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Hemoglobin Estimation by Copper Sulphate and Hemocue Comparative Study in Relation to Donor Deferal

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Abstract

Background: Accurate hemoglobin (Hb) measurement plays a vital role in determining whether individuals are eligible to donate blood. A variety of methods are commonly employed for this purpose, including the copper sulfate technique, Hemocue devices. However, it is important to evaluate and compare these techniques to determine which are most dependable and effective for use in blood donor screening. Aim and Objective: The objective of this study was to evaluate and compare various hemoglobin estimation techniques namely Hemocue and copper sulfate in relation to donor deferral. **Materials and Methods:** This retrospective study involved1700eligible blood donors; after obtaining informed consent, data collection took place between 1stSeptember 2022to 31stNovember 2022. Capillary blood samples were used to measure hemoglobin levels using the copper sulfate ($CuSO_4$) and Hemo Cue methods. **Results:** The study participants had a median age of 32 years. Using the $CuSO_4$ gravimetric method, 1.5 % of donors were incorrectly classified as eligible, and 3.5% were wrongly deferred. The method demonstrated a specificity of 80.5 %, sensitivity of 96.1 %, positive predictive value of 98.2%, and negative predictive value of 65.1%. In comparison, the HemoCue® method showed better diagnostic accuracy with specificity, sensitivity, positive predictive value, and negative predictive value of 90.8%, 98.7%, 99.1%and 87.1% respectively. **Conclusion:** The $CuSO_4$ method is cost-effective and can provide reliable results when stringent quality control measures are in place. It can continue to serve as the initial screening tool; however, to minimize unnecessary donor deferrals, follow-up testing with a more accurate method such as HemoCue is recommended.

Keywords: Blood donation, Copper sulphate, Hemoglobin estimation, HemoCue, Automated analyser

Introduction

Blood donation is a critical component of healthcare, providing a reliable supply for transfusions during surgeries, emergencies, and the management of chronic illnesses. One of the primary criteria for determining a donor's eligibility is their hemoglobin (Hb) concentration. Ensuring that donors have adequate Hb levels is crucial for protecting both donor health and transfusion recipient outcomes (1).

While various methods exist to assess hemoglobin levels in donors, no single approach has been universally recognized as the standard for use in donation settings. Numerous studies have investigated the diagnostic accuracy of these rapid tests in identifying anemia and low hemoglobin levels (2,3,4). According to the Drugs and Cosmetics Act of 1940 and the Directorate General of Health Services Technical Manual (2003), individuals are considered eligible for whole blood donation only if their hemoglobin concentration is at least 12.5 g/dL (5).

When properly conducted, hemoglobin (Hb) screening accurately identifies individuals who meet the eligibility criteria for blood donation (6,7,8). The primary purpose of Hb screening is to safeguard donor health by preventing those with anemia from donating, thereby avoiding further deterioration of their condition. A secondary aim is to ensure that blood transfusion recipients receive an adequate hemoglobin dose with each red blood cell unit transfused (9) and to identify methods with high sensitivity and accuracy, while minimizing false deferral (rejecting eligible donors) and false-pass (accepting anemic donors) rates against a laboratory reference standard.

Different methods are commonly used to measure hemoglobin in prospective blood donors. These include Copper sulphate method, HemoCue, Automated hematology analyser.

CuS04 method is based on the principle of specific gravity 1.053.

The HemoCue hemoglobin photometer is a compact, battery-operated device commonly used for point-of-care hemoglobin estimation, particularly in mobile blood donation settings and critical care units [3]. It is based on principle of absorbance measurement of whole blood at an Hb/HbO2 isobestic point; dual wavelengths for Hb measurement and turbidity compensation.

Automated hematology analyzers are widely regarded as the reference ("gold standard") method for hemoglobin estimation. These systems employ spectrophotometric techniques that analyze lysed blood samples to deliver precise and quantitative hemoglobin values. The results are highly reliable and reproducible, making these analyzers the benchmark in many clinical and laboratory studies.

Our goal is to evaluate and compare the accuracy of two widely used hemoglobin estimation methods with automated hematology analyser.

