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COMPARATIVE STUDIES OF HAEMOGLOBIN IN BLOOD DONORS USING HEMOCUE, COPPER SULPHATE, AUTOMATED ANALYSER, AND PACKED CELL VOLUME IN FEDERAL MEDICAL CENTER, ABEOKUTA

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ABSTRACT

Background: Accurate hemoglobin (Hb) estimation is essential in determining the eligibility of blood donors. Various methods such as the copper sulfate method, Hemocue, automated analyzers, and packed cell volume (PCV) are widely used. However, there is a need to compare these methods to assess their reliability and effectiveness in blood donation screening.

Objective: This study aimed to compare hemoglobin estimation methods, including Hemocue, copper sulfate, automated analyzer, and PCV, to determine their accuracy and reliability in screening blood donors at the Federal Medical Center, Abeokuta.

Materials and Methods: This hospital-based, cross-sectional study was conducted among 171 blood donors at the Federal Medical Center, Abeokuta, Nigeria, between October and November 2020. Blood samples were collected and analyzed using the copper sulphate gravimetric method, Hemocue Hb 201 photometric method, automated analyzer (Coulter Ac.T diff2), and PCV. Data were analyzed using SPSS version 22, and a p-value of <0.05 was considered statistically significant.

Results: Hemocue had the highest sensitivity (98.2%) and specificity (98.2%) compared to the other methods. The copper sulphate method demonstrated low sensitivity (8.8%) but high specificity (93.9%). Statistically significant correlations were found between Hemocue, automated analyzer, and PCV methods ($p<0.05$). Bland-Altman plots indicated no proportional bias between Hemocue and PCV but revealed bias between PCV and the automated analyzer.

Conclusion: Hemocue and automated analyzers showed superior performance in hemoglobin estimation compared to the copper sulfate method. Hemocue's accuracy and rapid results make it the preferred method for donor screening, while the copper sulfate method may result in misclassification.

Keywords: Hemoglobin estimation, Hemocue, Copper sulfate, Automated analyzer, Packed cell volume, Blood donors.

INTRODUCTION

Blood donation is a critical component of healthcare services, as it ensures the availability of blood for transfusions in a variety of clinical settings, including surgeries, trauma care, and the management of chronic diseases such as anemia and hemophilia. Hemoglobin concentration (Hb) is one of the primary criteria used to assess the suitability of blood donors because it serves as an indicator of anemia and the oxygen-carrying capacity of the blood. Ensuring that donors have adequate hemoglobin levels helps to protect the health of both donors and recipients (World Health Organization [WHO], 2020).

The measurement of hemoglobin is essential in blood donation screening because low levels of hemoglobin can indicate anemia, which may disqualify an individual from donating blood. Moreover, a transfusion with blood that has low hemoglobin concentration may not achieve the desired therapeutic effect, making hemoglobin estimation crucial for donor eligibility and recipient safety (de Korte et al., 2019). According to WHO (2020), blood donors must meet specific hemoglobin thresholds, typically 12.5 g/dL for females and 13.0 g/dL for males, to ensure that blood donation does not negatively affect the donor's health.

Different methods are employed to measure hemoglobin levels in prospective blood donors. These methods include the copper sulfate method, HemoCue, automated hematology analyzers, and packed cell volume (PCV). Each of these methods has unique advantages and limitations that affect their reliability, accuracy, and usability in blood donation centres.

The copper sulfate method is widely used in resource-limited settings due to its simplicity and low cost. In this method, a drop of blood is placed in a copper sulfate solution, and the rate of sinking is used as an indicator of hemoglobin concentration (Lamb et al., 2021). Blood with adequate hemoglobin levels will sink at a specific rate, while blood with low hemoglobin will float or sink slowly. Although cost-effective, the copper sulfate method is semi-quantitative and subject to operator error, as well as temperature and density fluctuations (Mitchell et al., 2020). It remains useful in developing countries where more advanced technologies may not be available.

The HemoCue system is a portable, point-of-care testing device that measures hemoglobin levels using a small sample of capillary or venous blood. The device provides quick and reliable results and is commonly used in clinical and field settings (Butler et al., 2019). HemoCue devices are widely regarded as accurate, with studies showing their strong correlation with results obtained from automated analyzers (Schnebele et al., 2021). However, factors such as sample size and blood handling can affect the accuracy of HemoCue results (Patton et al., 2022).

Automated hematology analyzers are considered the gold standard for hemoglobin estimation, providing precise, quantitative measurements through advanced technology (Bain, 2021). These analyzers typically operate using spectrophotometric techniques to measure hemoglobin concentrations, offering highly accurate results (Weiss et al., 2019). However, the cost and maintenance requirements of automated analyzers can be prohibitive for many blood donation centers, particularly in low-income settings (Brugnara, 2020).

Packed cell volume (PCV), also known as hematocrit, measures the proportion of blood volume occupied by red blood cells and is another indirect method of assessing hemoglobin concentration. The relationship between PCV and hemoglobin concentration is well established, with PCV typically being about three times the hemoglobin concentration in grams per deciliter (WHO, 2020). PCV measurement is relatively simple and inexpensive, but its accuracy can be affected by dehydration and other factors (Bain, 2021). Nonetheless, it remains a valuable tool in many blood donation settings where access to more advanced equipment may be limited.

The accuracy, cost-effectiveness, and feasibility of these hemoglobin measurement methods have been the subject of several studies, particularly in the context of blood donation centers. In resource-constrained environments like Nigeria, the choice of hemoglobin estimation method often depends on the availability of technology and the cost of implementation (Mitchell et al., 2020). Comparative studies have shown that while automated hematology analyzers provide the most accurate results, the HemoCue system and PCV measurements offer acceptable alternatives in settings where automated analyzers are not available (Brugnara, 2020).

A study by de Korte et al. (2019) compared the performance of HemoCue, copper sulfate, and automated analyzers in blood donation centers. The study found that while HemoCue and automated analyzers offered similar accuracy, the copper sulfate method was less reliable, often underestimating hemoglobin levels. Another study by Schnebele et al. (2021) demonstrated that PCV measurements correlated well with automated analyzers but were less accurate in individuals with abnormal red cell morphology.

In the context of Nigeria, the importance of using cost-effective and reliable methods for hemoglobin screening in blood donation centers cannot be overstated. The Federal Medical Center in Abeokuta, like many other health facilities in the country, faces challenges such as limited access to advanced medical technologies, necessitating the use of methods like copper sulfate and PCV (Mitchell et al., 2020). Therefore, it is crucial to conduct comparative studies to evaluate the performance of different hemoglobin estimation methods and determine which provides the best balance between accuracy, cost, and practicality in Nigerian healthcare settings.

