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Rate of False Deferral and False Pass of Prospective Blood Donors Screened by Copper Sulphate Gravimetric Method at Hossana Blood Bank, Hossana, South Ethiopia.

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This is to certify that the thesis prepared by Eshetu Guracha, entitled:

Rate of False Deferral and False Pass of Prospective Blood Donors Screened by Copper Sulphate Gravimetric Method at Hossana Blood Bank, Hossana, South Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Chairman of the Department or Graduate Program Coordinator

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Abbreviations

AHD	Alkaline Hematin Detergent
AOR	Adjusted odds ratio
BTS	Blood Transfusion Services
CuSO4	Copper Sulphate
DIID	Donation-induced iron deficiency
EDTA	Ethylenediamine tetra acetic acid
ENBTS	Ethiopian national blood transfusion service
FBC	Full blood count
Hb	Hemoglobin
HCS	Hemoglobin color scale
HiCN	Cyanmethemoglobin
NPV	Negative predictive value
PPV	Positive predictive value
SNNPR	South Nations Nationalities and People Region
SOPs	Standard operating procedures
SPSS	Statistical package for social science
STTD	Short term temporary deferral
WBD	Whole blood donors
WHO	World Health Organization

Operational definitions

False deferral: - excluding donors from donation while their hemoglobin is above the cut-off value.

False pass: - making donors to give blood while their hemoglobin is below the minimum acceptable level.

Hemoglobin cut-off value: - a minimum acceptable level of hemoglobin for blood donation (12.5 g/dl for female and 13.5 for male).

Voluntary non-remunerated donor :- donor who gives blood with his/her own free will and receives no payment, either in the form of cash or in kind.

Replacement donors:- individuals who give blood when it is required by a member of

their own family or community.

Paid donors:- a person who give blood in return for payment or other benefits.

True positive:- donors who pass both the copper sulphate screening test and the reference method.

True negative: donors who fail both the copper sulphate screening test and the reference method.

False positive: donors who pass the copper sulphate screening test but fail the reference method.

False negative: donors who fail the copper sulphate screening test but pass the reference method.

Sensitivity: The probability of passing by copper sulphate while passing by the reference method.

Sensitivity = Tp / (Tp + FN)

Specificity: The probability of failing by copper sulphate while failing by the reference method.

Specificity = TN / (TN + FP)

Positive predictive value: The probability that a person actually passes given that he or she tests positive (pass). PPV = Tp / (TP + Fp)

Negative predictive value: The probability that a person is truly failed given that he or she tests fail. NPV = TN / (FN + TN)

Agreement: Measurement of the extent to which the two method (copper sulphate test and cell-dyn 1800) give the same result (pass or fail) for the same donor.

Abstract

Background: The primary responsibility of a blood transfusion service (BTS) is to provide a safe, sufficient and timely supply of blood and blood products so as to ensure that blood donation causes no harm to the donor. The Copper Sulphate (CuSO4) screening test has been found to erroneously include anemic people and exclude eligible people and its performance is not well studied in poor settings like Ethiopia.

Objectives: To determine the rate of false deferral and false pass of prospective blood donors screened by the copper sulphate gravimetric test from April to June 2016 at Hossana blood bank, Ethiopia..

Methods: A cross- sectional study was carried out from April to June 2016 in Hossana blood bank. Blood sample from 422 voluntary donors were collected and analyzed by $CuSo_4$ gravimetric test and automated hematology analyzer (Cell-dyn 1800). The data was entered in Epi-Data 3.1 and analyzed by SPSS 20 and interpreted accordingly. P value less than 0.05 was considered as statistically significant. Agreement between the two methods was determined using the Kappa coefficient.

Result: The overall deferra1 rate was 20.1% by the reference method and varies by blood collection site for the copper sulphate method (15.4% and 16.8% using capillary and whole blood, respectively). False pass rates were 9.2% and 7.6% and false fail rates were 4.5% and 4.3% using capillary and venous blood samples, respectively. The $CuSo_4$ method using capillary blood sample had sensitivity of 94.4%, specificity 54.1%, PPV 89.1% and NPV 70.8%, while using venous blood sample it gave sensitivity of 94.7%, specificity 62.4%, PPV 90.9% and NPV 74.6%. The $CuSo_4$ method had moderate with Kappa value of 0.53, (95% CI, 0.425-0.625, $P<0.01$) and substantial (Kappa value of 0.61, 95% CI (0.507 to 0.698), ($P<0.01$.) level of agreement with the reference method using capillary and venous blood samples, respectively.

Conclusion: The high rate of false pass warrants the search for an alternative simple method to replace the old copper sulphate method. Until replaced, CuSO4 method which moderately agrees with the reference method can be retained as a primary screening tool by applying strict quality control method.

Introduction

1.1 Background

Blood transfusion is an indispensable component of health care. It contributes to save millions of lives each year in both routine and emergency situations. It permits increasingly complex medical and surgical interventions and dramatically improves the life expectancy and quality of life of patients with a variety of acute and chronic conditions [1].

Determination of the hemoglobin (Hb) concentration in prospective blood donors is a well-established initial screening test routinely performed before donation, which protects both donors' and recipients' health. The rationale for performing this test is to ascertain that the loss of a unit of blood would not lead to symptoms of anemia in the donor following the blood donation, and to ensure that the unit of packed red blood cells collected for the patient meets or exceeds an objective standard in terms of Hb content [2].

Blood Transfusion Services (BTS) are the vital part of modern health care system without which efficient medical care is impossible. The aim of BTS should be to provide effective blood and blood products, which are as safe as possible and adequate to meet patients' need. Many measures have been taken to make the blood transfusion safe by establishing blood transfusion committee. The most important is the selection of blood donors by donor selection criteria [3].

Blood donors are grouped into voluntary donors, replacement donors, and paid donors. The safest of these is the voluntary donor. In Ethiopia proportion of voluntary blood donation increased from 10% in 2012 to 92.1% in 2014 achieving the WHO regional target of 80% voluntary blood donation. The primary responsibility of a blood transfusion service is to provide a safe, sufficient and timely supply of blood and blood products. In fulfilling this responsibility, the BTS should ensure that the act of blood donation is safe and causes no harm to the donor. It should build and maintain a pool of safe, voluntary non-remunerated blood donors and take all necessary steps to ensure that the products derived from donated blood are efficacious for the

recipient, with a minimal risk of any infection that could be transmitted through transfusion [4-6].