- Copper sulfate specific gravity screening ($CuSO_4$ method)
- HemoCue hemoglobin photometer

The comparison focuses on how effectively each method reflects the actual hemoglobin levels of blood donors, using the automated analyzer as the reference standard.

Aim and Objective

The objective of this study was to evaluate and compare various hemoglobin estimation techniques namely Hemocue and copper sulfate in relation to donor deferral.

Materials and Methods

This retrospective analysis was carried out on 1,700 randomly selected, voluntary, non-remunerated altruistic blood donors over a three month period. All participants were fully informed of the aim of the study and their consent was obtained. Ethical clearance was obtained from the institute board.

Eligibility of Participants

Inclusion Criteria

- Healthy individuals presenting to the blood bank during the study period.
- Adults aged 18–65 years, of both sexes.
- Individuals weighing more than or equal to 45 kg.
- Female donors who were not menstruating at the time.
- Female donors not accepted during pregnancy and accepted after 1 year of lactation.

Exclusion Criteria

- Individuals who declined to participate were not included.
- Donors younger than 18 years or older than 65 years, or those weighing less than 45 kg, were excluded.
- Individuals with any health condition deemed unsafe for donation.
- Donors who had undergone major surgery within the past year or minor surgery in the past six months.
- Any donor currently on specific medications such as antibiotics.

Copper sulphate solution principle:

The copper sulphate method is based on the principle of specific gravity. A drop of blood dropped into copper sulphate solution of specific gravity 1.053 becomes encased in a sac of copper proteinate which prevents any change in specific gravity for about 10-15 seconds. If the drop of blood has a higher specific gravity than the solution it will sink within 10-15 seconds, if not then the drop will hesitate, remain suspended or rise to the top of solution.

Copper sulphate solution preparation:

The Copper sulphate working solution shall be prepared by dissolving 8.33 gms of pure air dried crystal Copper sulphate in 100 ml distilled water likewise for 1000 ml of distilled water add 83.3 gms of pure air dried Copper sulphate crystal and keep in jar or white can. Date of preparation of Copper sulphate solution is written on the white can. Check the specific gravity of the working solution. It should be 1.053. if not, adjust it using either Copper sulphate crystals or distilled water. If the solution appears cloudy or shows precipitate, discard the solution.

Each donor's hemoglobin level was measured using all three methods, and it was observed that the $CuSO_4$ and HemoCue methods differed by 0.2–0.5 g/dL when compared to the automated hematology analyzer. Therefore, the automated hematology analyzer was considered the gold standard method against which all other methods were assessed.

Sample Collection Procedure

Capillary blood samples were collected from retrospective blood donors by pricking the index or middle finger of the left hand using a sterile, dry lancet after disinfecting the area with ethanol. The donor was seated comfortably, and the finger was gently massaged to promote blood flow. The initial drop of blood was discarded, while the second and third drops were used—one collected into a capillary tube for the $CuSO_4$ gravimetric method and the other into a microcuvette for the HemoCue method, with the testing order alternated between samples (10,11). Additionally, two milliliters of venous blood were drawn into EDTA-anticoagulated vacutainer tubes and promptly measured using Mindray hematology analyzer.

The $CuSO_4$ method is based on the principle that a drop of whole blood, when introduced into a copper sulfate solution of a specific gravity (1.053), will sink and maintain its density for about 15 seconds if the hemoglobin concentration is adequate.

The HemoCue includes disposable microcuvettes pre-loaded with dry reagents and a dedicated photometer designed specifically for hemoglobin measurement. The microcuvettes were stored in a dry environment at room temperature, and once the container was opened, it was sealed tightly and kept under the same conditions to preserve their quality and shelf life. The chemical reaction within the microcuvette is a modified azide-methemoglobin method. In this process, sodium deoxycholate lyses the red blood cells, releasing hemoglobin. Sodium nitrite then converts the hemoglobin to methemoglobin, which reacts with sodium azide to form azide methemoglobin.The absorbance is measured at two wavelengths (570 nm and 880 nm) in order to compensate for turbidity in the sample.