MATERIALS AND METHODS

Study Design

This was a hospital-based analytical cross-sectional study carried out among family/replacement and voluntary non-remunerated blood donors at Federal Medical Center (FMC) Idi-Aba, Abeokuta, Ogun State. The study was carried out between October and November 2020.

Study Population

The subjects included in this study were new prospective males and females within the age range of 18-55 years blood donors at the Federal Medical Center, Abeokuta, Ogun State, Nigeria after obtaining informed consent and administration of pre-donation questionnaire to participating subjects. This study was carried out using a continuous convenient sampling technique.

Sample Size Determination

The sample size was determined using the Cochran formula for estimating proportions in a population outlined by Uduma et al. (2023):

$$n = \frac{Z^2(Pq)}{e^2}$$

where n = minimum sample size

Z = 1.96 at 95% confidence level,

P = known/expected prevalence

e = error margin tolerated at 5% = 0.05

q = 1 - p

The existing prevalence of Sickle cell anaemia is 13.7%.

P = 13.7% = 0.137

q = 1 – p

= 1 – 0.137

= 0.863

$$n = \frac{(1.96)^2(0.137 \times 0.863)}{(0.05)^2}$$

$$n = \frac{3.8416 \times (0.118231)}{0.0025}$$

$$n = \frac{0.454}{0.0025} = 177$$

A total of 177 blood specimens were collected from consenting blood donors at Federal Medical Center, Abeokuta, Ogun state.

Ethical Consideration

Ethical clearance was obtained from the Babcock University Health Research Ethics Committee (BUHREC) before the commencement of the study. Informed consent was obtained from each subject. The purpose and nature of the study as well as the method of the sample collection was explained to them properly. All information collected in this study were given code numbers and no name was recorded.

Eligibility of Subjects

Inclusion Criteria

- ✓ Healthy whole blood donors who came to the blood bank during the period at the Federal Medical Center, Abeokuta
- ✓ Male and female subjects within the age range of 18-50
- ✓ Male and female subjects that weigh at least 45 kg
- ✓ Women who are not currently seeing their monthly flow or have not menstruated in 2 weeks

Exclusion Criteria

- Any prospective subject that refused to participate in this study was excluded
- Subjects of less than 18 years or more than 55 years of age, weigh less than 45 kg
- Unhealthy subjects
- Subject that had undergone any major surgery in the last year or minor surgery in the last six months
- Any subject taking any specific medications like antibiotics, aspirin, antithyroid drugs etc.,
- Subjects with hemolytic anemia such as sickle cell disease.

Specimen Collection

Blood specimen was collected from each subject via venous puncture and finger prick method with the assistance of a trained phlebotomist. Specimen were not stored because they needed to be worked on as soon as possible and in order to avoid false positive or false negative results.

Techniques For Hemoglobin Estimation

The hematological parameter that was investigated in this study was Haemoglobin and a comparative study was carried out using the following methods;

1. Gravimetric method (Copper sulphate method)
2. Photometric method (HemoCue Hb photometer and Hematology autocell analyser)
3. Packed cell volume

Gravimetric Method (Copper Sulphate Gravimetric Method)

Principle Of Copper Sulphate Method

The principle is based on the specific gravity of blood. A blood droplet was allowed to fall into a copper sulphate solution of a specific gravity of 1.053 and the movement of the droplet was observed for at least 15 seconds.

Preparation of copper sulphate solution

In the preparation of 1% copper sulphate working solution, one gram of copper sulphate powder was weighed using a weighing balance and it was dispensed into a clean 100 ml beaker. 100 ml of distilled water was added to the beaker and it was stirred gently till the powder was completely dissolved. The copper sulphate was transferred into a beaker, corked and stored at room temperature.

Test Procedure for Copper Sulphate Method

One drop of blood (0.05 ml approximately) was collected from the site of an alcohol-swabbed finger of the blood donor. The working copper sulphate solution (of specific gravity 1.053) was taken in a clear beaker, having a depth of at least 3 inches. A drop of blood was then allowed to fall into a 30mL solution from a height of 1 cm. If the hemoglobin content is more than 12.5 g/dL, the droplet of blood sinks and if it is less than 12.5 g/dL, it floats. The result was declared only after 15 seconds of careful observation.

Hemocue Hb Photometric Method

The HemoCue Hb 201^{+} System is used for the quantitative determination of hemoglobin in blood using a specially designed analyzer, HemoCue Hb201^{+}, and specially designed HemoCue Hb 201 Micro-cuvettes. This is a non-dilution system where Sodium deoxycholate haemolyses the erythrocytes and hemoglobin are released. Hemocue 201 is factory-calibrated against the ICSH reference method and therefore needs no further calibration. Sample material included venous or arterial whole blood. The measurement range is 0–25.6 g/dL (0–256 g/L, 0–15.9 mmol/L). Results are produced within 60 seconds. Only 10 µL of venous blood was needed to carry the test

Principle of Hemocue Method for Hemoglobin Estimation

The hemoglobin concentration in blood is determined as azidemethemoglobin utilizing a microcuvette with a dry reagent system and a dual photometer. The erythrocyte membranes are disintegrated by sodium deoxycholate, releasing the hemoglobin. Sodium nitrite converts the hemoglobin iron from the ferrous to ferric state to form methemoglobin, which then combines with sodium azide to form azidemethmoglobin. Measurements are taken at 570nm and 880nm. (Srivastava et al., 2014).

Test Procedure of Hemocue Method for Hemoglobin Estimation

A fresh, well-mixed anticoagulated venous blood sample was used. The samples were mixed on a mechanical mixer for 2 minutes. A drop of blood from the anticoagulated bottle was dispensed onto a hydrophobic surface of the micro cuvette. Excess blood was wiped off from the outside of the cuvette using a clean tissue, making sure not to touch the open end of the cuvette. The cuvette was examined visually for air bubbles in the optical eye. The filled cuvette was placed into the cuvette holder and positioned in the measuring position. The result was then displayed within 60 seconds.