The pre-donation assessment of donor hemoglobin remains the best approach as there are no rapid, simple and direct bedside methods for determining iron status. The aim of hemoglobin screening is to ensure that the prospective donor is not anemic. The lower limit of acceptable hemoglobin for blood donation should be set at a level that prevents the selection of anemic individuals as blood donors and also minimizes the exclusion of healthy donors. Hemoglobin screening safeguards anemic individuals from donating blood and also protects returning donors from donation-induced iron deficiency (DIID), the depletion of iron stores by repeated donations. Collecting a unit of blood from a donor with a normal hemoglobin level also provides good quality blood components, with adequate and consistent hemoglobin content in the collected blood [6].

International and national guidelines commonly recommend minimum hemoglobin levels of 12.5 g/dl for females and 13.5 g/dl for males to be qualified as donors. In some countries (Canada, United states, Malaysia and India) the same hemoglobin level (12.5g/dl) is used for males and females. Ethiopian national blood transfusion service (ENBTS) uses 12.5 g/dl for females and 13.5 g/dl for males. This standard is intended to protect both the donor and the recipient. It is used to assess the general health of a donor, to make sure the donor can tolerate the loss of a unit of blood without developing symptomatic anemia, and to ensure that the red cell content of a unit of blood meets a minimum standard [7-9].

Screening for anemia is essential for blood donation to protect the donor and optimize the aim of red cell transfusion in the recipient. The copper sulphate specific gravity method is accepted method for mass screening of blood donors. This method was described in 1945 by Phillips *et al* for determining the specific gravity of whole blood and plasma [10-12].

The copper sulphate method is performed by allowing a drop of whole blood to fall in to the copper sulphate solution with specific gravities of 1.053 and 1.055, which are equivalent to12.5g/dl and 13.5g/dl of hemoglobin and used to screen female and male blood donors respectively. The whole blood maintains its own density, for approximately 15 seconds. If the drop of blood is denser than the specific gravity of copper sulphate it will go down indicating that the donor's hemoglobin level is acceptable. If the drop of blood floats on the copper sulphate solution, the donor's hemoglobin level is not good enough for blood donation. Although it is quick, easy and inexpensive method, it has its own limitation in that it does not give quantitative result, has subjective end point, and is difficult to quality control [13,14].

1.2 Statement of the Problem

Anemia is a major public health concern in developing countries like Ethiopia. World health organization estimated that anemia affected over 1.62 billion people, representing 24.8% of the world population. The degree of iron deficiency anemia in Ethiopia has not yet been well documented at a national level [15,16].

Blood transfusion is a lifesaving therapy for instance, whole blood is used in acute blood loss with hypovolumia and exchange transfusion; red cell concentrate is used to treat anemic patients; and platelet transfusion is indicated to prevent hemorrhage in patients with thrombocytopenia or platelet function defects. Moreover, fresh frozen plasma transfusion is indicated in settings where the patient is known to have multiple clotting factor deficiencies with active bleeding. Whereas cryoprecipitate is used in cases of hypofibrinogenemia, which most often occurs in the setting of massive hemorrhage or consumptive coagulopathy, von willebrand factor deficiency, factor VIII (hemophilia A) and factor XIII deficiency [17,18].

Approximately 8 million units of blood are currently needed to meet transfusion demand for a population of nearly 800 million in Africa. However, only 3 million units of blood are collected satisfying a mere 40% of this estimated need [19].

Deferral through failure to accord with selection criteria is highly unwelcomed by blood donors. Low hemoglobin (the most common cause of donor deferral worldwide), abnormal blood pressure or pulse, medication and fainting are the main non-infectious cause of deferral. Temporary deferral had negative impact on blood donor return rates for upcoming blood donation. Improper donation rejection due to low hemoglobin causes not only the current loss of blood units, but also possible shortage of blood supply in future [20-21].

A retrospective cohort analysis of allogenic whole blood donation conducted from 2000-2005 by Custer *et al* revealed that only 25 percent of temporarily deferred donors returned during the 5-year follow-up period compared to 47 percent of eligible donors. For repeat donors, 81% and 86% of deferred and eligible donors returned, respectively [22]. Also Halperin *et al* revealed that short term, temporary deferral (STTD) have very negative impact on blood donor return

rates and subsequent blood donations. When compared to STTD, non deferred donors were 29% more likely than the donors with STTD to return over the next 4.22 years, and non deferred donors donate 81% more whole blood units over the same period. Each deferral causes the loss of at least one donation from a willing donor. The impact of the deferral is multiplied because, once deferred, many donors do not return to attempt another donation [23- 25].

Studies have shown that the Copper Sulphate (CuSO4) screening test has been found to erroneously include anemic people and exclude eligible people, because it does not measure the actual hemoglobin but it is based on the principle of specific gravity [26].

1.3 Rationale of the study

The finding of this study will give information about how many fit blood donors are inappropriately excluded and ineligible blood donors are accepted for blood donation by the copper sulphate donor screening test. Thus, it will help to plan and implement additional, sensitive and specific test method for hemoglobin determination. By so doing, it will ultimately help minimize unnecessary deferrals and hence donor dissatisfactions. As the method conducted in several blood bank laboratories in Ethiopia this study can be considered as a base line.

2. Literature Review

A recent study by Mohan *et al* (2016) in India found that the specificity and PPV of copper sulphate was higher than Hemocue (83% and 80% versus 70% and 59% respectively) with similar sensitivity (100%). And the false positive rate of $CuSo_4$ was 19.2% while the false positive rate of Hemocue was 40.35%; false negativity was zero for both method [27].

Gupta et *al* (2015) in India compared the specific gravity (CuSo4) donor hemoglobin estimation method with hemocue and automated cell counter methods. The result revealed that the copper sulphate gravimetric test was found to be less specific with specificity of 55.1%, sensitivity 96.8%, positive predictive value (PPV) 91.8% and negative predictive value (NPV) 81.42%. The test also inappropriately passed 3.8% (19/500) donors, out of these 17 donors had hemoglobin(Hb) value between 11.0-12.4g/dl when tested by reference method (ERMA PCE 210, Tokyo), while1.4%(07/500) of donors were falsely deferred by $CuSo_4$ method [28].. Kim *et al* conducted a cross sectional study on evaluation of two Non invasive Hemoglobin testing devices in Korea in 2015 and they found that sensitivity and specificity of $CuSo_4$ donor screening test was 81.8% and 95.2% respectively [29].

