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A COMPARATIVE STUDY OF HEMOGLOBIN ESTIMATION IN DEFERRED BLOOD DONORS USING COPPER SULPHATE METHOD, HEMOCUE AND AUTOMATED ANALYZER IN A TERTIARY CARE HOSPITAL

Laboratory Medicine

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ABSTRACT

Background & Objectives: Blood is a valuable human drug. Hemoglobin levels determine donor's eligibility for blood donation. Accurate, economical, easy to use and portable testing method should be equipped for donor hemoglobin estimation to ensure better results and reduction of deferrals to enhance donor safety and transfusion outcomes. **Methods:** This study was conducted in the Blood Centre at a Tertiary Care Hospital in Navi Mumbai from July to December 2025. Samples from blood donors (n=60) deferred by Copper Sulphate (CuSO4) Gravimetric Method were collected and screened by Hemocue (Hb 201) and Nihon Kohden Haematology Analyser (Standard method). The results were compared and statistical analysis was applied. **Results:** Of the 60 participants, majority were under 35 years of age and showed male preponderance. The mean HB values were 12.1 + 0.7 for CuSO4 method, 12.5 + 0.6 for Hemocue and 12.6+ 0.5 for Analyser respectively. Hemocue showed higher correlation (0.95), sensitivity (88.3%), specificity (90.1%), PPV (89.2%) and NPV (89.5%) in comparison to CuSO4 (0.78; 75.2%; 84.5%; 80.3%; 81.0%) in relation to Analyser. **Interpretation & Conclusions:** Hemocue proves to be a better tool for Hemoglobin screening than CuSO4 and is safe to use, portable, cost-effective, sensitive which helps in better donor screening and reduces deferrals. Also, better counselling strategies need to be implemented for nutritional awareness regarding Hemoglobin based deferrals.

KEYWORDS

Blood Transfusion, Blood Donor Screening, Hemoglobin Estimation, Donor Deferral, Hemocue

INTRODUCTION

Blood donation is a vital part of healthcare system, as it guarantees the readiness of blood and its components for transfusions in clinical settings, such as surgeries, trauma care, and the management of medical illness.[1] One of the main goals of a blood transfusion services is to provide safe, high-quality blood products for patient treatment and in order to achieve this, we must refrain from drawing blood from an anaemic donor. Pre-donation hemoglobin screening is the primary test performed on blood donors to ascertain their eligibility for blood donation. Haemoglobin (Hb) screening is compulsory to safeguard blood donors' health and ensure optimal blood supply to recipients.[2]

An accurate pre-donation test may reduce the risk of making a blood donor anaemic because the Hb of a blood donor decreases by 1–1.5 g/dL after giving a single unit of whole blood.[3]

The Drug and Cosmetic Act of 1940 states that a minimum acceptable haemoglobin (Hb) level for blood donation is 12.5 g/dl, or a hematocrit (HCT) of 38% for both males and females. Despite the existence of numerous techniques for estimating haemoglobin, no single method has shown to be the most suitable and perfect for a blood donation setup.[3]

For estimating hemoglobin in blood donors, there are a number of alternatives, including the Copper Sulphate (CuSO4) gravimetric method, HemoCue, WHO colorimetric scale, and Cyanmethemoglobin method. Although the Automated Haematology Analyser (AHA) is the gold standard for measuring haemoglobin (Hb) in blood donors, point-of-care tests such the (CuSO4) gravimetric and HemoCue techniques are used in settings with limited resources.[4,5]

Such a process is not feasible for screening such a vast number of donors' blood samples in a developing nation like India. However, a less precise and less expensive approach can produce inaccurate results, which could result in either the loss of eligible donors. An ideal test for a blood donation situation should be quicker to complete, require less skill to administer, be portable, and—above all—be affordable.[1-5]

Our study's goal is to determine which of the widely accessible screening tests used in blood centers is best by contrasting them with a common technique for estimating haemoglobin levels. The study's main objective was to compare the CuSO4 gravimetric method and HemoCue against a standard haematology analyser in order to determine if HemoCue method should take the place of the conventional CuSO4 method for donor haemoglobin screening.

MATERIALS AND METHODS

This study was a unicentric, prospective observational study conducted at a tertiary care hospital's blood centre in Navi Mumbai over a period from July to December 2025. Institutional Ethics Committee clearance was obtained prior to the commencement of the study.