Automated Hematology Cell Analyzer for Hemoglobin Estimation

The automation method using hematological autoanalyzer is a quantitative automated hematology analyzer for in-vitro diagnostic use, which can determine 20 hematological parameters. It directly measures hematocrit, total WBC counts, RBC counts, hemoglobin (HGB), PCV, platelets count, absolute lymphocytes count and absolute mixed count while parameters like MCH, MCV, MCHC, and red cell distribution width are calculated.

The principle of electrical impedance is applied. Haemoglobin is measured using the photometric method which consists of a photodiode, a cuvette with a length of 15mm and a filter wavelength of 535nm (bandwidth 20nm). The blood sample was aspirated through the aspiration needle by gently inserting the aspiration needle into the sample tube and then pressing the whole blood start plate behind the aspiration needle. After about 45 seconds the result was displayed on the sample menu.

Quality Control

The working $CuSO_4$ solution was prepared (specific gravity 1.053) and was standardized before use according to the standard operating procedure (SOP). The HemoCue (HemoCue® Hb 201 System) instrument is factory-calibrated and its quality control is done quarterly. The automated blood cell counter (Coulter Ac. T diff2 Hematology Analyzer) is calibrated annually and its quality control was done before use with the stabilized control reagents provided

Reference Range

Hemoglobin concentration value:

Adult male: 14-18g/dL

Adult female: 13-15g/dL

CuSO4:

Blood sinks indicate Hb>12.5g/dL (Pass)

Blood sinks indicate Hb<12.5g/dL (Fail)

PCV

Adult male: 42-52%

Adult Female: 35-47%

Data Analysis

The collected data was entered into Microsoft Excel. Results of copper sulphate were interpreted as pass or fail at the Hb cut-off of > 12.5 g/dl. The sensitivity, specificity, positive and negative predictive values (PPV and NPV) of each method was calculated. Statistical analysis was then carried out using SPSS (Statistical Package for Social Sciences) version 22 (IBM Incorporated). A p-value less than 0.05 was considered statistically significant.

RESULTS

The socio-demographic characteristics (Table 1) of the respondents show that the majority (45.6%) were aged 31-40 years, with the highest percentage being Christians (69.6%) and Yoruba (91.2%). Most respondents had tertiary education (54.4%), and 50% were single.

In terms of hemoglobin concentration and hematocrit levels, Table 2 shows that the mean hemoglobin value using the Hemocue 201 method was 13.19 g/dL, slightly lower than the ACT hematolyzer method at 13.43 g/dL. The calculated Hb from PCV was 13.33 g/dL, demonstrating consistency across methods.

Comparison between donor types (Table 3) indicates that voluntary donors had a lower mean hemoglobin concentration (12.27 g/dL) compared to family/relative donors (13.25 g/dL) using the Hemocue method, although this difference was not statistically significant ($p > 0.05$). However, PCV values showed a significant difference ($p = 0.033$), with voluntary donors having lower PCV percentages than family donors.

Table 4 examines the association between the CuSO4 method and Hb concentration fit for donation, showing no significant association ($p = 0.525$). However, both the ACT hematolyzer (Table 5) and Hemocue methods (Table 6) were significantly associated with donation fitness, with p-values < 0.001, indicating these methods are reliable in determining fitness for blood donation.

The sensitivity and specificity of the Hemocue and ACT hematolyzer methods are high (98.2% for both) (Table 7), with excellent predictive values, confirming their accuracy in hemoglobin determination compared to CuSO4, which had low sensitivity (8.8%).

Correlation analysis (Table 8) revealed a strong positive correlation between the different hemoglobin estimation methods, with Hemocue and PCV showing the highest correlation ($R = 0.969$, $p < 0.001$). Reliability analysis (Table 9) further confirms that all methods had high intra-class coefficients, particularly between PCV and Hemocue (0.982, $p < 0.001$), indicating excellent reliability.

Bland-Altman plots (Figures 2-4) show agreement between the methods, although there was proportional bias between PCV and the ACT hematolyzer, as well as between Hemocue and the ACT hematolyzer, indicating that these methods' agreement may depend on the actual hemoglobin value measured.

Table 1: Socio-Demographic Characteristics of the Respondents

Characteristics	Category	Frequency (n=171)	Percentage (%)
Age (years)	18-25	51	29.8
	26-30	32	18.7
	31-40	78	45.6
	41-50	9	5.3
	>50	1	0.6
	Total	**171**	**100.0**
Religion	Christianity	119	69.6
	Islam	52	30.4
	Total	**171**	**100.0**
Marital status	Single	87	50.0
	Married	82	48.0
	Divorced	2	1.2
	Total	**171**	**100.0**
Ethnicity	Yoruba	156	91.2
	Ibo	10	5.8
	Hausa	3	1.8
	Others	2	1.2
	Total	**171**	**100.0**
Educational level	Primary	1	0.6
	Secondary	75	43.9
	Tertiary	93	54.4
	None	2	1.2
	Total	**171**	**100.0**

Table 2: Variation of different hematocrit and hemoglobin concentration methods among study subjects

Method	Number of participants (N)	Mean (X)	Standard deviation (s)
Hemocue 201 Hb (g/dl)	171	13.19	1.31
ACT hematolyzer Hb (g/dl)	171	13.43	1.59
PCV (%)	171	40	3.94
Calculated Hb from PCV/3	-	13.33	-

*N= Total Number of subjects

X=Mean Hemoglobin value

s= Standard Deviation

Table 3: Comparison of different methods of estimating Hemoglobin concentration in relation to Donor type

DONOR TYPE	**HEMOGLOBIN ESTIMATION METHOD**						
	HemoCue® (g/dL)	**Ac.T Hematolyzer(g/dL)**	**PCV (%)**	**$CuSO_4$**			
				Failed <12.5g/dL	**Passed >12.5g/dL**	**Total**	**Total**
Relative/ Family Donor	13.25±1.25	13.49±1.54	40.34±3.81	10(6.2%)	150(93.8%)		
Voluntary Donor	12.27±1.79	12.55±2.16	37.73±5.08	2(18.2%)	9(81.8%)		
T	1.77	1.421	2.152	-	-	-	-
P value	0.104	0.184	0.033*	0.134	0.134	-	-
χ2	-	-	-	2.246	2.246	-	-

*** χ2 =Chi square**

P value=

T=Difference represented in units of standard error

Table 4: Association between $CuSO_4$ method of hemoglobin determination and Hb Concentration fit for Donation among study subjects

	$CuSO_4$				
Hb Concentration fit for Donation	**Failed <12.5g/dL**	**Passed >12.5g/dL**	**Total**	**χ2**	**P value**
Not fit (PCV <40%)	5	52	57	0.403	0.525
Fit (PCV ≥40)	7	107	114		
Total	12	159	171		