In a study conducted in India by Akhtar *et al* on 3000 voluntary blood donors, 120 were deferred by the copper sulphate screening test. Out of the deferred donors 39(32.5%) were accepted by applying portable hemoglobin photometer (Hemocue). Additionally by testing the 81 donors deferred by Hemocue using both automated hematology analyzer and Hemocue 65.4%(53) were accepted and only 28(34.6%)donors were temporarily deferred. In this study out of 120 donors 92(76.7%) were falsely deferred by the copper sulphate method [30]

Study by Malukani *et al* (2014) in India demonstrated that copper sulphate method gave good results with overall 8% (16/200) false results, sensitivity of 96.36%, but specificity of 71.43%, PPV of 94.08% and NPV of 80.65%. The copper sulphate test inappropriately passed 5% (10/200) donors. Out of these, 6 donors had hemoglobin value between 12.4-12.0g/dl when tested by the reference method [31].

Another study by Mathur *et al (2012)* in India evaluated the result of Hb by copper sulphate test against the digital hemoglobinometer. It indicated that, by applying digital hemoglobinmeter on 3163 donors who were deferred by copper sulphate method, 1196 (37.81%) of donors were found to have Hb above the minimum acceptable level and become eligible for blood donation [32].

A research report by Parihk S *et al* (2010) in India on comparative analysis of a Novel Alkaline Hematin Detergent -575(AHD-575) and copper sulphate gravimetric methods on hemoglobinometry against the cyanmethemoglobin standard reference method, showed that the copper sulphate method had 4.75% (19/400) false pass and 8.5% (34/400) false fail rates. Its sensitivity is 73.6%, specificity 89.6%, PPV 60.9% and NPV 93.9% [33].

On the other hand, a study conducted by Tondon R *et al* (2009) in India showed that copper sulphate donor screening test gave good results with overall 7.9%(80/1014) false results with sensitivity of 98.8% but specificity of 58.1% with a PPV of 92.3% and NPV of 90.7%. The $CuSo_4$ screening test inappropriately passed 6.9% (70/1014) donors. Out of these 65 donors had Hb values between 12.4-12.0 g/dl when tested by the reference method [34].Whereas a study by Sawant RB *et al (*2007) in India found that out of 400 donors 116(29%) of them who failed by the copper sulphate test had Hb level > 12.5 g/dl (minimum acceptable level) [35]

Nadarajan *et al* (2002) investigated anemia and iron status among blood donors in a blood transfusion unit in Malaysia. It was alarming to note that 10 out of the 23 iron deficient individuals who had been screened to have hemoglobin level above minimum acceptable level by the copper sulphate method actually had hemoglobin level below 12.5 g/dl at donation when tested by automated hematology analyzer (ABX Vega Retic) using venous blood sample. Forty of all donors, comprising 21.3% (40/187) of the donors studied, had hemoglobin level below 12.5g/dl although they had passed the copper sulphate screening test [36].

Evaluation of four rapid methods for hemoglobin screening of whole blood donors in mobile collection settings by Gomez-Simon *et al* (2007) in Spain estimated that as many as 83% and 69% of prospective donors with unacceptable hemoglobin levels passed the copper sulphate test

and Hemocue, respectively. Inversely, the percentage of prospective donors with acceptable hemoglobin levels who were deferred from donation based on the copper gravimetric test and HemoCue, was only 1%, and of 3%, respectively . The estimated relative risk for a donor with low hemoglobin concentration, either male or female, for being inappropriately bled would be 6.08 if screened by the copper sulphate procedure; and 3.2 if tested with HemoCue [37].

In a study done in United Kingdom (UK) by applying statistical analysis on inappropriate results of current hemoglobin screening test, the false-pass and –fail rates for women and men respectively, were 11.2 and 6.3 percent (women) and 5.2 and 1.8 percent (men) for copper sulphate method [26].

A WHO hemoglobin color scale (HCS) validation study by Lewis SM (2002) which was done by applying two different approaches at several blood transfusion centers in United Kingdom to assess rate of false rejection and acceptances by using Automated hematology cell analyzer (Sysmex KX 21, Sysmex America, Inc. Lincolnshire) as a reference method . The result showed that, when all centers use their own customary Hb cut-off value for blood donor selection, the HCS gave 3.7% false results (1.5% false reject,2.2% false accept) compared to the copper sulphate method by which there were 6.1% false result(5.2% false reject, 0.9% false accept). When all centers reduce the Hb cut –off value to 12.0g/dl, the HCS showed 1.6 % false reject compared to 5.2% by copper sulphate. Thus HCS had the advantage over copper sulphate in reducing donor rejection rate [38].

A study of 250 donors in United kingdom reported in 2000 revealed that the specificity and sensitivity of gravimetry against venous automated full blood count (FBC) were 89% and76% for men and 95% and 45% for women respectively. But this study is compromised by using an immediate post donation venous sample (the hemoglobin which is known to be reduced by approximately 5%) to compare with gravimetric reading [39].

The inaccuracy of the specific gravity method for donor screening is reported to be worsen by other conditions as demonstrated in one case report by Newman B in 1997. It was shown that

gravimetry can pass severely anemic donors whose whole blood specific gravity is increased by pathology like paraprotenaemia or leukocytosis [40].

A review on the biological variation in human hemoglobin levels, and the current controversies in the establishment of blood donor Hb by Cabel RG (1995) in United States, suggested that the primary drawback of copper sulphate method was inappropriate deferral. Approximately 50% of deferred donors by the copper sulphate screening recovered by testing the same or a new blood sample with different method. Nearly all blood centers that use copper sulphate screening retest donors who fail testing, and those who passed the cutoff the second test are accepted [41].

Semie W *et al* conducted a cross sectional study at Jimma in 1998 to evaluate the accuracy of copper sulphate screening method for anemia in pregnancy against the standard cyanmethemoglobin method. They used two copper sulphate solutions with different strength Sp. G. 1.044 to detect hemoglobin level <8g/dl and Sp.G.1.049 to detect hemoglobin level<11.0g/dl in pregnancy. Copper sulphate solution with Sp.G. of 1.044 has sensitivity 95%, specificity 98.4%, PPV 75% and NPV 99.4%. Moreover copper sulphate solution with Sp.G. of 1.049 has sensitivity of 96.2%, specificity 91%, PPV 82.0% and NPV 99% [42].