During the six-month study period, donors were screened with the means of donor selection criteria as prescribed by NBTC, DGHS, Circular No. IM1102/09/2022-NBTC/BTS[6] dated 24th January 2025. The potential blood donors deferred during screening because of inadequate hemoglobin levels by means of the CuSO gravimetric method (below 12.5 g/dl, labelled as Fail) were invited to participate, and 60 consenting donors were thus enrolled and included in the study.

Their capillary (finger-prick) samples were tested using Hemocue machine (Hb 201, Hemocue Pvt. Ltd.), and additional 2ml venous samples (in EDTA - Ethylene Diamine Tetraacetic Acid vacutainer) withdrawn from these participants were tested with Nihon Kohden 3-part Hematology analyzer.

Principle of the Tests [4,7-12]

a. Copper Sulphate Method

This is a semi quantitative method is based on the blood's specific gravity. A blood droplet is dropped into a copper sulphate solution with a specific gravity of 1.053, and the droplet's mobility is tracked for a minimum of 15 seconds.

b. HemoCue Method

This is a quantitative, non-dilution method where Sodium deoxycholate haemolyses the erythrocytes and haemoglobin is released. Hemoglobin is converted to methaemoglobin by sodium nitrite which, together with sodium azide, gives azide methaemoglobin and the absorbance measured by two wave lengths of 570 nm and 880 nm.

c. Automated haematology cell analyser

This method measures both methaemoglobin and oxymethaemoglobin using a non-cyanide haemoglobin analysis technique.

Laboratory procedure [4]

Laboratory Procedure: As described below, the donor's finger was pierced just once, and two drops of blood were taken for haemoglobin quantification using two distinct techniques (copper sulphate and Hemocue). Blood is solely obtained in an EDTA vial for the purpose of estimating haemoglobin using an automated haematology analyser.

For the copper sulphate procedure, the blood donor's finger is swabbed with alcohol to obtain one drop of blood (about 0.05 ml). A transparent beaker with a minimum depth of three inches is used to hold the working copper sulphate solution (specific gravity 1.053). Next, a drop of blood is dropped from a height of one centimetre into a 30millilitre solution.

The blood droplet floats if the haemoglobin level is less than 12.5 g/dL and sinks if it is greater than 12.5 g/dL. But only after 15 seconds of close scrutiny is the outcome announced. For the hemocue method, the blood donor's finger is swabbed with alcohol to create a tiny drop of blood (about 0.01 ml).

The manufacturer's self-filling disposable cuvette is used to collect the drop of blood, which is then put into the machine. Within 60 seconds, the findings are shown. The measurement range for haemoglobin is 0–25.6 g/dL.

The Automated Haematology Cell Analyser uses two millilitres of blood drawn during phlebotomy coupled with dipotassium EDTA. After fully mixing each specimen tube on a mechanical mixer for at least two minutes, the analyser is used. The outcomes appear in less than 60 seconds.

STATISTICALANALYSIS

The mean ± standard deviation (SD), proportions, and p-value were used to express all the results. The goal was to compare the specificity, sensitivity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and accuracy of the two methods—the HemoCue technique and the Copper Sulphate method—with the Automated Haematology Analyser reference standard. SPSS 20.0 was used to evaluate the data.

RESULTS

During our study period of 6 months, a total of 60 donors were enrolled. The donors spanned across all groups and genders. The donors ages varied from 18 to 57, with majority falling within the 28-37 age range. 40 of the 60 donors who were deferred because of their low hemoglobin content were males (66.6%) and the remaining 20 donors were females (33.3%). The age and gender wise distribution of these donors is summarised in the Table 1 and Figure 1.

Table 1: Age and Sex-wise Distribution of Participants (n=60)

Age Group (Years)	Total Donors (n=60)	Male (n=40)	Female (n=20)
18–27	18 (30%)	12 (30%)	6 (30%)
28–37	20 (33.3%)	14 (35%)	6 (30%)
38–47	14 (23.3%)	9 (22.5%)	5 (25%)
48–57	8 (13.3%)	5 (12.5%)	3 (15%)
Total	60	40	20