Capillary blood samples were tested onsite for hemoglobin (Hb) using the $CuSO_4$ gravimetric method and the HemoCue system (10,11).

Quality Control:

The $CuSO_4$ working solution, with a specific gravity of 1.053, was prepared and standardized according to the standard operating procedure (SOP) before use. The HemoCue device HB 301 system comes factory-calibrated against the ICSH reference method, and is built – in selftest, liquid controls. Mindray BC -20 hematology analyser is standardized using following methods- 1.Internal Quality Control (IQC), 2. External Quality Assurance (EQA) / Proficiency Testing, 3.Instrument Maintenance & Calibration.

Data Analysis

Data entry, cleaning, and storage were carried out using Microsoft Excel 2021. To ensure data quality, the data collection tools were thoroughly reviewed. The dataset was then exported to Epi Info version 7.2.2.6 for statistical analysis, with a backup copy created to safeguard against any potential data loss during the analysis process. Categorical variables were summarized using frequencies and percentages. The diagnostic performance of $CuSO_4$ and HemoCue methods was assessed by calculating sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) using the automated hematology analyzer as the reference or gold standard method (12,13).

Results of copper sulphate were interpreted as selected or deferred at Hb cut-off of>12.5 g/dl. The sensitivity, specificity, positive and negative predictive values (PPV and NPV) of each method was calculated.

Results

The donors were aged between 18 and 60 years. Out of the 1700 participants, most were male, comprising 84.47% of the sample, whereas females made up 15.53%. The deferral rate was significantly higher among female donors at 73.25%, compared to 26.75% among male donors. [Table 1].

Table 1. Demographic Characteristics of Donors.

Gender	Selected	Defered	Total
Male	1390(90.96%)	46(26.75%)	1436(84.47%)
Female	138(9.04%)	126(73.25%)	264(15.53%)
Total	1528(89.88%)	172(10.12%)	1700(100%)

The hemoglobin levels among blood donors ranged from a minimum of 9.5 g/dl to a maximum of approximately 16.5 g/dl. Hemoglobin measurements obtained by all methods were analyzed, and a summary comparing these methods with the reference hematology analyzer is presented [Table 2].

Table 2.Deferral data of the three hemoglobin estimation methods in different levels of hemoglobin level according to cell counter method

HB Value	Automated analyser	CuSo4 Accepted	Deferred	Hemocue Accepted	Deferred
9.5–10.9	15	0	15	0	15
11.0-11.9	20	0	20	0	20
12.0-12.4	48	16	32	0	48
12.5–13.9	1280	118298		127010	
14.0–16.4	282	2757		282	0
>16.5	55	55	0	0	55
	1700	1528	172	1552	148

The HemoCue method demonstrated greater efficiency, with a sensitivity of 98.7%, specificity of 90.8%, positive predictive value (PPV) of 99.1%, and negative predictive value (NPV) of 87.1%. In contrast, the $CuSO_4$ method showed lower specificity at 80.5%, sensitivity of 96.1 %, PPV of 98.2%, and NPV of 65.1%. The $CuSO_4$ screening test incorrectly classified 27 out of 1700 donors (1.5 %) as eligible; among them, 1335 had hemoglobin levels between 12.5 and 13.9 g/dl and more than 16.5 g/dl when measured by the cell counter. Additionally, 60 donors (3.5%) were wrongly deferred by the $CuSO_4$ method. A comparison of the different methods used in this study is presented [Table 3].

Table 3: Performance characteristics of two methods for Hb estimation of blood donors

Result	CuSo4	Hemocue
True Positive	1501	1539
True Negative	112	129
False Positive	27	13
False Negative	60	19
Sensitivity	96.1%	98.7%
Specificity	80.5%	90.8%
Positive predictive value	98.2%	99.1%
Negative predictive value	65.1%	87.1%

Discussion

Precise measurement of hemoglobin levels in blood donors is essential for safeguarding the health of both donors and transfusion recipients. The semi-quantitative copper sulfate gravimetric method, due to its simplicity and low cost, has long been the conventional approach for screening blood donors in many centers. For years, it has been widely adopted across countries as the primary method for initial hemoglobin assessment in prospective donors. The method is cost-effective, quick, and does not require a venous blood sample. However, it demands thorough staff

training and continuous supervision. Strict quality control and validation are essential before it can be used for donor screening. Since it does not provide a quantitative hemoglobin value, there is always a risk of false acceptance or deferral.

