Original Article

Comparison of Haemoglobin Estimation of Blood Donors by Specific Gravity Method, HemoCue Method and Automated Haematology Cell Analyzer

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Abstract

Introduction: Pre-donation haemoglobin (Hb) screening is among the foremost test done on blood donors to determine whether an individual is fit to donate with the intention of preventing inadvertent donation from an anaemic donor. The aim of the study was to compare the efficacy of the three common Hb estimation methods, namely, copper sulphate ($CuSO_4$) method, HemoCue photometer and automated cell counter in reporting the Hb levels of blood donors. **Materials and Methods:** $CuSo_4$ specific gravity method, HemoCue and automated cell analyzer (Sysmex XN-550) were used to determine the Hb levels in blood samples of 500 donors. Descriptive statistics were used to analyse the demographic details of the donor. Kappa statistics were used to determine the level of agreement between the three methods of Hb estimation. **Results:** HemoCue was found to be more sensitive (86.21%), whereas $CuSo_4$(97.88%) was found to be more specific. Kappa agreement was good between $CuSo_4$ and Sysmex XN-550 (0.703), whereas it was moderate between HemoCue and Sysmex XN-550 (0.458). **Conclusions:** The $CuSO_4$ method is still viable for Hb estimation among blood donors. Thus, it can be utilised as the primary screening method; however, follow-up testing with HemoCue or automated cell analyzer can be done to minimise unnecessary deferrals and false acceptance.

Keywords: Blood donation, copper sulphate, haemoglobin estimation, HemoCue

Introduction

One of the fundamental purposes of a blood transfusion service is to offer safe and high-quality blood products for patients and to do so, we must avoid collecting blood from an anaemic donor. As a result, pre-donation haemoglobin (Hb) testing for blood donors is critical to protect the health of transfusion recipients.[1]

According to the Drugs and Cosmetic Act, 1940 and the criteria mentioned in the Directorate General of Health Services Technical Manual, 2003, only blood donors with Hb levels of ≥12.5gm/dl are eligible for whole blood donation.[2,3]

Despite various methods for Hb estimation, no single technique has emerged as the most appropriate and ideal for a blood donation setup. A highly accurate method in a blood donor setting is more likely to be expensive. In a developing country like India, it is not possible to use such a method for screening so many blood donors' samples. On the other hand, a less accurate and cheaper method may give false results which may lead to either donation of blood by an anaemic subject or loss of eligible donors. Therefore, there is a requirement to adopt a cost-effective and time-saving Hb estimation method that delivers accurate laboratory results.[1]

The copper sulphate ($CuSo_4$) specific gravity method has been traditionally used and is still being used for donor screening at many blood centres in India due to its easy availability and cost-effectiveness. However, it does not provide an acceptable degree of accuracy with many studies showing deferral of subjects who failed the test but were not anaemic.

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It is, therefore, important to determine anaemia amongst them using the standard diagnostic method so that there is no loss of any potential donors.[4,5]

Another method uses a new generation of Hb photometers, the HemoCue. HemoCue photometer calculates the Hb concentration in g/dl and displays the results as a digital reading in 15–45 s.[6] Similarly, the Sysmex XN-550 is an automated, compact and haematology analyzer designed to generate a full blood count with a standard five-part white blood cell differential and an immature granulocyte count, as well as an optional reticulocyte and optical platelet counts.[6]

The present study aims to compare the efficacy of three Hb estimation methods, namely, the $CuSO_4$ method, HemoCue photometer and automated cell counter (Sysmex XN-550) in reporting the Hb levels of blood donors.

Materials and Methods

Study population

This is a prospective observational study conducted over 6 months from January 2021 to June 2021 in a tertiary health care centre. This study was ethically approved by the Institutional Ethics Committee (SDUMC/KLR/IEC/745/2020–21). This study followed ethical standards delineated in the Helsinki declaration 1975, with an update in 2013.

Study size

A convenient sample of 500 human blood samples was obtained from blood donors. The inclusion criteria included consenting donors aged 18 years and above who provided blood samples of a minimum 2 ml volume. The exclusion criteria included all the samples that were seropositive, insufficient or haemolysed.

The procedure for sample collection

Capillary blood samples were collected by deep finger prick on the index or middle finger of the left hand using a dry sterile lancet (Unilet Excelite II, England) after disinfecting with ethanol and massaging the finger to facilitate blood flow. The first drop was wiped away and the second drop was used for testing by $CuSo_4$ method and HemoCue method (HemoCue AB, Ängelholm, Sweden). Two millilitres of venous blood samples were collected into EDTA Vacutainer tubes and were analysed on the automated cell counter as soon as possible.