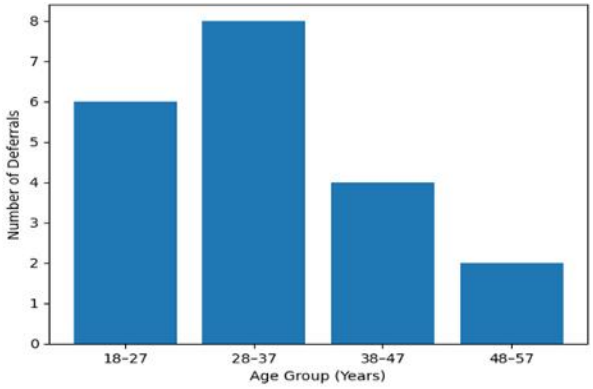

Figure 1: Age wise Distribution of Participants (n=60)

After Hb estimation by the three methods i.e, CuSO4 method, Hemocue & AHA, different values and levels of correlation were found while comparing each technique with standard method i.e., AHA. A somewhat positive correlation (r = 0.78) was shown between the copper sulphate technique and the Analyser, suggesting a decent but less exact agreement. Compared to the automated analyser, which was regarded as the reference standard, the HemoCue technique demonstrated a very significant positive correlation (r = 0.95), indicating great agreement and high dependability. The values have been summarised in Table 2 and represented in Figure 2.

Table 2: Mean Hb Values and Correlation with AHA (n=60)

Method	Mean Hb (g/dL) ± SD	Correlation (r) vs Analyzer
Copper Sulphate	12.1 ± 0.7	0.78
HemoCue	12.5 ± 0.6	0.95
Automated Analyzer	12.6 ± 0.5	-

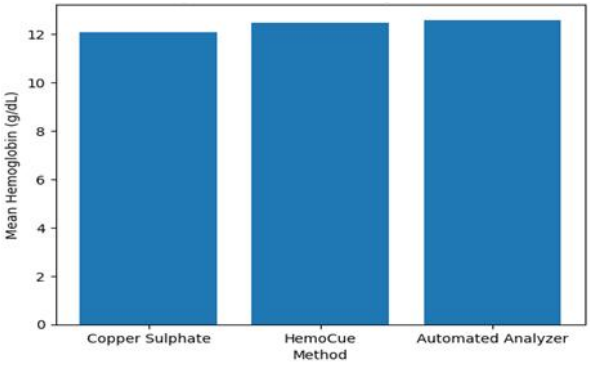

Figure 2: Mean Hb levels by CuSO4, Hemocue and Hematology analyser methods

The HemoCue approach showed better diagnostic performance than the copper sulphate method at a cut-off value of 12.5 g/dL. HemoCue demonstrated improved sensitivity (88.3%) and specificity (90.1%), demonstrating a greater capacity to accurately identify donors who are eligible and those who are not. The copper sulphate approach, on the other hand, showed a higher probability of misclassification due to its lower sensitivity (75.2%) and specificity (84.5%).

In a similar way, HemoCue had greater positive predictive value (PPV) and negative predictive value (NPV) (89.2% and 89.5%, respectively) than copper sulphate (80.3% and 81.0%). Overall, these results show that, at the specified cutoff, HemoCue offers more accurate and dependable haemoglobin estimate than the copper sulphate approach. The data of diagnostic accuracy has been condensed in Table 3 and Graphical represented in Figure 3.

Table 3: Diagnostic Accuracy of CuSO4 & Hemocue Methods (n=60)

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Copper Sulphate	75.2	84.5	80.3	81.0
HemoCue	88.3	90.1	89.2	89.5

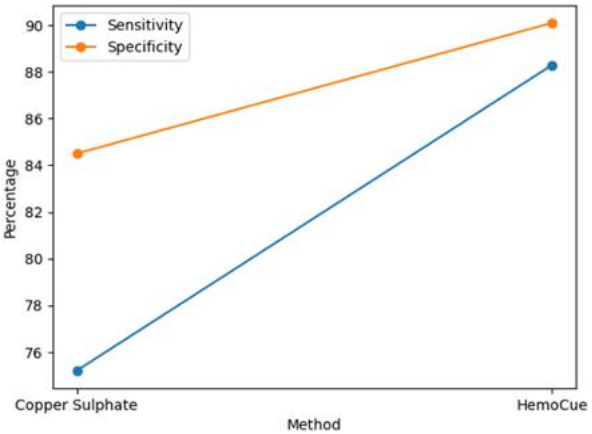

Figure 3: Diagnostic Accuracy of CuSO4 & Hemocue Methods (n=60)