Landis and Koch have proposed the following as standards for strength of agreement of Kappa coefficient: < 0.00 = Poor, 0.00-0.20 = Slight, 0.21-0.40 = Fair, 0.41-0.60 = moderate, 0.61-0.80= Substantial, 0.81-1.00 Almost Perfect [43].

Patel *et al* conducted cross sectional study in 2013 in United states on 150 prospective donors on capillary versus venous hemoglobin determination in the assessment of healthy blood donors and concluded that Capillary hemoglobin levels were significantly higher than venous hemoglobin values obtained using the same hemoglobin meter, and both capillary and venous assessments using the hemoglobin meter were found to be significantly higher than “reference” hemoglobin values obtained using the automated analyzer (Cell-dyn4000) [44].

Radtke *et al* conducted cross sectional study in 2005 in Germany on hemoglobin screening in prospective blood donors: Comparison of different blood samples and different quantitative

methods and found that the finger stick hemoglobin values tended to be higher than the venous hemoglobin concentrations [45].

Agnihotri N conducted cross sectional study in 2010 on Whole blood donor deferral analysis at a center in Western India and found that females have higher deferral rate than males due to low hemoglobin (77.9% in females vs 37.0% in males; *P*=0.000). Odds ratio for deferral in female donors was 15.4 (95% confidence interval 12.8 to 18.6), implying thereby that chance of deferral in females is nearly 15 times higher as compared to males [46].

A study by Mast AE *et al* (2010) in United States on demographic correlates of low hemoglobin deferral among prospective Whole Blood Donors showed that the dominant determinant of low hemoglobin deferral was gender; 1.6% of donation attempts made by males were deferred, while 17.7% of donation attempts made by females were deferred. Overall, women had on average 11 times higher odds for a low hemoglobin deferral compared to men [47].

Also Ngoma AM *et al* in Japan (2014) found that being female was associated with deferral due to low hemoglobin level. More importantly, females were deferred almost 35 times more than males [48].

Taken together, several studies documented different rate of false rejection as well as pass rates when the copper sulphate method is used for donor screening. As far as my knowledge goes, such published studies are lacking in our country.

3. Objectives

3.1. General objective

To assess rate of false deferral and false pass of prospective blood donors screened by the copper sulphate gravimetric test as compared to automated hematology analyzer from April to June 2016

3.2. Specific objectives

- To determine rate of false deferrals of prospective blood donors screened by the copper sulphate gravimetric test
- To determine rate of false pass of prospective blood donors screened by the copper sulphate gravimetric test
- To calculate the specificity, sensitivity, PPV, NPV, and kappa values of copper sulphate donor screening test against automation.
- To determine effect of sampling site variation on the rate of deferrals.

4. Hypothesis

HO: The rate of false deferral or false pass between copper sulphate and automated method will be similar.

5. Materials and methods

5.1. Study area

The study was conducted in Hossana town in South Nations and Nationalities People Region (SNNPR) which is located 232Km south of Addis Ababa. The town has a total area of 23sq.km and lies between $7^0$33’ N latitude and $37^0$51’06.60’’ E longitude. Hossana blood bank which is found in the town was established in 2013 and it’s catchment population is 5,412,601. The blood bank serves hospitals in five zones; these are Hadiya, Kambata, Tembaro, Wolyita, Siltie and Gurage zone. Selection of suitable blood donor, on site and field collection of blood from fit donors, ABO grouping and Rh typing, and screening of blood for transfusion transmitted infections are the main activities performed in the blood bank.

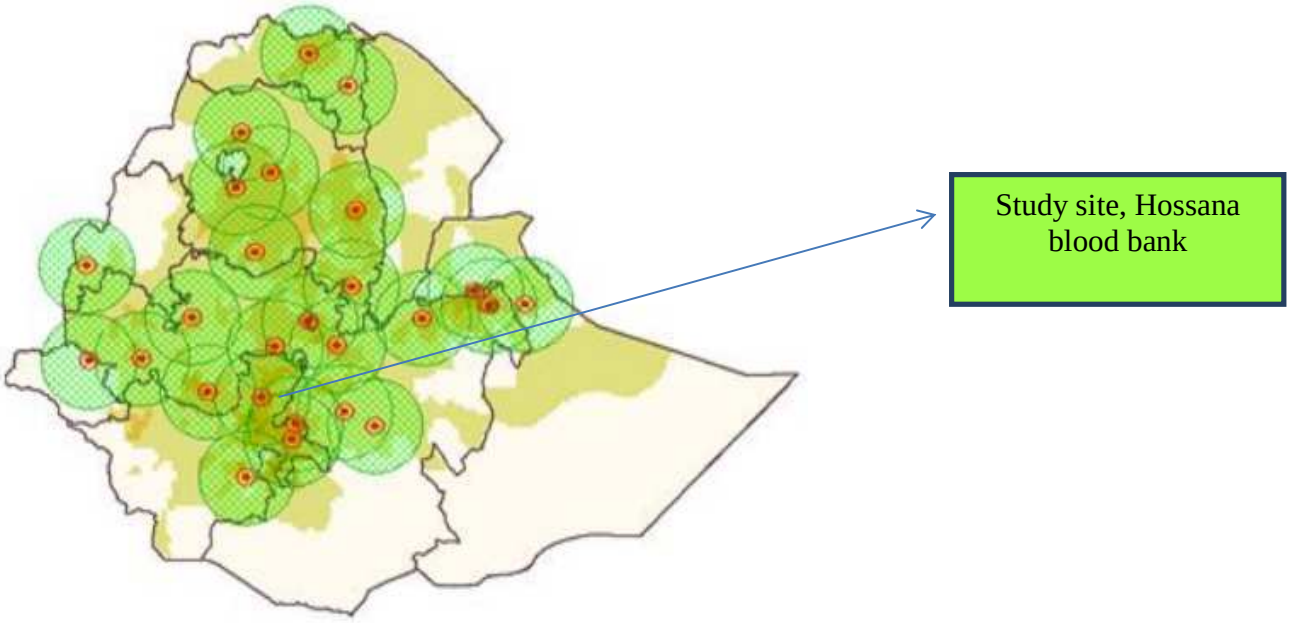

Figure 1 Regional blood bank sites, Hossana blood bank, study site

5.2. Study period

The study was carried out from April to June 2016.

5.3. Study design

A prospective cross sectional study was conducted in Hossana blood bank to determine the rate of false deferral and false pass of donors screened by copper sulphate gravimetric test.

5.4. Population

5.4.1. Source population

Non –remunerated voluntary blood donors who visit Hossana blood bank.

