

Comparison of Pre donation Hemoglobin Screening Methods - Implications of Quality and Cost

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ABSTRACT

Introduction: Pre donation hemoglobin screening is among the foremost test done on blood donors to determine whether an individual is fit to donate blood with the intention of preventing inadvertent donation from an anaemic donor. Despite the availability of various tests, no single technique has emerged as the most suitable for hemoglobin screening in blood donor. Primary aim of the study was to compare CuSO₄ gravimetric method and HemoCue against a standard hematology analyzer to ascertain whether HemoCue could replace the CuSO₄ method and also did a comparison of the cost effectiveness between two methods.

Material and Methods: Prospective observational study done in 54 donor's and 57 patient's EDTA samples. Sample analysis were done using CuSO₄ solution (specific gravity- 1.053), HemoCue HB-301(Ängelholm, Sweden) and HORIBA microsemi CRP Hematology analyzer(Horiba Ltd, Japan), which was the standard reference.

Results: 54 samples were >12.5gm/dL and 57 samples were <12.5 gm/dL. 87 (78.37%) of them were males and 24 (21.62%) of them were females. Mean age of the study population was 34.8 ± 1.74 (18-40). Mean Hb values were 13.47 ± 2.33 and 12.74 ± 2.31 in HemoCue and Analyzer respectively. Specificity and positive predictive value of CuSO₄ was higher than HemoCue (83% and 80% to 70% and 59% respectively) with similar sensitivity (100%).

Conclusions: Despite the incongruities, CuSO₄ gravimetric method can still be the test of choice for pre-donation Hb screening if the test is being done by trained staff following Standard Operating Procedures, especially in a resource limited country.

Keywords: Hemoglobin, Donor screening, CuSO₄, HemoCue, Quality, Cost, Kerala

INTRODUCTION

Pre donation hemoglobin screening is the first and foremost test done on blood donors to determine an individual's eligibility to donate blood. The intention is to prevent bleeding an anaemic donor. The minimum acceptable Hemoglobin (Hb) level for blood donation is 12.5 g/dl or Hematocrit (Hct) of 38% for both males and females, according to Drug and Cosmetic Act 1940.¹ There are various options like the CuSO₄ gravimetric method, HemoCue, Hemoglobin colorimetric scale and Cyanmethemoglobin etc for Hb estimation in blood donors.^{2,3} In recent years, non invasive screening methods have emerged (Occlusion Spectroscopy and Pulse co-oximetry)⁴⁻⁷ which avoids the fear of pain in the donor and which by itself can motivate blood donors to donate blood more often.⁸ In a blood donation scenario, an ideal test must have less turnaround time, less expertise needed to run the test, one which is portable and most importantly it must be cost effective.

Despite the availability of various tests at our door step, no single technique has emerged as the most suitable and ideal method for hemoglobin estimation in blood donor screening scenario. Unnecessary donor deferral (range from 0.4-16%) due to inaccurate Hb results will lead to permanent donor loss.⁹ Detection of an anemic donor will depend upon the reliability of the screening tests adopted. Rationale of this study was to identify the most suitable method amongst the commonly used screening tests in blood banks by comparing them with a standard method of Hb estimation. Primary aim of the study was to compare CuSO₄ gravimetric method and HemoCue against a standard hematology analyzer and to ascertain whether HemoCue should replace the traditional CuSO₄ method for donor Hb screening and we also did a comparison of the cost effectiveness of these two methods.-

MATERIAL AND METHODS

This was a prospective observational study done in a tertiary care hospital, Kerala. We undertook this study after obtaining clearance from Institutional Ethics Committee. Randomly collected EDTA blood samples from 54 donors and included 57 patients with known Hb of < 12.5g/dL to simulate a wide analytical Hb range. Patient population was selected to include Hb values ≤ 12.5g/dL which helped to compare the positive as well as negative predictive value of CuSO₄ and HemoCue. We analyzed the samples using CuSO₄ solution (specific gravity of 1.053), HemoCue HB-301(Ängelholm, Sweden) and HORIBA microsemi CRP Hematology analyzer(Horiba Ltd, Kyoto, Japan), which was taken as standard reference.

Inclusion criteria

- Whole blood donors who came to our blood bank during the time period and were declared fit by pre donation screening were randomly selected.
- Patients with Hb values recorded as ≤ 12.5 g/dL in the analyzer.
- Age group of 18-40 years.