*** χ2 =Chi square**

P value= probability value

Table 5: Association between Ac.T hemotolyzer for hemoglobin determination and Hb Concentration fit for Donation among study subjects

	ACT hemotolyzer hemoglobin determination				
Hb Concentration fit for Donation	**Failed <12.5g/dL**	**Passed >12.5g/dL**	**Total**	**χ2**	**P value**
Not fit (PCV <40%)	5	1	114	171.703	0.000*
Fit (PCV ≥40)	56	109	57		
Total	61	110	171		

*** χ2: Chi square**

p-value: probability of test

Table 6: Association between Hemocue® Hb 201 method for hemoglobin determination and Hb Concentration fit for Donation among study subjects

	HemoCue®				
Hb Concentration fit for Donation	**Failed** <12.5g/dL	**Passed** >12.5g/Dl	**Total**	**χ2**	**P value**
Not fit (PCV <40%)	56	1	57	188.84	0.000*
Fit (PCV ≥40)	2	112	114		
Total	58	113	171		

Table 7: Optimum Sensitivity and specificity of Hemocue 201 for hemoglobin determination, Ac.T hemotolyzer as a diagnostic screening tool for anemia

	Sensitivity (%)	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**AUC**	**Cut-off value (g/dL)**	**95% CI**	**P-value**
HemoCue 201 hemoglobin determination (g/dL)	98.2	98.2	99.1	96.6	0.957	10.5	0.929-0.986	0.000*
Ac.T hemotolyzer	95.6	98.2	99.1	91.8	0.971	10.5	0.944-0.998	0.000*
$CuSO_4$	8.8	93.9	41.7	67.3	N/A	N/A	N/A	N/A

*PPV: Positive Predictive Value

NPV: Negative Predictive Value

AUC: Area Under Curve

CI: Confidence Interval

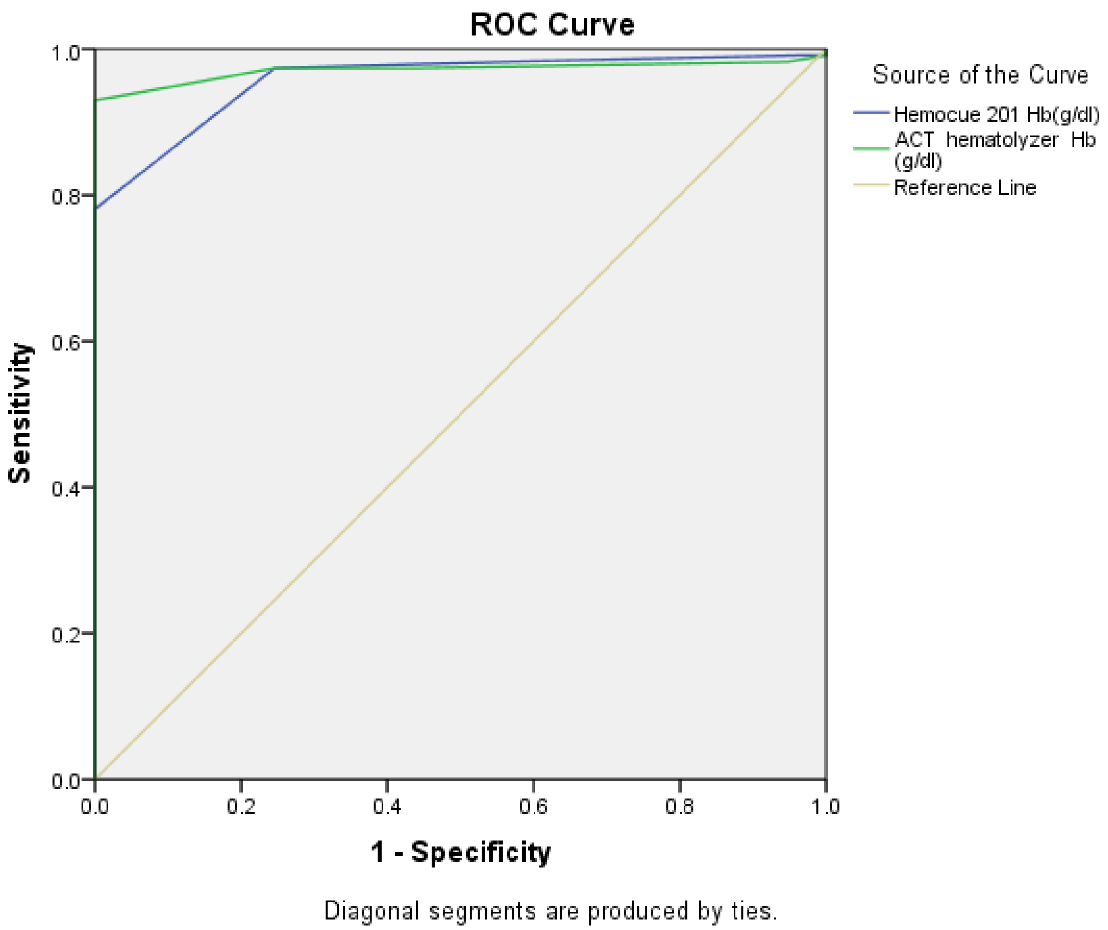

Figure 1: Area under curve of Hemocue 201 and ACT hemotolyzer in the determination of hemoglobin level

Table 8: Linear Correlational Analysis of Different Methods of estimating Hemoglobin concentration methods among study subjects

		Hemocue 201	ACT hematolyzer	PCV
Hemocue 201 (g/dL)	R	1	0.954	0.969
	p-value		0.000*	0.000*
ACT hematolyzer (g/dL)	R	0.954	1	0.959
	p-value	0.000*		0.000
PCV (%)	R	0.969	0.959	1
	p-value	0.000*	0.000	

Table 9: Reliability Analysis of Different Hb estimation methods among study subjects

Method	Intra class coefficient	Range	95% CI	p-value
PCV and ACT hematolyzer	0.970	0.016	0.960-0.978	0.000
PCV and Hemocue 201	0.982	0.120	0.946-0.977	0.000
ACT hematolyzer and Hemocue 201	0.937	0.104	0.915-0.954	0.000

