 5: Accuracy of Sahli's Method with Respect to Gold Standard Cyanmethemoglobin method.				
	Cyanmethemoglobin Method			
Sahli's Method				
		AnaemiaPresent	AnaemiaAbsent	Total
	AnaemiaPresent	39	1	40
	AnaemiaAbsent	8	52	60
	Total	57	53	100

Accuracy = 91%

Table 4-Comparison of Sahli's Method, Cyanmethemoglobin method and Automated cell counter

	Sahli's Method	Cyanmethemoglobin method	Automated Cell Counter
Range of HB Values	2.9-11.5 g/dl	5.4-14.4 g/dl	5.5-14.5 g/dl
Mean	7.4333 g/dl	10.3235 g/dl	10.427 g/dl
Repeatability Standard Deviation	1.94 g/dl	1.95 g/dl	1.95 g/dl
Method Prediction Range	3.55-11.32 g/dl	6.42-14.23 g/dl	6.54-14.23 g/dl

In our study, all three methods exhibit a similar level of repeatability, with standard deviations around 1.94–1.95. This indicates that all methods are consistent in their measurements with minimal variability.

Sahli's Method has a narrower prediction range (3.55–11.32), which might suggest lower reliability for measurements across the full spectrum of possible values. Cyanmet hemoglobin and Automated hemoglobin show wider and more similar prediction ranges (Cyanmet: 6.42–14.23, Automated: 6.54–14.32), indicating better capability to accommodate a broader range of hemoglobin values.

DISCUSSION

Haemoglobin estimation is used as a screening test for detecting anaemia. This is a frequently identified abnormality in our population. Hence, accurate Hb estimation is essential so that further tests can be done to ascertain its cause and treat the patient accordingly.

Few common methods for haemoglobin estimation are Sahli's acid haematin method, copper sulphate method, manual Drabkin's method, Hemocue method, automated haematology analysers, non-invasive Pulse co-oximetry method etc.³

Sahli's acid haematin method is quick, easy to perform, inexpensive, does not require technical expertise and can be used as a bedside procedure.

However it is less accurate, lacks a true standard, there is significant subjective variation in color matching and it does not measure all haemoglobins

i.e. oxyhaemoglobin, sulphaemoglobin as they are not converted to acid hematin.

With the advent of automated blood cell counter by Wallace Coulter in 1956, the paradigm of hematological investigations shifted. A cell counter not only assesses Hemoglobin very accurately, but also measures all red cell indices such as total RBC count, hematocrit, MCV, MCH, MCHC, RDW, etc.,

Total and differential Leukocyte count and platelet count and other indices. A variety of fully automated instruments are now commercially available. They work on the principle of impedance measurement, high frequency measurement, light scatter at different angles, Fluorescence flow-through cytometry etc.^{4,5}

In our study, there was only a small difference between the automated cell counting and the cyanmethemoglobin technique in the calculated hemoglobin values. but there was a sizeable divergence when cyanmethemoglobin was compared to the calculated hemoglobin values of the Sahli's acid-hematin method, All three methods exhibit a similar level of repeatability, with standard deviations around 1.94–1.95. This indicates that all methods are consistent in their measurements with minimal variability.

Sahli's Method has a narrower prediction range (3.55–11.32), which might suggest lower reliability for measurements across the full spectrum of possible values. Cyanmet HB and Automated HB show wider and more similar prediction ranges (Cyanmet: 6.42–14.23, Automated: 6.54–14.32), indicating better capability to accommodate a broader range of HB values.

The study done by Hema Anand *et al.*⁶, aimed to compare the efficacy of Hemoglobin Colour Scale method with Sahli's method and auto-analyzer for the hemoglobin estimation. The observed values of that study showed a systemically higher result for Hemoglobin Colour Scale method and Sahli's method than the autoanalyzer values.

In a study by drashti thakkar *et al*³ they found that on comparison of Sahli's method with cell counter, Sahli's method underestimates Hb by 0.746 g/dl as compared to cell counter.

In a study by Natarajan S *et al*⁷(2010) had found results of lower haemoglobin by 0.37gm/ dl using venous blood comparing Sahli's method with the coulter auto analyzer.

In a study Study by Bezarra da silva *et al*⁸comparing Sahli's method with cell counter did not find any significant difference between the two methods. They found mean difference of 0.2267g/ dl.

The study by P Balasubramaniam *et al.*⁹showed a p value of less than 0.001 for the Sahli's single time method and the Drabkin's method of hemoglobin estimation.

CONCLUSION

In our study, all three methods exhibit a similar level of repeatability which indicates that all methods are consistent in their measurements with minimal variability. The Automated hemoglobin and Cyanmet hemoglobin methods are likely more suitable for applications requiring higher reliability and coverage of a wide range of hemoglobin values. The Sahli's Method, while consistent, might be less reliable for extreme or high values due to its narrower range.

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REFERENCES

1. Morris SS, Ruel MT, Cohen RJ, Dewey KG, de la Brière B, Hassan MN, et al. Precision, accuracy, and reliability of hemoglobin assessment with use of capillary blood. *Am J Clin Nutr.* 1999;69:1243–8.
2. Patrick G. Gallagher Anemia in the pediatric patient *Blood.* 2022 ; 140(6): 571–593.
3. Drashti Thakkar¹, Nirali Shah² et al. Comparative Study of Different Haemoglobin Estimation Methods *International Journal of Contemporary Pathology*, 2021; 7,:1- 8
4. Coulter WH: Means for counting particles suspended in a fluid.U.S. Patent No. 2.656.508. 20.10.1953.
5. Tatsumi N, Tsuda I, Furota A, Takubo T, Hayashi M, Matsumoto H: Principle of blood cell counter development of electric impedance method. *Sysmex J Int* 1999;8-20.
6. Hema Anand, Rashid Mir, Renu Saxena. Hemoglobin color scale a diagnostic dilemma. *Indian Journal of Pathology and Microbiology.* 2009; 52(3):360-362.
7. Shrinivasan NM, Kasturba MP. Intra-operative point of care haemoglobin estimation: A comparison of three methods. *Shri Lankan Journal of Anesthesiology.* 2010;18(1): 15-19
8. Bezerra da Silva, J.F.1; Oliveira, T.N.1; Souza Filhol, S.D.; Alves, A.Q.2; comparative evaluation of haemoglobin using N/10 hydrochloric acid versus automated method. *Luso-Brazilian congress of the experimental pathology.* 12/11/2011.
9. Balasubramaniam P, Malathi A. Comparative study of hemoglobin estimated by Drabkin's and Sahli's methods. 1992; 38(1):8-9.