Exclusion Criteria

- Donor samples from Outdoor camps
- Samples kept for > 1 hour at room temperature.

CuSO₄ solution is a semi quantitative method which works by the principle of specific gravity (gravimetric) where Hb value

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> 12.5 g/dL has specific gravity of ≥ 1.053 , these samples will sink to the bottom of the solution as it has more specific gravity. If the Hb < 12.5 g/dL, it floats on top as the specific gravity is < 1.053. Whole blood is dropped from a height of 1cm above the solution surface and CuSO₄ will create a copper proteinate sphere around the blood drop. The working CuSO₄ solution should be taken in a clear beaker of 3 inch depth and results should be declared negative only after 15 seconds. Working CuSO₄ solution was prepared everyday and was changed every 6 hours or after every 25 tests according to the Standard Operating Procedure (SOP).^{10,11}

HemoCue works by the principle of Photometry. Blood drop is placed on a cuvette, which is patented to HemoCue and quantitative Hb values are shown in less than 15 seconds. Sodium deoxycholate hemolyses erythrocytes and haemoglobin is released. Sodium nitrite converts haemoglobin to methemoglobin which, together with sodium azide, gives azidemethemoglobin. The absorbance is measured at two wavelengths (570 nm and 880 nm) in order to compensate for turbidity in the sample. Horiba microsemi analyzer works by the principle of photometry for Hb estimation.

The tests were done by a trained person assigned to the job to avoid subjective bias, within one hour of sample collection. All QCs were done as per the SOPs and manufacturer’s recommendations. Results of CuSO₄ were recorded as either <12.5 or > 12.5 gm/dL.

STATISTICAL ANALYSIS

Sample size was 100 with 10% error chance calculated based on 95% CI and 80% power. All results were expressed as mean \pm standard deviation (SD), proportions and p-value was compared with alpha (α) level at 5%. The results were considered to be statistically significant when p is < 0.05. Independent t test was used to compare between the groups. Data were entered in Microsoft MS Office Excel, 2010 and results were statistically analyzed using software SPSS20 (Inc. Chicago)

RESULTS

A total 111 samples were taken; of which 54 samples were >12.5gm/dL according to Analyzer, and 57 samples were <12.5 g/dL. 87 (78.37%) of them were males and 24 (21.62%) of them were females. Hb ranged from 6.4 -17.8 g/dL. Mean age of the study population was 34.8 \pm 1.74 (18-40). Mean Hb values were 13.47 \pm 2.33 and 12.74 \pm 2.31 in HemoCue and Analyzer respectively with a difference in mean of 0.73 more in HemoCue.

23 Samples were false positive in HemoCue (40.35%) compared to the 11 (19.29%) in CuSO₄ on comparing with Analyzer which was significant (P =0.001) (table-1). CuSO₄ had a higher specificity (0.83>0.7) and positive predictive value (0.8>0.59) and the Likely hood ratio + (5.9>3.4) in comparison with HemoCue meaning CuSO₄ had a significantly lower false positive rate. Sensitivity and Specificity of both methods were similar compared to analyzer, but there was one donor sample initially passed in CuSO₄ method which failed by the other two tests. Hb values given by the HemoCue were always 0.5-1 g/dL greater than the values in Analyzer. From Table-2 and 3, the mean Hb values in different methods were comparable, though the range was much higher in HemoCue. Cost per test

analysis showed Indian Rupees (IR) 0.02-0.05 in CuSO₄, IR 25 in HemoCue and IR 10 in analyzer; CuSO₄ being the most economical test.

DISCUSSION

Low hemoglobin is one of the leading causes of donor deferral^{9,12}; so pre donation Hb screening has to be reliable in order to prevent un necessary donor deferrals and to avoid inadvertent phlebotomy from an anaemic donor and to ensure proper product quality.^{13,14} There are so many methods for Hb estimation in a whole blood donor, but we have to ensure the selected test is validated. We compared 54 samples of Hb > 12.5 g/dL and 57 samples with Hb < 12.5g/dL using CuSO₄ method, HemoCue Hb 301 and Horiba microsemi analyzer as the reference method.