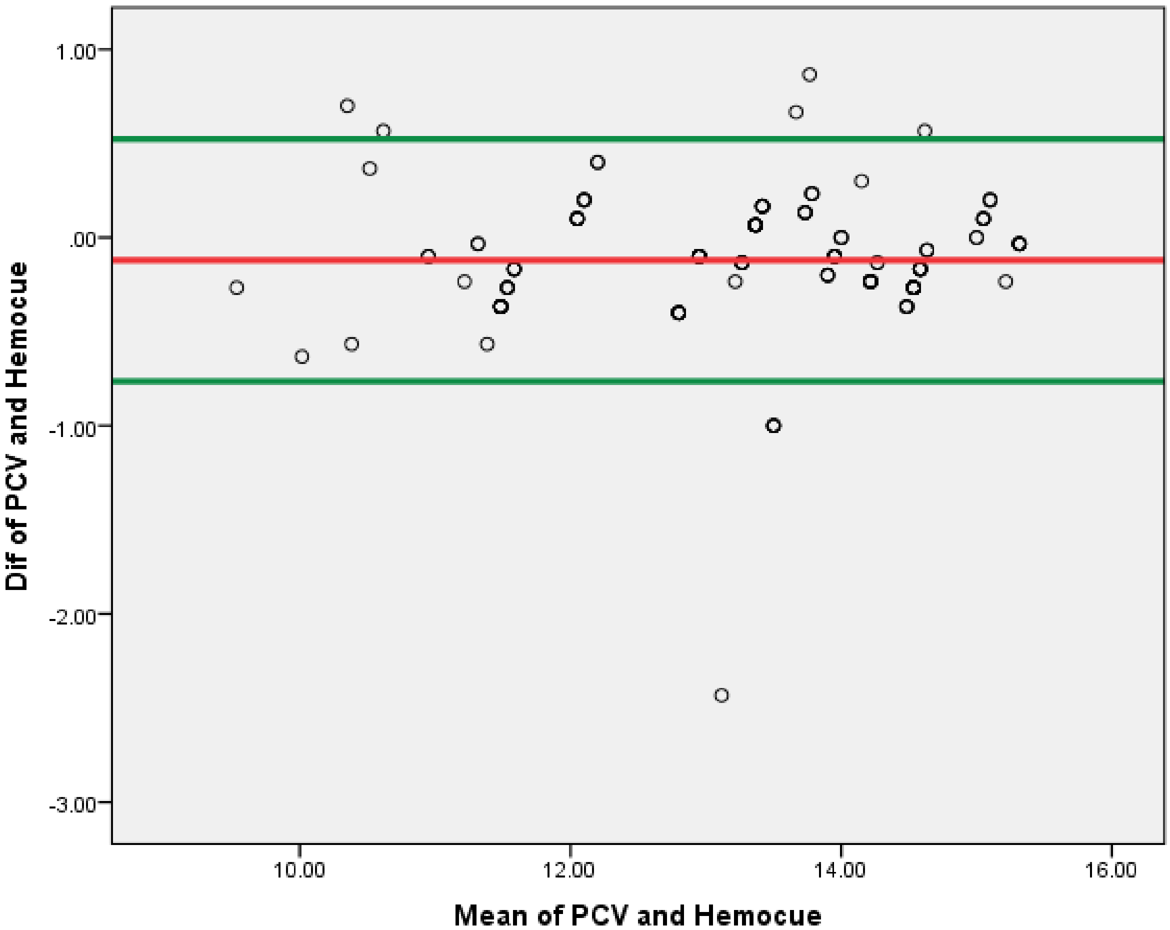

Figure 2: Bland Altman Plot of PCV against Hemocue method of Hemoglobin determination

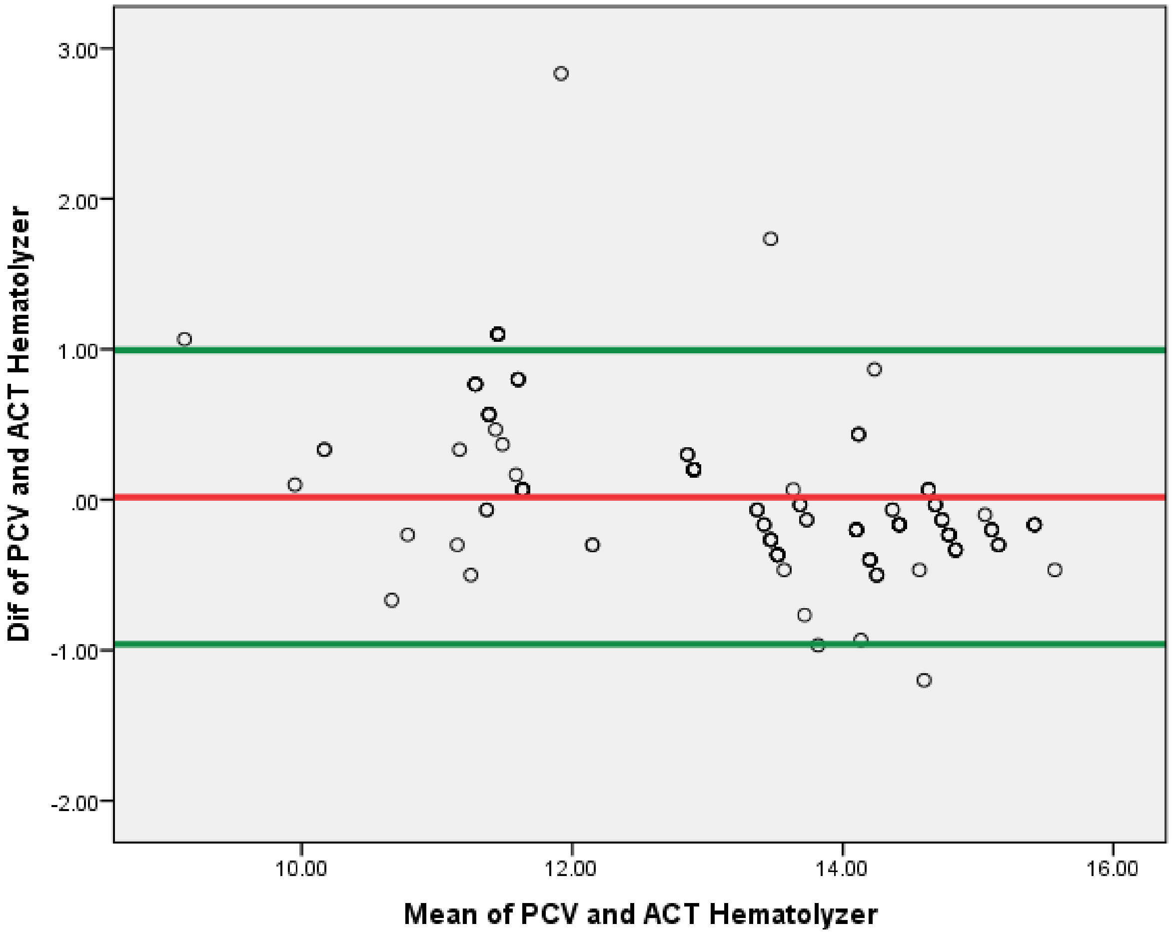

Figure 3: Bland Altman Plot of PCV against ACT hematolyzer method of Hemoglobin determination