Blood sampling and analysis of Hb were performed only by doctors and technicians who were trained for the instruments on a few pilot samples using the three methods before commencing the study.

Testing of all eligible donor samples was analysed immediately or within 30–60 min of collection. The blood sampling and analysis of Hb were first estimated by $CuSo_4$, followed by HemoCue, and finally by the automated cell analyzer. Results of $CuSO_4$ were interpreted as pass or fail at Hb cutoff of ≥12.5 g/dl while HemoCue readings were considered to pass when the readings were ≥12.0 g/dl and fail below 12.0 g/dl.

Every day, following the standard operating procedure, the working $CuSO_4$ solution was created (specific gravity 1.053) and standardised. Every day, the HemoCue photometer's functionality was tested by measuring the control cuvette according to the manufacturer's recommendations. Quality control and calibration of the automated haematology analyzer were performed according to standard operating procedures using stabilised control reagents provided by the manufacturer.

Statistical analysis

Data were entered into a Microsoft Excel data sheet and were analysed using SPSS 22 version software (SPSS Statistics - IBM Data Science Community USA). Categorical data were represented in the form of frequencies and frequency percentages. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each method were also calculated. Kappa statistics were used to check the level of agreement between the tests.

Results

Most of the donors were males (95.4%) and between 21 and 30 years (52.6%). Around 80% were voluntary blood donors. Furthermore, 70% of the samples were collected from outdoor camps as shown in Table 1. Table 2 shows the deferral data of the three methods against the cell counter method.

According to this data, the highest deferrals were seen in the $CuSo_4$ (7.6%) method followed by HemoCue (6.4%) and automated cell analyzer (6.2%). $CuSO_4$ falsely accepted four of 500 (0.8%) donors and falsely deferred 11 of 500 (2.2%) donors. The false acceptance with HemoCue was 5 of 500 (1%), and false deferral was 6 of 500 (1.2%) donors.

The measure of agreement between $CuSo_4$ and Sysmex XN-550 was found to be good with a $\kappa = 0.703$ ($P < 0.001$). Whereas the measure of agreement between HemoCue and Sysmex XN-550 was found to be moderate with a $\kappa = 0.458$ ($P < 0.001$).

Table 1: Demographic details of the blood donors and the site of sample collection

Variable	Categories	Frequency (%)
Gender	Female	23 (4.6)
	Male	477 (95.4)
Age (years)	<20	22 (4.4)
	21-30	263 (52.6)
	31-40	177 (35.4)
	41-50	38 (7.6)
Occupation	College students	200 (40)
	Services	150 (30)
	Agriculture	150 (30)
Type of donor	Voluntary blood donors	400 (80)
	Relative blood donors	100 (20)
Site of collection	Outdoor camps	350 (70)
	In-house camps	150 (30)

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

Hb value (g/dl)	Sysmex XN-550	$CuSo_4$ method		HemoCue	
		Pass	Fail	Pass	Fail
9-10.9	11	0	11	0	11
11-12.4	20	4	16	5	15
12.5-13.9	350	340	10	344	6
14-15.9	100	99	1	100	0
16-17	19	19	0	19	0

Hb: Haemoglobin, $CuSo_4$: Copper sulphate

The sensitivity of the HemoCue (86.21%) method was found to be higher than that of the $CuSO_4$ (75.86%) method, whereas specificity was higher in the case of $CuSO_4$ (97.88%) when compared to the HemoCue (91.30%) method.

The PPV was higher in the case of $CuSO_4$ (68.75%) when compared to HemoCue (37.88%), whereas the NPV was higher in the HemoCue (99.08%) method when compared to $CuSo_4$ (98.50%). Accuracy was found to be higher in $CuSO_4$ (96.60%) than HemoCue (91%).

Discussion

An acceptable Hb screening method should be available for blood collection to accept as many potential donors as feasible and avoid any unnecessary deferrals. Any new approach for Hb screening that is introduced must reduce time and money, and it must be validated against gold standard methods.