Comparing false positive rates in the current study (Figure-1),

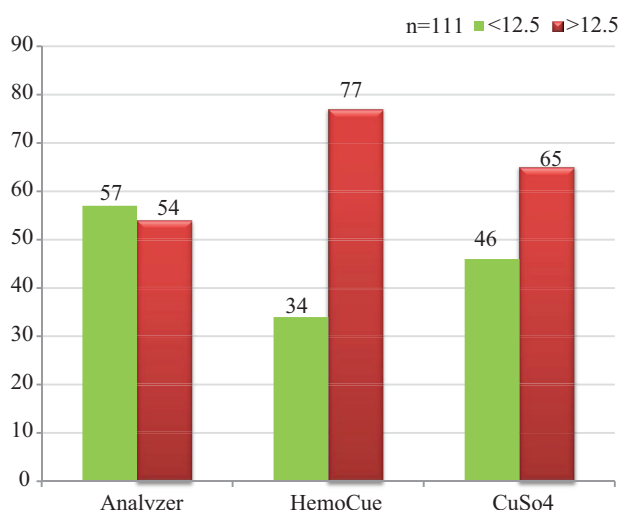


Figure-1: Comparison among CuSO₄ and HemoCue against Hematology analyzer

	Analyzer <12.5	Analyzer >12.5
CuSO ₄ <12.5	46 (80.7%)*	0
CuSO ₄ >12.5	11 (19.29%)	54
HemoCue<12.5	34 (59.69%)*	0
HemoCue>12.5	23 (40.35%)	54

* 80.7% in CuSO₄ concurred with Syandard Analyzer, where as only 59.7% in HemoCue was concurring with Analyzer in the <12.5 g/dL group.

Table-1: CuSO₄ and HemoCue compared with Analyzer

	HemoCue <12.5	HemoCue >12.5
CuSO ₄ <12.5	32	13
CuSO ₄ >12.5	1	65

Pearson Chi Square co efficient = 62.035, P value = 0.0001.

Table-2: CuSO₄ vs HemoCue

	CuSO ₄	HemoCue
Sensitivity	1	1
Specificity	0.83	0.7
Positive predictive value	0.8	0.59
Negative predictive value	1	1
Likelihood ratio+	5.9	3.34
Likelihood ratio -	0	0

Table-3: Comparison between CuSO₄ and HemoCue

HemoCue had a very high false positive rate; 23 (40.35%) compared to the 11(19.29%) by CuSO₄. False positive rate for CuSO₄ in another study was found out to be 7.9% in 1014 donations with sensitivity of 99.8%.¹⁵ In another study, 37% of deferred donors were acceptable with HemoCue method, mentioning deferral rates would be lesser if HemoCue replaces time tested CuSO₄.¹⁶ However in those studies, there were no comparisons made of HemoCue with a referral method. As we have seen in our study, HemoCue gives significantly higher mean Hb values when it was compared with a standard method.¹⁴⁻¹⁹ So the possibility of re accepting already deferred donors by HemoCue might actually include anemic donors which might ultimately affect the product quality and be harmful to the donor. Despite the presence of an auto calibration unit in HemoCue, the high rate of false positivity shows that more number of anemic donors will be chosen for whole blood donation. These falsely elevated Hb values (23 in HemoCue compared to the analyzer, Table-1) may be due to small air bubbles trapped in the cuvette or blood in the cuvette face or finger print while handling cuvette or humidity.²⁰ Inability to standardize a completely manual method like CuSO₄ could be a reason for the observed discrepancy (11 false positives, Table-1) compared to the automated hematology analyzer.¹⁶ Higher false positivity in the screening was found with samples close to <12.5 g/dL, and as we can see from the mean values, HemoCue had a higher mean Hb (0.73) compared to reference methods. This was similar to various studies conducted in India.²¹⁻²³ HemoCue had a higher mean Hb range (14.7± 1.49 to 13.8 ± 1.52) compared to the reference method. Gomez et al, 2007 stated that the reliability of portable hemoglobinometers at hemoglobin levels below 12.5g/dL was unacceptably low (ICC < 0.30), indicating that under the conditions employed in their study these devices might not allow discrimination of potential donors at borderline hemoglobin values.

As per our data on Positive Predictive Value of CuSO₄ (80%) and HemoCue (59%), donors declared fit by HemoCue might not actually have a satisfactory Hb for whole blood donation (Table-3). Theoretically HemoCue is superior to CuSO₄ having features like quantitative Hb estimation, avoiding problems of sample turbidity and equally good as a point of care testing method, however, based on this study, because the false positive rate was higher, selecting HemoCue as the only Hb screening method may not be a good option.