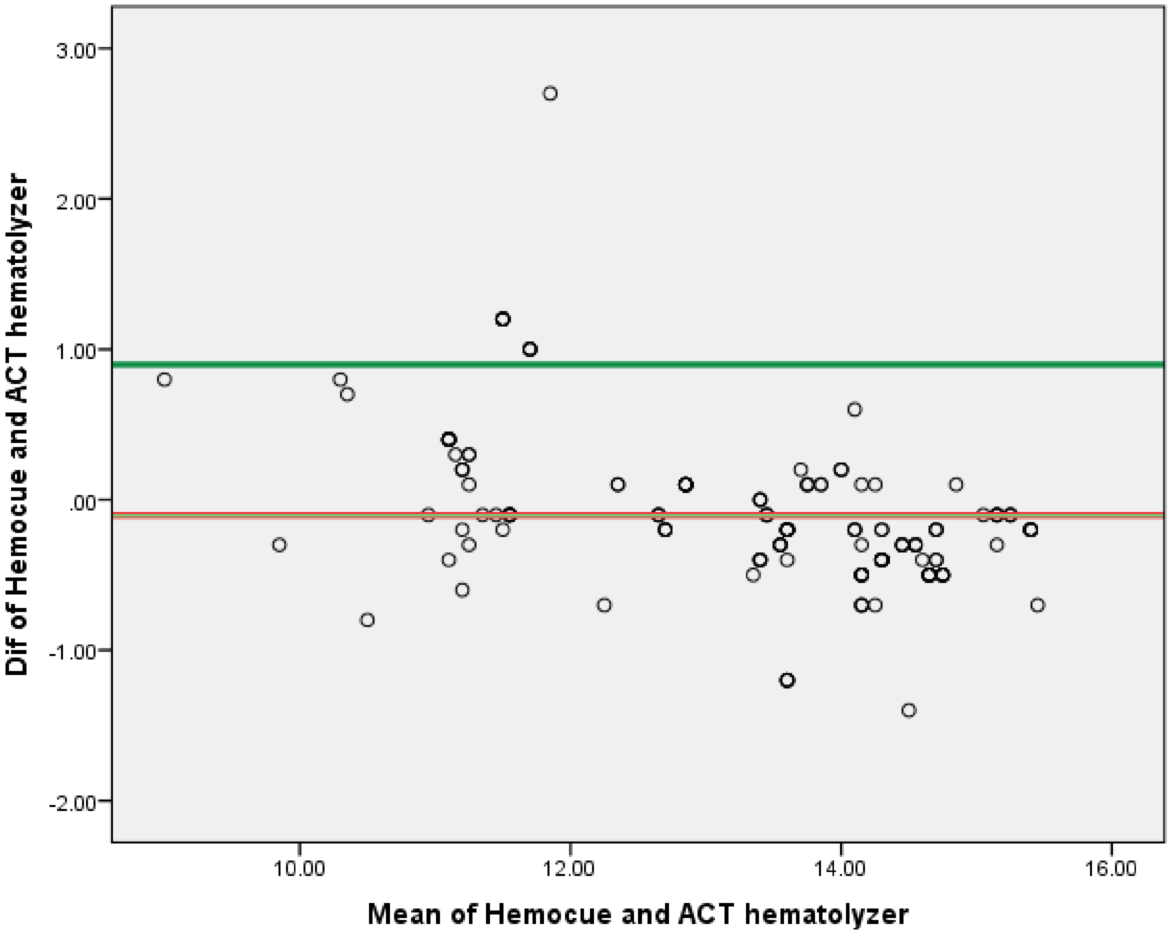

Figure 4: Bland Altman Plot of hemocue against ACT hematolyzer method of Hemoglobin determination

DISCUSSION

Hemoglobin estimation is a critical step in blood donor selection and anemia detection. This study compares hemoglobin concentration using four methods: HemoCue, copper sulfate, automated hematology analyzer (ACT Hematolyzer), and Packed Cell Volume (PCV). Understanding how these methods vary in their results and reliability is important, especially in settings like Nigeria, where blood donor services depend on quick and accurate assessment tools. The comparison of the results across these methods highlights significant findings relevant to transfusion services and health policy.

The HemoCue 201 Hemoglobinometer, widely used in low-resource settings, showed a mean hemoglobin concentration of 13.19 g/dL with a standard deviation of 1.31 among the study subjects. The ACT Hematolyzer, an automated analyzer, gave a slightly higher mean value of 13.43 g/dL and a standard deviation of 1.59. Despite this slight variation, the hemoglobin values from the two methods were not significantly different, with a T-value of 1.421 and a P-value of 0.184, indicating no statistically significant difference between the results obtained from HemoCue and the ACT Hematolyzer.

These findings are consistent with previous research. For instance, a study by Olatunji et al. (2021) compared HemoCue and automated analyzers in a tertiary hospital setting in Lagos, Nigeria. They found a similar mean hemoglobin difference of 0.3 g/dL between the two methods, with the automated analyzer yielding marginally higher values but still within clinically acceptable limits (Olatunji et al., 2021). This reinforces the reliability of HemoCue for field settings, as it provides comparable accuracy to more sophisticated laboratory analyzers.

The PCV-based hemoglobin estimate is a simpler method frequently used in resource-limited settings. In this study, the mean PCV was 40% with a standard deviation of 3.94. Calculating hemoglobin from the PCV (PCV/3) gave a mean hemoglobin of 13.33 g/dL, which closely aligns with the values from both HemoCue and the ACT Hematolyzer. This agreement between the methods is notable, as it suggests that simple hematocrit measurements can provide a reasonably accurate hemoglobin estimate in the absence of more sophisticated equipment.