The capillary method of Hb measurement in field situations for $CuSO_4$/HemoCue is more practical than the venous sampling approach,[7] but because our reference method was based on venous samples, only venous samples were employed in this work to maintain homogeneity and have near true values.[8] In addition, venous samples outperform capillary samples in terms of sensitivity, specificity, PPV, NPV and other performance metrics.[9,10]

Furthermore, because donor acceptance procedures are based on venous Hb standards rather than capillary Hb values, all these devices favoured venous sampling over capillary for Hb measurement. $CuSO_4$ has been a traditional method of donor Hb screening notwithstanding its disadvantages.[11] To ensure accurate results, a $CuSO_4$ solution with an appropriate specific gravity should be used, along with other technical procedures. Because each drop of blood added to the solution affects the specific gravity, the solution should be changed daily, or at least every 25 tests.[12] $CuSO_4$ falsely accepted four of 500 (0.8%) donors for blood donation in the present study, which is less compared with the 5% reported by Malukani *et al*.[13]

$CuSO_4$ falsely deferred 11 of 500 (2.2%) donors, which was in contrast to 29% reported by Sawant *et al*.[14] This discrepancy could be attributable to a variety of factors, including preparation, quality control and storage, as well as technical ability and diligence, which can differ from person to person. These factors were a source of concern when the semi-quantitative methodology was used.

The false acceptance with HemoCue was five of 500 (1%), which is lower than that (6%) reported by Patel *et al*.[15] HemoCue falsely deferred six donors (1.2%), whereas Patel *et al*. reported 3.3%.[15]

In the present study, HemoCue (86.21%) was found to be more sensitive than $CuSO_4$ (75.86%). This is in accordance with the study by Tondon *et al*.[16] whereas it contrasts with the study by Rout *et al*.[17] who found both the methods equally sensitive. The specificity of $CuSo_4$ (97.88%) was higher than that of HemoCue (91.30%). This is in contrast with the findings of Rout *et al*. and Tondon *et al*. who found the specificity of HemoCue higher than that of $CuSo_4$.[17]

When choosing a method for Hb estimation in blood donors, the PPV of the screening test is critical since the goal is to ensure the safety of blood donors while also avoiding unnecessary deferral of potential blood donors.[18] In the present study, PPV was found to be high in $CuSo_4$ (68.75%) than that of HemoCue (37.88%).[Table 3] This is in contrast with the findings of Rout *et al*. and Tondon *et al*.[16,17] NPV is important for donor safety. In the present study, NPV was similar for $CuSO_4$ (98.50%) and HemoCue (99.08%). This is in accordance with the study by Tondon *et al*. and Rout *et al*.[16,17]

In the case of measures of agreement, HemoCue when compared with Sysmex XN-500 showed moderate agreement ($\kappa = 0.458$). This is in accordance with the findings of Yadav *et al*. who also found moderate agreement between the two testing methods ($\kappa = 0.697$).[19] In contrast, the measure of agreement was good between the $CuSO_4$ method and Sysmex XN-550 with a $\kappa = 0.703$ in the present study.

It is also worth mentioning that the HemoCue apparatus costs around 35000 Indian Rupees (INR), whereas each disposable microcuvette costs around 30 INR. $CuSo_4$ powder (500 g) costs only 175 INR and can be used to test 2000–2500 samples (assuming 159.63 g is utilised for about 750–800 tests), costing around 0.06–0.08 INR for each test. Our findings show that HemoCue is roughly 500 times more expensive than the $CuSO_4$ technique.

Minimal inter-observer and inter-instrument errors were among the study's strengths. The equipment was subjected to adequate quality control and was regularly inspected. All three instruments were subjected to daily internal quality checks.

Limitations of the study

The study's shortcomings include the limited sample size and the fact that it was done in a single location. A larger sample size and a multicentre investigation could yield more conclusive results.

Conclusions

We have shown that the $CuSO_4$ approach is still valid. Therefore, it can be used as the primary screening method;

Table 3: Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of copper sulphate method and HemoCue in comparison with Sysmex XN-550

Statistic	$CuSo_4$		HemoCue	
	Value (%)	95% CI	Value (%)	95% CI
Sensitivity	75.86	56.46-89.70	86.21	68.34-96.11
Specificity	97.88	96.13-98.98	91.30	88.38-93.68
PPV	68.75	53.54-80.77	37.88	30.55-45.81
NPV	98.50	97.19-99.21	99.08	97.74-99.63
Accuracy	96.60	94.61-98.01	91.00	88.14-93.36

PPV: Positive predictive value, NPV: Negative predictive value, $CuSo_4$: Copper sulphate, CI: Confidence interval

however, to avoid unnecessary deferrals, follow-up testing can be done with HemoCue or an automated cell analyzer. This study may be useful to blood centres with limited resources, particularly for camp donations where mass Hb screening is performed.

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Conflicts of interest

There are no conflicts of interest.

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