5.4.2. Study Population

The study population was voluntary blood donors, who were willing to take part in the study.

5.5. Inclusion and exclusion criteria

5.5.1. Inclusion criteria

Voluntary blood donors, who were willing to participate and pass other donor selection criteria like Age limit, blood pressure, pulse , temperature, weight, were included in this study.

5.6. Study variables

5.6.1. Dependent variable

- Rate of false deferral and false pass

5.6.2. Independent variables

- Sampling sites variation (Venous versus capillary)
- Age,
- Sex
- Frequency of previous blood donation

5.7. Measurement and Data collection

5.7.1. Sample size determination

The maximum number of sample required for this study is determined by using single population proportion formula considering the following assumptions:

Where:-

n, = maximum sample size required for the study

Z,=standard normal distribution ($Z\alpha/2=1.96$) with confidence interval of 95% will be used

P,= since similar study was not done in Ethiopia, p value of 0.5 will be used

d,= absolute precision or tolerable margin of error(d)=5%(0.05)

q,=(1-p)

$$n = \frac{(z_{\alpha/2})^2 \cdot pq}{d^2}$$

$$n = \frac{(1.96)^2 * 0.5(1-0.5)}{(0.05)^2}$$

n= 384

The total sample size of study is 384+38(10% non response rate)=422.

5.7.2. Sampling method

Convenience sampling method was used.

5.7.3. Data collection procedure

Four milliliter of venous blood sample was collected in EDTA tube from the study participants following standard operating procedures (SOPs) for blood sample collection after obtaining their consent. Most of the samples were collected from mobile sites and transported to hospital lab in a cold box. The Hgb value of the sample was estimated by the copper sulphate method and the automated hematology analyzer (as a reference method). The results of the copper sulphate test was interpreted as pass or fail at hemoglobin cut off ≥12.5 g/dl for female and ≥13.5g/dl for male.

The effect of sampling site variation was determined by testing both capillary and venous blood sample with copper sulphate gravimetric method. Capillary blood was collected following SOP from ring finger using sterile lancet. The first drop of blood was wiped off and freely flowing capillary blood collected using capillary tube and copper sulphate test was performed using copper sulphate with specific gravity of 1.053 for females and 1.055 for males. Donors were classified as pass or fail based on the behavior (sinking or floating) of the sample in the copper sulphate solution.

5.7.4 Principle of copper sulphate gravimetric test method

The specific gravity measurements are carried out by letting drop of whole blood from 1cm above the surface of the solution fall in to a copper sulphate solution of either 1.053 or 1.055 specific gravity, which are equivalent to hemoglobin levels of 12.5g/dl and 13.5g/dl respectively. Each drop on entering a solution becomes encased in a coat of denatured plasma proteins and reaming as a separate mass without change of gravity. The momentum of the droplet takes it to

1-2cm below the surface within 5 seconds. The movement of the drop over the next 10 seconds is observed. If it continues to fall; the hemoglobin level is judged to be above 12.5g/dl or 13.5g/dl. If it rises, the hemoglobin level is taken as less than 12.5g/dl (minimum acceptable Hb level for female) or 13.5g/dl (minimum acceptable Hb level for male) [13].

5.7.5 Principle of Cell-Dyn 1800 Hematology Analyzer

Cell-Dyn 1800 hematology analyzer uses a modified methemoglobin method for the colorimetric determination of hemoglobin. A zero or blank reading is first obtained to provide a reference to which the sample signal is compared. Lyse reagent lyses the diluted red blood cells and convert the released hemoglobin to a chromogen. The sample is then transferred to the hemoglobin flow cell where Hb concentration is measured. A low energy light emitting diode (LED) is used as a light source and shines through the Hb flow cell and 540 nm narrow band width filter on to a photo detector. The Hb concentration is directly proportional to the absorbance of the sample. Reference and sample readings are compared to determine the Hb concentration of the sample. The result is expressed in gram of Hb per deciliter (g/dl) of whole blood. (Cell-Dyn System 1800 Operator's Manual. 2003.)(Annex V)

5.7.6. Hematological analysis

Blood sample (both capillary and venous) collected from the study participants was first, tested by the copper sulphate gravimetric method to make sure whether its hemoglobin is above or below the minimum acceptable level for blood donation. Then all venous blood samples tested by first test, were transported to Nigst Eleni Mohammad memorial hospital laboratory and analyzed by automated hematology analyzer within 12 hours (since most of the samples were collected from mobile sites, and it takes time to transport the sample to lab. The storage time has no significant effect on hemoglobin value, since hemoglobin concentration was found to be stable up to72 hours stored at 4^0C and room temperature [49, 50]. The results obtained by the two tests were registered on data collection form prepared for this purpose.(Annex IV and V)

5.8. Data Quality Assurance

Per-analytic

- Blood sample was collected and processed according to the standard operating procedures.
- Standard and working copper sulphate solutions were prepared and standardized daily following SOPs (blood bank SOPS) before using it.
- Copper sulphate solution was checked for suitability in donor screening by observing the behavior (sinking or floating) of drops of blood of known hemoglobin concentration.
- Blood sample was homogenized before running the test.

Analytic

- Quality control of automated hematology analyzer was done as per SOPs using control sample before running the sample used for the study purpose.
- During analysis the manufacturer procedures and SOPs were followed strictly.
- Thirty ml of copper sulphate solution was used for only 25 tests as per the protocol

Post-analytic

- The result of copper sulphate test was registered as pass or fail and also the exact value of hemoglobin by the analyzer obtained in print out.

5.8.1 Precision (Repeatability) test:

The objective of this test was to determine the analyzer capacity to reproduce the results for a certain parameter in a given sample. Analysis was carried out in normal conditions, by the same operator, within a short period of time, same location and with the same reagents. In order to fulfill the manufacturer's recommendation, samples to the analytical ranges of normal values and no flags were used. The prepared sample was analyzed 20 consecutive times in the open mode of Cell-Dyn 1800. The mean, the standard deviation (SD) and the coefficient of variation (CV)

were calculated for hemoglobin. Prior to the analysis, samples were well homogenized by inverting 8-10 times. Table 1 summarizes the calculated coefficient of variation values of Cell-Dyn 1800 for hemoglobin. As shown in the table, the precision (repeatability) values of hemoglobin lie within the verification specification of the manufacturer.