However, the use of PCV to estimate hemoglobin concentration is not without criticism. Studies have shown that hemoglobin estimates based on PCV can be influenced by factors such as hydration status and erythrocyte size. According to Salami et al. (2019), hemoglobin values derived from PCV may not always be accurate in conditions where red cell morphology is abnormal, such as in cases of microcytic or macrocytic anemia. Nonetheless, in healthy blood donors, as in the current study, the calculated hemoglobin values were sufficiently reliable.

The copper sulfate ($CuSO_4$) method remains a common, rapid screening tool for blood donors, especially in low-resource settings. In this study, the $CuSO_4$ test indicated that 6.2% of family donors and 18.2% of voluntary donors failed the test, with hemoglobin levels below 12.5 g/dL. These results are in line with global trends where voluntary donors are more likely to have lower hemoglobin concentrations compared to family or replacement donors (World Health Organization, 2020).

While the $CuSO_4$ method is efficient for quick screening, it lacks precision, which is reflected in the significant number of false positives and negatives reported in earlier studies. For instance, Silva et al. (2022) reported that the $CuSO_4$ method misclassified up to 12% of blood donors when compared to automated analyzers. Thus, while $CuSO_4$ is useful for initial screening, it should be supplemented with more accurate methods such as HemoCue or automated analyzers when feasible.

The study also explored hemoglobin variations based on donor type. Family donors had higher mean hemoglobin concentrations (13.25 g/dL with HemoCue and 13.49 g/dL with the ACT Hematolyzer) compared to voluntary donors (12.27 g/dL with HemoCue and 12.55 g/dL with the ACT Hematolyzer). This difference was statistically significant with the PCV method (P = 0.033), where family donors also had higher PCV values (40.34%) compared to voluntary donors (37.73%).

These findings are consistent with the general observation that family or replacement donors tend to have higher hemoglobin levels than voluntary donors. This could be attributed to socio-economic factors, nutritional status, and underlying health conditions that may vary between the two groups. A study by Adeolu et al. (2023) in Abuja, Nigeria, found similar results, where voluntary donors, especially young males, had a higher incidence of anemia compared to family donors.

The variation in hemoglobin levels across donor types and the differences between estimation methods have significant implications for blood donation programs. The use of the $CuSO_4$ method as a primary screening tool, though effective in resource-limited settings, may lead to unnecessary deferrals, particularly among voluntary donors. The adoption of portable devices like HemoCue could improve the accuracy of hemoglobin estimation and reduce deferral rates, which in turn could enhance blood supply, especially in regions with high demand for blood products like Nigeria.

Moreover, the slight differences in hemoglobin values between methods indicate that a uniform standard for hemoglobin screening should be established, especially in resource-limited settings where varying methods are used. Policies could focus on incorporating automated analyzers in larger blood donation centers while using reliable alternatives like HemoCue in smaller centers.

A total of 171 donors were assessed, with 12 (7.02%) failing and 159 (92.98%) passing the hemoglobin threshold. The chi-square (χ^2) value was 0.403, with a p-value of 0.525, indicating no statistically significant association between hemoglobin concentration and fitness for donation using the $CuSO_4$ method.

These results are in line with previous research that highlighted the limitations of the $CuSO_4$ method. Although widely used in resource-limited settings due to its simplicity and low cost, the method lacks precision, particularly at hemoglobin levels near the 12.5 g/dL threshold (Allain et al., 2019). This explains the poor discriminatory capacity seen in the present study, where donors with hemoglobin levels below 12.5 g/dL were passed as fit for donation. Previous studies have documented the $CuSO_4$ method's tendency for false negatives (those failing the test despite meeting donation requirements) and false positives (those passing the test while being unfit) (De Silva et al., 2021).

The automated analyzer provided more accurate results, as reflected in Table 5. Out of 171 participants, 61 failed and 110 passed the hemoglobin cut-off. The chi-square value was 171.703, with a p-value of 0.000, demonstrating a statistically significant association between hemoglobin concentration and fitness for donation. The automated analyzer showed high sensitivity and specificity, reliably distinguishing between eligible and ineligible donors.

This finding aligns with research advocating for the use of automated analyzers for hemoglobin estimation in blood donation settings. Automated systems, such as the Ac.T hematology analyzer, offer precise, reproducible results and are generally considered the gold standard for hemoglobin determination (Ravichandran et al., 2022). Moreover, automated analyzers minimize the subjectivity inherent in manual methods and improve the safety of blood donation practices (Sayers et al., 2020).

The Hemocue® method (Table 6) also displayed a statistically significant association between hemoglobin concentration and donor eligibility, with a chi-square value of 188.84 and a p-value of 0.000. Out of 171 donors, 58 failed, while 113 passed. Hemocue® showed strong agreement with the automated analyzer in identifying donors who were either fit or unfit for donation.

The Hemocue® system is a popular point-of-care device known for its accuracy and portability. Multiple studies have validated the Hemocue® system against more sophisticated laboratory analyzers, concluding that Hemocue® offers reliable results in blood donation screening (Sloop et al., 2021). A study by Ozdemir et al. (2020) also highlighted its low margin of error when compared to automated analyzers, making it a suitable alternative in settings where full laboratory automation is not feasible.

The findings from this study are consistent with earlier reports that point to the limitations of the $CuSO_4$ method and the superiority of both automated analyzers and the Hemocue® system. For instance, a study conducted in Tanzania compared $CuSO_4$ with Hemocue® and automated analyzers, showing that $CuSO_4$ had a higher rate of false positives and negatives compared to the more advanced methods (Mziray et al., 2019). Similarly, a comprehensive review of hemoglobin determination methods in low-resource settings confirmed that while $CuSO_4$ remains commonly used, its accuracy is often compromised, leading to unreliable donor selection (Kassam et al., 2018).

Moreover, the automated analyzer and Hemocue® Hb 201 have been validated in a variety of contexts. Studies in developed and developing countries alike have shown that these methods outperform manual or semi-automated systems in both speed and reliability (Sloop et al., 2021). Hemocue® in particular has gained traction as a portable, easy-to-use option in blood donor screening and primary healthcare settings (Ravichandran et al., 2022). This is in line with the current study's findings, where both the Hemocue® and automated analyzer methods produced statistically significant associations with accurate hemoglobin concentration.