DISCUSSION

Pre-donation haemoglobin screening must be trustworthy in order to prevent unnecessary donor deferrals, avoid unintentional phlebotomy from an anaemic donor, and guarantee appropriate product quality because low haemoglobin is one of the main causes of donor deferral. Haemoglobin (Hb) estimate is required by blood donor screening as the sole laboratory test to be carried out before the blood donation procedure.[1-4]

The Revised Guidelines for Donor Selection and Referral (Im11012/09/2022-NBTC/BTS)** mandates that a minimum of 12.5 gm/dl haemoglobin is essential for whole blood donors. In order to optimise donor safety and balance for sufficient availability, the cut-off Hb criteria have been developed for balancing blood safety along with both donor and patient safety.[3-6]

Demographic analysis revealed that 66.7% of deferred donors were male and 33.3% female, with the majority (38/60, 63.3%) in the 18–37 years age group. This pattern is similar to Shiraz et al. (2019)[4] who reported 81.25% of deferrals were male and 18.75% female, and Choudhari et al.[2] (2022) who found 95.4% male donors overall. The higher proportion of male deferrals in our study (despite males generally having higher Hb) may reflect the fact that more males present for donation in our setting; however, the proportion of female donors who were deferred (33.3% of all deferrals) is disproportionately higher given that females constituted a smaller percentage of total donors. Female donors were lesser due to lower number of women willing to donate blood in general, attributed to apprehension regarding blood donation, no previous history of donation, fear of needles, physiological variations such as menstruation, previous delivery or active breast feeding status, pallor, etc. This highlights the need for targeted nutritional interventions for female donors of reproductive age.

In our study, the mean hemoglobin values were 12.1 ± 0.7 g/dL (CuSO), 12.5 ± 0.6 g/dL (HemoCue), and 12.6 ± 0.5 g/dL (Automated Analyzer). The CuSO method consistently underestimated Hb by approximately 0.5 g/dL compared to the analyzer, leading to unnecessary deferrals. This observation is consistent with the findings of Tondon et al. (2009)[13] who reported that CuSO resulted in a mean underestimation of 0.4–0.6 g/dL, and Mohan et al. (2024)[3] who found that HemoCue overestimated Hb by 0.73 g/dL compared to the analyzer, while CuSO had a lower false-positive rate (19.3% vs. 40.4% for HemoCue). In contrast, Shiraz et al. (2019)[4] reported that HemoCue gave higher mean values (13.9 g/dL) than the analyzer (13.8 g/dL), with a difference of only 0.1 g/dL, suggesting that inter-study variability may be due to differences in donor populations, sample handling, or device calibration. The findings of our study in contrast with other studies are mentioned in Table 4.

Table 4: Comparative findings of present study with other studies [1,2,4]

Parameter	Present Study (2026)	Shiraz et. al. (2019)[4]	Choudhari et al. (2022)[2]	Opeyami et al. (2024)[1]
Sample size (n)	60 (deferred donors)	100	500	171
Mean Hb ± SD (g/dL) – CuSO	12.1 ± 0.7	Not reported as mean	Not reported as mean	Not reported
Mean Hb ± SD (g/dL) – HemoCue	12.5 ± 0.6	13.9 ± 1.5	Not reported as mean	13.19 ± 1.31
Mean Hb ± SD (g/dL) – Analyzer	12.6 ± 0.5	13.8	Not reported	13.43 ± 1.59
Correlation (r)- HemoCue vs. Analyzer	0.95	Not reported	κ = 0.458 (moderate agreement)	0.954
Correlation (r) – CuSO vs. Analyzer	0.78	Not reported	κ = 0.703 (good agreement)	Not applicable
Sensitivity – HemoCue	88.3%	98.28%	86.21%	98.2%
Specificity – HemoCue	90.1%	86.66%	91.30%	98.2%
Sensitivity – CuSO	75.2%	96.42%	75.86%	8.8%
Specificity – CuSO	84.5%	68.75%	97.88%	93.9%
PPV – HemoCue	89.2%	97.67%	37.88%	99.1%
NPV – HemoCue	89.5%	92.85%	99.08%	91.8%
PPV – CuSO	80.3%	94.18%	68.75%	41.7%
NPV – CuSO	81.0%	78.57%	98.50%	67.3%
False deferral rate – CuSO	Higher (lower specificity)	31.25% false passes	2.2% falsely deferred	Not applicable
Cost per test – CuSO	Very low (~ 0.05)	5	~ 0.06–0.08	Not reported
Cost per test – HemoCue	25–30	25	~ 30 (cuvette)	Not reported
Main conclusion	HemoCue more accurate; reduces unnecessary deferrals	HemoCue appropriate; CuSO as backup in poor areas	CuSO still viable as primary screening	HemoCue superior; CuSO poor sensitivity

