Original Article

# Comparison of Hemoglobin Levels by Copper Sulphate Method and Automated Hematology Analyzer in Blood Donors

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ABSTRACT

Objective: To contrast the Hb results obtained using CuSO4 method and automated analyzer and to investigate how donor deferral is affected by the CuSO4 method's inaccuracy.
Methodology: The study was conducted in Blood bank of Holy Family Hospital, Benazir Bhutto Hospital, District Headquarters Hospital and Hematology Laboratory, Pathology Department of Holy Family Hospital, Rawalpindi.
Capillary and venous blood sample were tested by using Hb screening appropriate standardized CuSO4 test (1.053) alongside the automated hematology analyzer, which was regarded as the industry standard reference technique.
Results: Results of copper sulphate were interpreted as Pass or Fail at Hb cut off of ≥12.5 g/dl. By this method, a total of 20 (16.6%) blood donors were deferred, out of which 15 were male and 5 were female. The percentage of deferrals was greater in women. (62.5%) in contrast to men. (13.3%) By Automated Hematology Analyzer (Sysmex-KX21) method, only 15 blood donors were deferred, out of which 11(10%) were male and 4 (50%) were female. The sensitivity of the CuSO4 was (95.24%), specificity (100%), PPV (100%), NPV (75%) and the sensitivity of automated hematology analyzer was (100%), specificity (100%), PPV (100%), NPV (100%).
Conclusion: The accuracy of CuSO4 was (95.83%) and the accuracy of the automated hematology analyzer is (100%). The inaccuracy of CuSO4 was 4.17%.
Keywords: Hemoglobin, Copper Sulphate, Blood Donors.

## Introduction

An establishment that collects, stores, and preserves blood donated by donors for use in future transfusions is known as a blood bank. The term "whole blood" refers to a single, clearly defined product—that is, unseparated venous blood with an authorised preservative added. Most blood for transfusion is collected as a whole blood. The protein molecule known as haemoglobin is found in red blood cells and is responsible for transporting oxygen from the lungs to the body's tissues and returning carbon dioxide from those tissues to the brain. Within the erythrocytes (red blood cells) of the blood is haemoglobin. Mature erythrocytes are devoid of any internal organelles, such as the nucleus and mitochondria, so these cells essentially serve as a bag to carry haemoglobin. Myoglobin, the other oxygen-binding protein present in vertebrates, stores oxygen in bodily tissues so that it is available to the tissues when needed. Similar to myoglobin, haemoglobin is primarily made up of four polypeptide peptide units.[1] Haemoglobin is composed of four interconnected globin chains.[2] Two alpha and two beta haemoglobin chains make up the typical adult haemoglobin (Hb) molecule. The haemoglobin molecule in foetuses consists of two gamma and two alpha chains. The adult haemoglobin structure is formed when the gamma chains are replaced gradually by beta chains after birth. Six months of age marks the completion of this transition. [3] The 141 amino acids found in each alpha chain and the 146 amino acids found in each beta chain are arranged in a specific order. Compared to dissolved oxygen in blood, hemoglobin's oxygen binding capacity of 1.34 mL O2 per gramme increases the total blood oxygen capacity by a factor of seven. Four Adult haemoglobin has a molecular weight of 64458 Dalton.[5] The haeme molecule is a crucial central structure found in every globulin chain. Iron is a component of the home molecule and is essential for the blood's transportation of carbon dioxide and oxygen. The red hue of blood is also caused by the iron found in haemoglobin.[6,7] There are two types of haemoglobin:

desaturated haemoglobin (deoxyhemoglobin) and saturated haemoglobin (oxyhemoglobin). When oxygen attaches itself to the haemoglobin haeme unit in red blood cells during physiological respiration, oxyhaemoglobin is produced. The type of haemoglobin without bound oxygen is called deoxygenated haemoglobin.[8,9] Red blood cell morphology is maintained by haemoglobin, which is another essential role. Red blood cells resemble a donut without a hole in the middle because of their spherical form and narrow cores. Therefore, abnormal haemoglobin structure can cause red blood cells to change in shape and lose their capacity to circulate through blood arteries.[10]