Mendrone et al studied Hb screening in female population in detail and found out that HemoCue reduces the risk of accepting anemic female blood donors without increasing the deferral of non anaemic donors.²⁴ This was contradictory to our findings, which clearly showed that even though donor deferral rates could be lower, anemic donors could have been accepted by HemoCue. This can be more detrimental for the female donor population; selecting unfit female donors based on HemoCue can lead to worsening of the anemia on a reproductive age group female population.²⁵

In our study, sensitivity of both methods were 100%, however CuSO₄ had a higher specificity (83% compared to 70% for HemoCue).^{21,23,26} In this study, specificity (83%), positive predictive value (80%) and likelihood ratio+ (5.9 > 3.34) of CuSO₄ is much higher as compared to HemoCue (Table-3), which was contradictory to the findings of Gupta et al, who

showed HemoCue had higher sensitivity, specificity, true positives, positive predictive value. CuSO₄ had a specificity of 58.1%, positive predictive value 92.3% and negative predictive value of 90.7% in a study conducted in a different part of India.²¹ The false positive rate of CuSO₄ is 19.2% where as the false positive rate of HemoCue is 40.35%. False negativity was zero for both methods. So, overall in this study CuSO₄ method was superior to HemoCue method and both tests were statistically significant (*P* value<0.005).

Except one donor sample (Hb =10.8) all other false positives in CuSO₄ were between 12-12.5 g/dL which was similar to the findings of James et al.²⁷ One sample in CuSO₄ which had showed a value > 12.5 g/dL however showed that the Hb value was 10.8 g/dL in the analyzer and 11.8 g/dL in HemoCue. This was from a male donor who was bled 450 ml of whole blood. We recalled the donor, advised him on the dietary modifications, iron supplements, further tests he needed to do including a physician's consultation. He had no relevant history in the past. Even though CuSO₄ solution had a lesser false positive rate; this was one incident where it failed to pick up an anemic donor. In a study conducted by Gomez et al, 13 donors were inappropriately bled by using CuSO₄ solution, postulating that gravimetric method has a low sensitivity for anemia.¹⁸ Mannarino et al had described a case of anemic donor with an Hb value of 6.2 gm/dL accepted for phlebotomy with a globulin value of 15.4 gm% and "M" bands in electrophoresis who was later diagnosed with Multiple Myeloma. Plasma protein content and Hb are directly proportional, for every 1 gm rise in protein; Hb increases by 0.7gm or vice versa.²⁸

Comparing the cost per test, CuSO₄ was the most affordable with 0.02 INR/ test, HemoCue at 25 INR/test and analyzer at 10 INR /test. 500 gm of CuSO₄ cost only 175 rupees and 159.63 gm can be used approximately for 700-800 tests. Considering average 800-1000 donations in our centre per month, running cost of HemoCue will be approximately INR-25000 whereas the cost of CuSO₄ will be very much cheaper.¹⁵ This is apart from the initial capital investments.⁵ As a blood donor screening method, its always better to select an affordable and reliable test which can also be used as a point of care testing in outdoor blood donation camps. CuSO₄ has withstood the test of time over the years, as a simple and affordable technique for Hb screening. In a resource poor country like ours, selecting HemoCue as a pre donation standard method for Hb estimation does not come across as a viable alternative.

Good Manufacturing Practice in CuSO₄

The stock solution and working CuSO₄ with a specific gravity of 1.053 solutions were prepared according to AABB technical manual.² The 30 ml working solution is replaced every 6 hours or after performing 25 samples test, whichever is earlier.^{2,11} Allow the drop to fall gently from a height of 1cm. CuSO₄ solution will not change the specific gravity of blood for 15 seconds, so whenever doubtful, always wait for 15 seconds to make a decision.¹¹

Limitations

The study was limited by low sample size. More studies are required with larger sample sizes comparing traditional methods with a standard reference method. To get more precise details, we could have analyzed capillary and venous samples

for comparisons.

CONCLUSIONS

Despite the incongruities, CuSO₄ gravimetric method can still be the test of choice for pre-donation Hb screening if the test is being done by trained staff following Standard Operating Procedures, especially in a resource limited country. Because approximately 8 million¹⁶ donations are collected annually in India, even a small percentage of false acceptance or false deferral by the Hb screening represents a significantly large number of individuals. Since CuSO₄ has stood the test of time, only doubtful samples need to be retested using more expensive quantitative methods, saving time and money. Furthermore, use of validated newer non invasive methods should also be encouraged as a donor screening tool in our population to motivate blood donors to donate blood more often by removing the fear of pain associated with blood donation.

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