The correlation analysis showed that HemoCue had a very strong positive correlation with the automated analyzer ($r = 0.95$, $p < 0.001$), whereas CuSO showed only moderate correlation ($r = 0.78$, $p < 0.01$). These results align with Choudhari et al. (2022)[2] who reported a good agreement ($\kappa = 0.703$) for CuSO and moderate agreement ($\kappa = 0.458$) for HemoCue with their reference analyzer, and with Opeyami et al. (2024)[1] who found correlation coefficients of 0.954 between HemoCue and the automated analyzer. The strong correlation of HemoCue supports its utility as a reliable point-of-care screening tool.

Regarding diagnostic accuracy at the 12.5 g/dL cutoff, our study demonstrated that HemoCue had a sensitivity of 88.3% and specificity of 90.1%, compared to CuSO with 75.2% sensitivity and 84.5% specificity. These values are comparable to those reported by Shiraz et al. (2019)[4] who found HemoCue sensitivity of 98.3% and specificity of 86.7%, and CuSO sensitivity of 96.4% and specificity of 68.8%. The slightly lower sensitivity in our study for HemoCue may be due to our exclusive inclusion of deferred donors (all had Hb < 12.5 g/dL by initial CuSO screening), creating a more challenging test population near the cutoff. Opeyami et al. (2024)[1] reported even higher values for HemoCue (sensitivity 98.2%, specificity 98.2%), while Mohan et al. (2024)[3] found CuSO had higher specificity (83%) than HemoCue (70%), which contrasts with our findings and those of Shiraz et al[4]. This discrepancy may be explained by differences in the proportion of borderline samples and operator experience.

The positive predictive value (PPV) and negative predictive value (NPV) in our study were 89.2% and 89.5% for HemoCue, and 80.3% and 81.0% for CuSO . These are consistent with Shiraz et al. (2019)[4] who reported PPV of 97.7% and NPV of 92.9% for HemoCue. The slightly lower PPV in our study again reflects our deferred donor population. Importantly, our false deferral rate (donors incorrectly identified as ineligible) was lower with HemoCue, which is critical for donor retention.

From a cost-effectiveness perspective, CuSO remains the cheapest method (approximately 0.02–0.05 per test) which is the only advantage compared to HemoCue (25–30 per test) and automated analyzers (10–25 per test), also reported by Tondon et al. (2009)[13] and Mohan et al. (2024)[3]. However, the cost of unnecessary deferrals—including loss of potential donors, recruitment costs, and reduced blood supply—must be considered as evidenced by other studies as well.[1-5,13-14] Our data suggest that HemoCue, while more expensive per test, reduces false deferrals by approximately 13% compared to CuSO (based on specificity difference), potentially offsetting its higher per-test cost in high- settings.

CONCLUSION

In conclusion, HemoCue demonstrates superior accuracy, sensitivity, and specificity compared to the CuSO method for hemoglobin screening in deferred donors. While CuSO method remains an economical option for primary screening in resource-limited settings it can cause redundant false deferrals. HemoCue should be employed as a confirmatory test for deferred donors, along with its implementation

in routine practices due to its portable, economical and easy-to use function. Additionally, the demographic analysis revealed that donor deferrals were more common among younger age groups and male donors, emphasizing generic need for pre-donation nutritional awareness programs and regular health monitoring.

LIMITATIONS

A limitation of our study is the relatively small sample size (n=60), which may affect generalizability. Additionally, we used only venous samples for HemoCue and the analyzer, whereas capillary samples are often used in field settings. Future larger multicenter studies should also evaluate newer noninvasive devices and assess the long-term impact of implementing HemoCue on donor retention and blood supply.

ETHICAL APPROVAL: The study protocol was approved by the Institutional Ethics committee and informed consent was obtained from all participants.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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