The first line of defense for blood safety is the selection of donors. [11] More voluntary blood donors must be recruited in order to guarantee a steady supply of blood and blood components, as evidenced by the rising demand for blood and blood components in developing nations like Pakistan. To ensure that the donor and recipient safety procedures are met, as well as to ensure an adequate blood supply, ongoing oversight of the donor selection process is necessary. [12] In the year 2013, the Ministry of National Health Services established the blood transfusion authority in Islamabad. An oversight body that registers and licensed blood banks under its purview is the Islamabad Blood Transfusion Authority (IBTA). The gathering and examination of information about blood transfusion services is one of the authority's primary responsibilities. Within the Capital Territory of Islamabad, there are currently 19 blood banks that are licensed and in operation. All blood banks that remain are hospital-based and rely on family replacement donations, with the exception of one.[13] Prior to donation The primary test for choosing blood donors is hemoglobin estimation, which is done primarily to avoid collecting blood through anemic doors. For this reason, it is imperative to employ a precise and trustworthy hemoglobin determination method.[14] To prevent any unintended effects on either the donor or the recipient, the donor must be in good health. [15] As a result, all blood banks must adhere to established standards for accepting blood donors. It's crucial to ascertain the donor's hemoglobin level prior to donation. Although there are several ways to measure hemoglobin, the copper sulphate (CuSO4) method is the most widely used method for estimating hemoglobin for blood donation. [16]

When estimating hemoglobin (Hb), the copper sulphate specific gravity test frequently yields unreliable results.

In order to estimate hemoglobin (Hb), we decided to compare it with the other automated hematology analyzer.[17] The copper sulphate (CuSO4) method works on the basis that a drop of whole blood added to a solution of CuSO4 that has been given a specific gravity will hold its own density for about 15 seconds. A specific gravity of 1.053 is expected for the test solution.[18]

In males and females, normal hemoglobin levels vary. Blood hemoglobin levels in males are typically 13–16 g/dl, while those in females are typically 12–15 g/dl. [19] 12.5 g/dl of hemoglobin is equal to a specific gravity of 1.053. In order to estimate pre-donation hemoglobin, the CuSO4 solution's specific gravity of 1.053 is used.[20,21] The donor is approved for blood donation if the drop of blood sinks in less than 15 seconds and their hemoglobin content is greater than 12.5 g/dl. On the other hand, the donor is declined if the blood drop falls halfway and then rises with a hemoglobin level of less than 12.5 g/dl. Verify the donor's hemoglobin if the drop descends gradually, pauses, and eventually reaches the bottom of the jar. Hemoglobin will be tested again using an automated cell counter if the donor is unable to pass the CuSO4 test.[22,23] The CuSO4 method has certain drawbacks. Incorporating air bubbles or using an insufficient height to drop the blood, for instance, can lead to an underestimation of hemoglobin, which may cause donors to be postponed needlessly. False results may also occur from donors with low or high protein levels.[25]

## Methodology

The study was conducted at the blood banks of Holy Family Hospital, Benazir Bhutto Hospital, and District Headquarters Hospital, three tertiary care hospitals in Rawalpindi. Holy family is an 1150 beds hospital, was established in 1948 by the Christian mission of Philadelphia at Murree Road, *Rawalpindi.* Benazir Bhutto Hospital is also known as the Rawalpindi General Hospital is hospital located on ever-busy Murree Road, Rawalpindi. District headquarters hospital is located at Kashmiri Bazaar Road, Rawalpindi. In addition to serving as a trauma patient referral centre, it offers medical care to Rawalpindi's inner city. There is a connection between Rawalpindi Medical College and all three hospitals.

Random blood donors were selected who visited the blood bank from September 2019 to December 2019. In the course of the study, most (~90%) blood Donors could be friends, family, or replacement donors for the recipients. Prospective donors complete a survey and get checked out physically Performed by trained staff. Those who were apparently healthy aged between 18-60 years and weigh above 50 kg were qualified for donation.

In this study, 120 arbitrary male and female donors were selected at random from the blood banks of three different hospitals. From Holy Family Hospital, Benazir Bhutto Hospital, and District Headquarters Hospital in Rawalpindi, 40 donors were chosen at random. Participants in the study ranged in age from 18 to 46. Therefore, 40 cases from each hospital were included in the study.