The results of this study underscore the importance of accurate hemoglobin determination in ensuring donor and recipient safety. Given the statistically significant differences between the methods tested, the $CuSO_4$ method may no longer be advisable in settings where more precise alternatives like Hemocue® or automated analyzers are available. The failure of $CuSO_4$ to reliably classify donors as fit or unfit increases the risk of collecting blood from donors with suboptimal hemoglobin levels, potentially jeopardizing both donor health and blood supply quality.

Conversely, the adoption of either the automated analyzer or Hemocue® would improve the accuracy of blood donation screening, reducing the likelihood of false classifications. These technologies are particularly valuable in high-volume blood banks where rapid and reliable results are necessary.

The sensitivity and specificity results for the HemoCue 201 and Ac.T hematolyzer methods were notably high, with values of 98.2% and 95.6% sensitivity, and 98.2% specificity for both methods (Table 7). These results demonstrate that both methods are highly reliable for detecting anemia in blood donors. Furthermore, the positive predictive value (PPV) and negative predictive value (NPV) were robust, indicating that these methods are excellent for both confirming and ruling out anemia. These findings are consistent with earlier research, which suggests that both HemoCue and automated analyzers provide accurate and reliable hemoglobin measurements, particularly when compared to traditional techniques such as $CuSO_4$ (Atsma et al., 2012; Cable et al., 2011).

The CuSO4 method, on the other hand, showed significantly lower sensitivity (8.8%) and specificity (93.9%), confirming its poor diagnostic value as a screening tool for anemia. This result is in line with previous studies that have criticized the $CuSO_4$ method for its lack of precision and the high risk of false negatives (Hodges et al., 2017). Given the critical role of hemoglobin screening in blood donation, reliance on the $CuSO_4$ method may result in underdiagnosis of anemia, leading to the inappropriate acceptance of donors who are not eligible (Kitchen et al., 2013).

The results of the linear correlational analysis (Table 8) further emphasize the strong agreement between the HemoCue 201, Ac.T hematolyzer, and PCV methods, with correlation coefficients (R) ranging from 0.954 to 0.969. These findings are statistically significant (p-value = 0.000), suggesting a high level of consistency across these methods in hemoglobin estimation. Previous research supports these findings, noting that automated analyzers like the Ac.T hematolyzer are superior to conventional methods in terms of precision and reproducibility (Dassanayake et al., 2019). Similarly, the HemoCue device has been validated in numerous studies for its ease of use and reliability in point-of-care settings (Bucher et al., 2019).

The Bland-Altman analysis further confirmed the interchangeability of HemoCue 201 with PCV (Figure 2) but highlighted a significant proportional bias between the Ac.T hematolyzer and PCV (Figure 3). The significant negative correlation (coefficient = -0.194, p-value = 0.000) suggests that discrepancies between the two methods depend on the actual haemoglobin value. This bias indicates that the Ac.T hematolyzer might systematically under or overestimate haemoglobin levels compared to PCV at certain hemoglobin concentrations, a trend that has been observed in other studies comparing automated analyzers to manual techniques (Morris et al., 2015).

The reliability analysis of the different hemoglobin estimation methods (Table 9) showed high intra-class correlation coefficients (ICC) for all pairwise comparisons, with the highest correlation observed between the PCV and HemoCue 201 methods (ICC = 0.982). This indicates that both methods are highly reproducible and reliable for hemoglobin determination. The strong reliability of the HemoCue 201 method corroborates previous findings that point-of-care devices offer dependable alternatives to traditional laboratory methods, especially in resource-limited settings where quick decision-making is crucial (Lukudu et al., 2017). On the other hand, while the Ac.T hematolyzer also demonstrated high reliability, the observed proportional bias in the Bland-Altman plot suggests that the method's performance should be interpreted with caution at certain hemoglobin levels.

These results align well with other research that has compared the accuracy of different hemoglobin estimation methods. For instance, Kitchen et al. (2013) found that automated analyzers such as the Ac.T hematolyzer outperformed traditional methods like CuSO4 in terms of both sensitivity and specificity. Similarly, Dassanayake et al. (2019) highlighted the limitations of manual techniques, including CuSO4, noting their susceptibility to operator errors and environmental conditions. However, the high sensitivity and specificity of the HemoCue 201 method observed in this study are slightly higher than those reported in some earlier studies, where sensitivities ranged from 90% to 95% (Bucher et al., 2019). This discrepancy could be due to differences in sample populations, environmental conditions, or calibration protocols.

A notable point of divergence from previous research is the proportional bias identified between the Ac.T hematolyzer and PCV methods. While studies like that of Morris et al. (2015) also observed variability between automated and manual methods, the degree of bias in this study suggests a potential area for further investigation. It may be necessary to explore whether specific calibration adjustments or procedural refinements can reduce this bias.

The implications of these findings for blood donor screening are significant. The high sensitivity and specificity of the HemoCue 201 and Ac.T hematolyzer methods make them ideal candidates for routine use in blood donation centers. However, the proportional bias between the Ac.T hematolyzer and PCV methods suggests that care must be taken when interpreting results at specific hemoglobin levels. Additionally, the poor performance of the $CuSO_4$ method calls into question its continued use, especially in high-volume blood donation settings where accurate screening is critical.

CONCLUSION

This study compared hemoglobin estimation methods—HemoCue 201, ACT hematolyzer, Copper Sulphate ($CuSO_4$), and Packed Cell Volume (PCV)—among blood donors at Federal Medical Center, Abeokuta, Nigeria. HemoCue and ACT hematolyzer methods demonstrated high sensitivity, specificity, and strong correlations with PCV, making them reliable alternatives for hemoglobin estimation. The $CuSO_4$ method showed lower sensitivity, indicating its limitation for precise hemoglobin screening. Overall, automated methods such as HemoCue and ACT hematolyzer were more accurate and consistent compared to traditional methods like $CuSO_4$.

RECOMMENDATIONS

1. **Adopt Automated Hemoglobin Estimation Methods**: Given the accuracy and reliability of HemoCue and ACT hematolyzer, health institutions should consider using these methods for routine blood donor screening to improve safety and efficiency.
2. **Phase Out $CuSO_4$ Method**: The low sensitivity and specificity of the $CuSO_4$ method make it less effective for hemoglobin screening. It is recommended to phase out its use in favor of more reliable automated methods.
3. **Training and Quality Control**: Continuous training of laboratory personnel on the use of automated hemoglobin analyzers and regular quality control checks should be emphasized to maintain accuracy in results.
4. **Further Research**: Comparative studies involving a larger population and other diagnostic tools are recommended to validate the findings and explore other potential hemoglobin estimation methods.

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