Table 1 Imprecision (within sample variation) of cell-dyn 1800 hematology analyzer for hemoglobin determination

Parameter	**Mean**	**Standard deviation (SD)**	**Coefficient Variation(%CV)**	**Manufacture's specification**
Hemoglobin	15.650	0.2236	0.050	≤ 1.2

5.9. Data analysis and interpretation

The data obtained from the two tests was entered in epi-data version 3.1 and exported to SPSS 20. The exported data was cleaned, analyzed and interpreted accordingly. The specificity, sensitivity, PPV and NPV of copper sulphate donor screening test were calculated. Agreement between the two methods was determined using Kappa. P-Value < 0.05 was considered statistically significant.

5.10. Ethical considerations

Before the research work, ethical clearance and approval was obtained from Addis Ababa University, Medical Laboratory Science department research ethics committee. The study aim, risks, benefits and right for withdrawal anytime from the study was explained to the study participants and informed consent was obtained. Samples were coded and confidentiality of data was maintained throughout the study by locking hard copies and password protecting electronic files.

5.11. Dissemination of the result

The final paper will be presented and submitted to Addis Ababa University, College of Health Science Medical Laboratory Science Department, SNNPR regional blood bank, Ethiopian national blood transfusions service and Hossana blood bank so as to influence for using sensitive and specific methods of hemoglobin screening in blood donation setting. It will also be presented for scientific community elsewhere and manuscript will be submitted to peer reviewed national or international journals.

6. Result

The study participants were 422 voluntary blood donors. Among them, 217 (51.4%) were females. The study population aged between 18-63 years with a median (±SD) age of 21 ± 6.3. Majority 311(73.7%) of study participants were between the age range of 18-24 years and 102 (24.2%) were in the age range of 25-44 years. Large proportion of the donors, 293 (69.4%), were first time donors [Table 2].

Table 2. Demographic characteristics and donation status of study participants at Hossana Blood bank, South Ethiopia, April-June, 2016

Variable	N	%
Sex		
Male	205	48.6
Female	217	51.4
Age group		
18-24	311	73.7
25-44	102	24.2
>44	9	2.1
Donation Status		
First time donation	293	69.4
Repeat donation	129	30.6

The overall deferral rate, as determined by the reference method using the recommended Hb cut off values of 12.5 gm/dL for females and 13.5 gm/dL for males was 20.1% (85/422). As shown in Table 3, the deferral rate was higher among female donors 58 (68.2%) as compared to male donors 27(31.8%) and among those aged 18-24 years which was 82.4% (70/85). When calculated by donation status, from the overall 85 deferrals, the rate was higher in first time donors 52(61.2%) compared to repeat donors 33 (38.8%) [Table 3].

Table 3. Rate of pass/fail based on demographic characteristics and donation status as determined by the reference method (Cell-Dyn 1800) at Hossana Blood bank, South Ethiopia, April-June, 2016

Variable	Pass	Fail	Total
Sex			
Male	178(52.8%)	27(31.8%)	205(48.6%)
Female	159(47.2%)	58(68.2%)	217(51.4%)
Total	337	85	422
Age group			
18-24	241 (71.5%)	70 (82.4%)	311 (73.7%)
25-44	89 (26.4%)	13 (15.3%)	102 (24.2%)
>44	7 (2.1%)	2 (2.4%)	9 (2.1%)
Total	337	85	422
Donation Status			
First time donation	241(71.5%)	52(61.2%)	293(69.4%)
Repeat donation	96(28.5%)	33(38.8%)	129(30.6%)
Total	337	85	422

Figure 2 depicts hemoglobin level distribution of donors as determined by the reference method (Cell-Dyn 1800). The minimum and maximum hemoglobin values among blood donors were 7.3 g/dl and 18.4g/dl, respectively with an overall mean (SD) value of 14.3 (1.9) gm/dL.

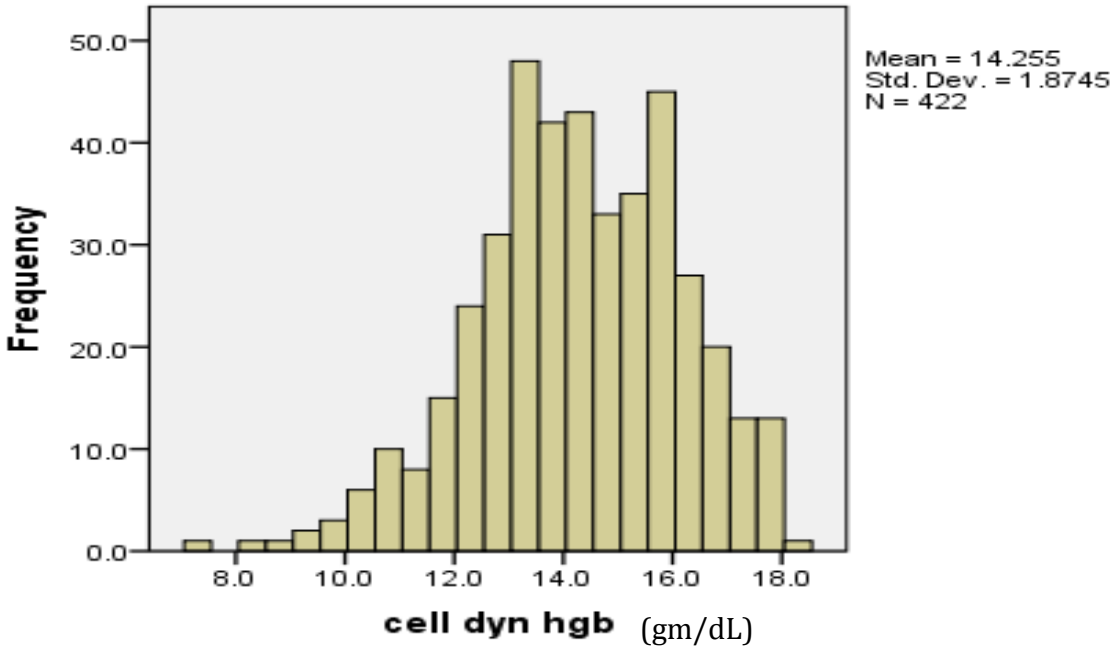

Figure 2. Hemoglobin level distribution of donors at Hossana Blood bank, as determined by reference method (Cell-Dyn 1800), South Ethiopia, April-June, 2016

Hemoglobin values with reference method were analyzed and comparison of the pass and fail rate of copper sulphate donor screening test using both capillary and venous blood sample against the reference method (Cell dyn 1800) was summarized in Table 4. The cut off value recommended by WHO was hemoglobin level of not less than 12.0 g/dl for females and not less than 13.0 g/dl for males as the threshold [6] and our country uses 12.5 gm/dL for females and 13.5 gm/dL for males to determine pass/fail status. As shown in the table, among those with Hb value less than 12.5 gm/dL none were rated as “Pass” by the reference method while 25 and 18 of prospective donors in this category were rated as “Pass” by CuSo4 method using capillary and venous blood samples, respectively. The overall deferral by blood collection site also varies; 65 (15.4%) versus 71 (16.8%) for capillary and venous samples, respectively which is lower than the 85 (20.1%) deferral by the reference method.