Every willing study participant, who was between the ages of 18 and 50, was required to sign informed consent forms before they could participate in the study. All study participants who were over 65 years old or who were under 18 but declined to give consent. Women who were menstruating or pregnant were not allowed to participate.

At every site, a project nurse or staff member conducted counselling to make sure all study participants were aware of the purpose, design, goals, and potential risks of the study. Every study participant gave their informed consent, and the nurse at each location administered the survey. This questionnaire asks about the medical history, sexual preferences, surgical history, number of donations and transfusions, and tattooing and body piercing history of the potential donors. This reduces the possibility of receiving contaminated blood, preventing the waste of donated blood and equipment. Following donation, blood is tested in Pakistan; samples that test positive for TTIs and are determined to be contaminated are disposed of.

Blood samples were collected using safe, tried-and-true procedures in accordance with the study's design and methodology. Every specimen was gathered for every study. each of the forty samples from the hospital's individual blood banks.

Participant after written consent was acquired and informed. At each site, a staff member with the necessary training was assigned to gather peripheral blood. All samples were run through the Sysmex-KX21 hemoglobin analysis system at the Hematology Laboratory, Holy Family Hospital, Rawalpindi, and tested for hemoglobin using the cuso4 method.

2ml of blood was taken from blood donor under aseptic conditions in EDTA tube. Samples were then transported to the Hematology laboratory, Holy family Hospital (HFH).

## Results

A total of 120 blood donors were included in study. Donor's age ranged from 18-45 years. The gender distribution among 120 donor population consists predominantly of males, 112 (93%) with comparatively less females, 8 (7%).

Results of copper sulphate were interpreted as Pass or Fail at Hb cut off of ≥12.5 g/dl. By this method, a total of 20 (16.6%) blood donors were deferred, out of which 15 were male and 5 were female. The deferral rate was higher among females (62.5%) as compared to males (13.3%). (Table I)

Table I: Frequency of Donations accepted or deferred by copper sulphate method.

| Gender | Pass (no.) | Fail (no.) | Total (%) |
|---|---|---|---|
| Male | 97 | 15 | 112 (93%) |
| Female | 3 | 5 | 8 (7%) |
| Total | 100 | 20 | 120 (100%) |

By Automated Hematology Analyzer (Sysmex-KX21) method, only 15 blood donors were deferred, out of which 11(10%) were male and 4 (50%) were female. Figure 1 shows the frequency of blood donors accepted or deferred by Automated Hematology Analyze.

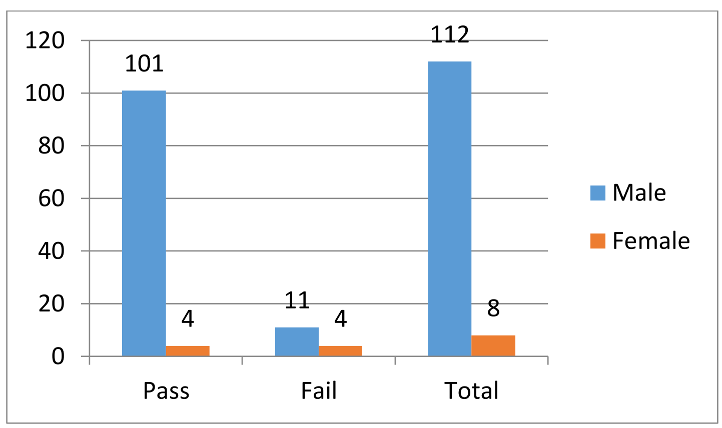


Figure 1. Frequency of Donations accepted or deferred by automated hematology analyzer method.

By the comparison of $CuSO_4$ and automated hematology analyzer the tests results of the $CuSO_4$ screening test were true positive 100, true negative 15, false positive 00, false negative 05 and by the automated hematology analyzer where test was true positive 105, true negative 05, false positive 00, false negative 00.

By comparison of $CuSO_4$ and automated hematology analyzer the tests results of the sensitivity of the cuso4 was (95.24%), and their specificity was (100%), PPV (100%), NPV (75%) and the sensitivity of automated hematology analyzer was (100%), and their specificity was (100%), PPV (100%), NPV (100%).