The primary objective of this study was to assess and compare the accuracy of two widely used hemoglobin estimation methods—Copper Sulfate ($CuSO_4$) and HemoCue photometer—in determining hemoglobin levels in blood donors. This cross sectional study included 1700 randomly selected voluntary, non-remunerated, altruistic donors and was conducted over a three-month period. The $CuSO_4$ method and Hemocue provided outcomes, classified as either accepted or rejected. For each method, sensitivity, specificity, and both positive and negative predictive values were calculated.

In our study, the $CuSO_4$ method incorrectly passed 1.5% of donors, most of whom had hemoglobin levels within 1.0 g/dL of the threshold when compared to the reference values. This finding was lower as compared with the results reported by Rashmi et al. [14] and similar with result reported by James et al. [15]. Similarly, Boulton et al. [16] also noted a similar rate of inappropriate passes using the $CuSO_4$ method, with most discrepancies falling within 1.0 g/dL of the gender-specific threshold.

Polycythemia is a hematologic condition characterized by an elevated red blood cell (RBC) mass, commonly reflected in increased hemoglobin (Hb) and hematocrit (Hct) levels. In the context of blood donation, polycythemia is of particular relevance due to both donor health implications and the quality of blood products collected.
In most blood donor settings, a hemoglobin concentration of >18 g/dL in males or >16.5 g/dL in females typically results in temporary deferral of donation. This precaution is primarily to safeguard donor well-being, as polycythemia can significantly increase blood viscosity, thereby elevating the risk of thrombosis, stroke, and cardiovascular complications. Additionally, the hyperviscous blood may compromise the quality of the donation by impeding flow during collection, potentially leading to incomplete or clotted units.

When high Hb is identified, it is critical to reconfirm the value to exclude pre-analytical errors. Donors with persistently high Hb should be evaluated for contributing factors such as medications, supplements (e.g., testosterone, anabolic steroids), or undiagnosed medical conditions. In such cases, donation should be deferred, and the individual should be referred for further medical evaluation.
Further diagnostic work-up for suspected polycythemia includes a complete blood count (CBC) to assess the red cell mass, serum erythropoietin (EPO) levels to differentiate between primary and secondary causes, and JAK2 V617F mutation testing—which is positive in approximately 95% of cases of polycythemia vera, a myeloproliferative neoplasm. Additionally, oxygen saturation should be measured to rule out chronic hypoxic states, such as those caused by lung disease or sleep apnea,

which can lead to secondary polycythemia.

Another advantage of hemocue over other photometric methods isits built-in turbidity control, which allows for more accurate measurements in lipemic samples [16]. While HemoCue is a reliable tool for hemoglobin testing in blood donors, its high cost is a significant limitation. Additionally, proper staff training is essential, as errors such as air bubbles or fingerprints and blood on the cuvette surface can lead to inaccurate results.

In our study, the HemoCue method showed a sensitivity of 98.7%, in comparison with the findings of Sawant et al. [17] and Rashmi et al. [14].HemoCue is user-friendly, requires minimal training, and provides immediate results. It is particularly valuable in both clinical and epidemiological contexts, where capillary blood sampling through finger prick offers a simpler, less resource-intensive alternative to venous sampling and is generally more acceptable to both patients and the broader community.

Conclusion

The method chosen for hemoglobin screening in blood donors should be both reliable and cost-effective. The $CuSO_4$ method continues to be a dependable option and can serve as the primary screening tool. However, using HemoCue as the initial screening method may be financially burdensome for some blood centers. An efficient approach could involve re-evaluating donors who are deferred by the $CuSO_4$ method using HemoCue to confirm whether deferral is truly necessary. This strategy may be particularly beneficial for resource-limited blood centers, especially during donation camps where large-scale hemoglobin screening is required.

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