Table 4. Deferral rates of donors by Hemoglobin categories determined by reference method (Cell-Dyn 1800) and CuSo4 screening method at Hossana Blood bank, South Ethiopia, April-June, 2016.

Hemoglobin value	**True pass and fail (by reference method)**		**$CuSo_4$ using capillary blood sample**		**$CuSo_4$ using venous blood sample**	
	Pass	Fail	Pass	Fail	Pass	Fail
<9.5	0	5	0	5	0	5
9.5-10.9	0	16	4	12	3	13
11.0-12.4	0	44	21	23	15	29
12.5-13.9	101	20	96	25	97	24
14.0-15.9	153	0	153	0	153	0
16.0-17.0	56	0	56	0	56	0
>17.0	27	0	27	0	27	0
Total	337	85	357	65	351	71

The performance characteristics of the copper sulphate screening method were evaluated against the Reference method (Cell-Dyn 1800) using both capillary and venous blood samples (Table 5). Accordingly, the copper sulphate screening test using capillary blood sample inappropriately passed 39/422 (9.2%) donors, while 19/422 (4.5%) of donors were falsely deferred. When using venous blood sample, the copper sulphate screening test inappropriately passed 32/422 (7.6%) donors and falsely deferred 18/422 (4.3%) donors.

The copper sulphate screening test using capillary blood sample had sensitivity of 94.4%, specificity 54.1%, PPV 89.1% and NPV 70.8%, while using venous blood sample it had sensitivity of 94.7%, specificity 62.4%, PPV 90.9% and NPV 74.6%.

Table 5. Performance characteristic of CuSo4 donor screening test against Cell-Dyn 1800 automated hematology analyzer at Hossana Blood bank, South Ethiopia, April -June, 2016.

Result	$CuSo_4$ using capillary blood	$CuSo_4$ using venous blood
True positive	318	319
True negative	46	53
False positive	39	32
False negative	19	18
Sensitivity	94.4%	94.7%
Specificity	54.1%	62.4%
PPV	89.1%	90.9%
NPV	70.8%	74.6%

Kappa coefficient was calculated to determine the level of agreement between the copper sulphate and the reference method. The cut off proposed by Landis and Koch as standards for strength of agreement of Kappa coefficient, was used in this study: < 0.00 = Poor; 0.00-0.20 = Slight; 0.21-0.40 = Fair; 0.41-0.60 = moderate; 0.61-0.80 = Substantial, 0.81-1.00 Almost Perfect [43]. Accordingly, the copper sulphate donor screening test using capillary blood sample was in "moderate" level of agreement with the reference method: Kappa (0.53), 95% CI (0.425 to 0.625), P<0.01. Using venous blood sample it was in "Substantial" level of agreement with the hematology cell analyzer: Kappa (0.61), 95% CI (0.507 to 0.698), P<0.01.

Effort was made to see if any of the factors (sex, age, and previous donation status) could affect the rate of deferrals or pass by the copper sulphate methods by sites of blood sample collection. As shown in Table 6, status of pervious donation had no statistically significant association with adjusted odds ratio (AOR) of 1.375, 95% CI (0.790 to 2.394), P=0.261. Females were 3.75 times more likely to fail the copper sulphate test using venous blood sample than the males, AOR =3.75, 95% CI (2.001, 7.008), P <0.01. Age of donors had no statistical significant association, (AOR 0.464,95% CI0.198 to 1.090, P=0.078)

When using capillary blood sample, females were 2.67 times more likely to fail the copper sulphate test than the males, AOR = 2.67, 95% CI(1.450 to 4.923), P<0.002. Whereas, age [AOR =0.468, 95% CI (0.199 to 1.096), P=0.08] and frequency of pervious donation [AOR =1.191, 95% CI (0.672 to 2.110), P=0.557] had no statistically significant associations with rates of passing and failing the copper sulphate test.

Table 6. Effect of age, sex and frequency of previous donation on pass/fail rate of copper sulphate test using capillary and venous blood sample at Hossana Blood bank, South Ethiopia, April-June,2016.

		Capillary blood $CuSo_4$ test		Venous blood $CuSo_4$ test	
Variables	frequency	AOR (95% CI)	P-value	AOR (95% CI)	P-value
Gender					
Male	205(48.6%)	1		1	
Female	217(51.4%)	2.672(1.450-4.923)	0.002	3.745(2.001-7.008)	**0.01**
Age(years)					
18-24	311(73.7%)	1		1	
25-44	102(24.2%)	0.468(0.199 -1.096)	0.080	0.464(0.198-1.090)	0.078
>44	9(2.1%)	0.531(0.199-1.096)	0.550	0.465(0.056-3.894)	0.480
Previous donation					
First time	293(69.4%)	1		1	
Repeat	129(30.6%)	1.191(0.672-2.110)	0.557	1.375(0.790-2.394)	0.261

7. Discussion

To accept suitable donors and to prevent any inappropriate deferrals, there should be an appropriate Hb screening method [38]. The copper sulphate method being very easy, inexpensive, and not requiring venous blood, remains as a method of choice in many resource limited countries for primary Hb screening of prospective donors for many years. It needs to undergo strict quality control and validation before it is used to screen donors [30]. The method always has a chance of erroneously including anemic people and excluding eligible people because it does not give quantitative results but has subjective end points based on the principle of specific gravity [28].

This study determined the rate of false deferral and false pass of prospective blood donors screened by Copper Sulphate Gravimetric method at Hossana Blood Bank, South Ethiopia, using Cell-Dyn 1800 as a reference method. The study demonstrated $CuSo_4$ method using capillary and venous blood sample inappropriately passed 9.2% and 7.6% of donors, respectively; of which majority (75%) were within 1.0g/dl of threshold against the reference values. This finding was slightly higher than the observation made by Malukani *et al* (5%) in India using Sysmex kx 21 as a reference method [31]. The false pass rate in our study using venous blood was higher than the observation made by Tondon *et al* (6.9%) in India using ABX micros 60 as a reference method [34].