Table II: comparison of $CuSO_4$ and automated hematology analyzer the tests results

| Technique | True Positive | True Negative | False Positive | False Negative |
|---|---|---|---|---|
| Copper sulphate method | 100 | 15 | 00 | 05 |
| Automated Hematology Analyzer | 105 | 05 | 00 | 00 |

## Discussion

Donor selection is one of the most crucial stages in enhancing the safety of blood and blood products. The blood donors who are widely accepted are those who donate blood voluntarily and without payment. The donor is motivated by nothing other than the desire to assist another person, so they provide accurate information. Many nations have succeeded in achieving this objective. Over three million units of blood are used annually in Pakistan. Eighty-two percent of the blood is donated directedly, with the majority coming from family members. Still, the younger pupils are prepared to give blood in an emergency. Blood banks are accountable for safeguarding donors, which includes keeping them from becoming anemic.

When used for Hb screening, the copper sulphate (CuSO4) specific gravity test frequently yields unreliable results. This led us to compare its hemoglobin (Hb) estimation results with those of the other automated hematology analyzer.

This study compared the Hb results obtained using the automated analyzer and the CuSO4 method in order to determine how donor deferral is affected by the method's inaccuracy. Both the automated hematology analyzer, which was regarded as the standard reference method, and the Hb screening appropriate standardized CuSO4 test (1.053) were used to test capillary and venous blood samples. This study involved the selection of 120 blood donors. The copper sulphate results were interpreted as Pass or Fail at the ≥12.5 g/dl Hb cut off. This procedure resulted in the deferment of 20 blood donors (16.6%), of whom 5 were female and 15 were male. The deferral rate was greater in women (62.5%) than in men (13.3%). Through Hematology Analyzer Automation (Sysmex-KX21) method, only 15 blood donors were deferred, out of which 11(10%) were male and 4 (50%) were female. The sensitivity of the CuSO4 was (95.24%), specificity (100%), PPV (100%), NPV (75%) and the sensitivity of automated hematology analyzer was (100%), specificity (100%), PPV (100%), NPV (100%).

Just 8% of the 1,014-donor population in this study were women. The gender distribution of the population was dominated by men. Twelve two percent of the screened donors were men, making up 124 of the 167 (16.5%) donors who were deferred owing to low Hb. With 34 (79.07%) of the potential female donors having Hb levels below 12.0 g/dl, low Hb levels resulted in the deferment of over half (43/81). The comparison of the various techniques used in the present study against the reference hematology analyzer is summarized in. For 1014 venous samples tested using each method, we evaluated the Hb values (mean ± standard deviation). When compared to the reference, the results of the HemoCue and HCS Hb values were fairly similar. In contrast, the reference Hb values (13.8 ± 1.52 g/dl) were 0.24 lower than the mean Hb value of HemoCue (14.7 ± 1.49 g/dl). HCS had mean Hb values of 13.3 ± 1.18 g/dl. It was discovered that HemoCue was the most sensitive method (sensitivity 99.4%; specificity 84.4%).

CuSO4 also produced good results, with an overall false result rate of 7.9% (80/1014) and a sensitivity of 98.8%, specificity of 58.1%, PPV of 92.3%, and NPV of 90.7%. 6.9% (70/1014) of donors were incorrectly passed through the CuSO4 screening test. Of these, 65 donors' Hb values, as determined by the reference method, ranged from 12.4 to 12.0 g/dl. 41 (24.5%) of the total deferrals (4.0% of donors who appeared to be in good health) had Hb values less than 11.0 g/dl. With a sensitivity of 87.2% and a specificity of 81.8%, HCS yielded inaccurate estimates for 256 samples (25.2%) when applied to this method. The range of 10–12 g/dl was found to have the maximum (63.5%) incoherent results when comparing HCS values in 2 g/dl increments. variations in the upper and lower 95% limits of agreement (LOA) and means.

## Conclusion

CuSO4 had an accuracy of 95.83%, while the standard reference method, the Automated Hematology Analyzer, had an accuracy of 100%. CuSO4's error rate was 4.17%.

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