Other studies have also demonstrated high rates of inappropriate pass by the copper sulphate method. For instance, James *et al* in UK reported 16.4% using Bekman Coulter as a reference method [26]. Nadarajan *et al* (21.3%) in Malaysia using ABX Vega retic [36] and Gomez-Simon *et al* (83%) in Spain using Coulter Electronics [37] also observed more inappropriate pass by the $CuSo_4$ method, respectively. On the other hand, less inappropriate pass rate compared to the current study was observed by Gupta *et al* (3.8%) in India using ERMA PCE 210 cell analyzer [28], Lewis SM (0.9%) in UK using Sysmex KX 21 [30] and Parikh *et al* (4.75%) in India using cyanmethemoglobin standard as reference method respectively [33]. Mohan *et al* in India observed higher false pass rate (19.2%) than ours but Zero false fail rate using HORIBA microsemi CPR hematology analyzer as reference method [27]. The observed differences

between the various studies can be explained by the subjectivity of end point determination by the copper sulphate method, though instrument variations cannot be totally excluded. Moreover, the small sample size and different target population used by Mohan. *et al* might explain the difference with this study [27].

In this study, the copper sulphate method when using capillary blood sample tends to give more false pass rate as compared to venous sample, this difference might be due to greater hemoglobin value in capillary blood [37, 44, 45].

The $CuSo_4$ gravimetric method has also been found to give inappropriate failure and approximately 50% of deferred donors could be recovered by testing with different method [29]. The current study revealed that copper sulphate method by using capillary and venous blood sample inappropriately deferred 4.5% and 4.3% of donors, respectively. Finding of this study using capillary blood sample was almost similar with that of Lewis SM (5.2%) in UK using sysmex KX 21 as reference method [38]. But Mathur *et al* (37.81%) in India [32], Sawnt *et al* (29%) in India using coulter counter as reference method [35] and Akhtar *et al* (76.7%) in India [30] found more false deferral rate as compared with our finding. This difference might be due to using donors who fail the primary copper sulphate screening test as their study participants.

Also Parikh *et al* (8.5%) in India using cyanmethemoglobin [33] and James *et al* (8.1%) in UK using Bekman Coulter [26] as reference method observed slightly higher false deferral rates respectively. As compared to our finding Malukani *et al* (3%) in India using sysmex kx 21 [31], Tondon *et al* (1.3%) in India using ABX micros 60 [34], Gupta *et al* (1.4%) in India using ERMA PCE 210 cell analyzer [28] Gomez-Simon *et al* (1%) in Spain using coulter Electronics [37] as a reference method observed less false deferral rates, again underscoring the subjectivity of the old copper sulphate method of donor screening.

The calculated sensitivity of copper sulphate method for capillary and venous blood sample in the current study was 94.4% and 95%, respectively. This finding is slightly lower than that of Malukani *et al* (96.36%) in India using Sysmex kx 21 as a reference method [31]. Gupta *et al* (96.8%) in India using ERMA PCE 210 cell analyzer [28], Tondon *et al* (98.8%) in India using

ABX micros 60 and Mohan et al (100%) in India using HORIBA microsemi CRP Hematology analyzer as a reference method [27], respectively observed high level of sensitivity and Parikh *et al* (73.6%) in India using cyanmethemoglobin [33] and Kim *et al* (81.8%) in Korea using Sysmex KX-21N as a reference method found low level of sensitivity, respectively [29]. This difference might be due to using manual cyanmethemoglobin method as a reference by Parikh *et al*.

Specificity of copper sulphate gravimetric method when using capillary and venous blood sample was 54.1% and 62.4% respectively. Parikh *et al* (89.6%) in India using cyanmethemoglobin [33], Mohan *et al* (83%) in India using HORIBA microsemi CRP Hematology analyzer [27] , Kim *et al* (95.2%) in Korea using Sysmex KX-21N [29] and Malukani *et al* (71.43%) in India using sysmex kx 21 as a reference method [31] observed high level of specificity respectively as compared to ours. Gupta *et al* (55.1%) in India, on the other hand, using ERMA PCE 210 cell analyzer as a reference method [28] observed almost similar level of specificity with our copper sulphate method using capillary blood sample. Gupta *et al* (55.1%) in India using ERMA PCE 210 cell analyzer [28] and Tondon *et al* (58.1%) in India using ABX micros 60 [34] as a reference method observed low level of specificity as compared to copper sulphate method using venous blood sample.

An earlier study from Ethiopia by Semie *et al* [42] observed similar level of sensitivity and high level of specificity as compared to our finding. This difference might be due to using different strength of copper sulphate solution (Sp. G. 1.044 and Sp.G.1.049) and different cut off value for hemoglobin (<8g/dl and <11.0g/dl).

Based on Landis and Koch's standard [43] the copper sulphate donor screening test using capillary blood sample was in moderate level of agreement with the reference method: Kappa (0.53), 95% CI (0.425 to 0.625), P<0.01. Using venous blood sample it was in substantial level of agreement with the hematology cell analyzer: Kappa (0.61), 95% CI (0.507 to 0.698), P<0.01. Considering the routine practice of use of capillary blood for donor screening using the copper sulphate method in the blood bank, and the Ethiopian Federal Ministry of Health's plan of recruiting large number of voluntary donors, the moderate level of agreement observed for the

traditional copper sulphate method with the reference method is of concern. As deferral may have a negative impact on donor satisfaction [25], effort has to be made to have reliable method of screening to maximize donors' satisfaction and maintain them in the donation program.

Status of pervious donation and age of donors had no statistical significant association with rates of deferral or pass. Females are 3.75 times (using venous blood sample) and 2.67 times (using capillary blood sample) more likely to fail the copper sulphate test than the males due to low hemoglobin level. The most important cause might be low iron stores in women due to menstruation and pregnancy. Agnihotri in India (OR 15.4 with 95% CI of 12.8 to 18.6) [46], Mast *et al* in United States (OR 11.27 with 95% CI of 10.09 to 12.59) [47] and Ngoma *et al* in Japan (OR 35.48 with 95% CI 27.74 to45.38) [48] observed higher odd ratio for deferral due to low hemoglobin in females compared to our finding. This difference might be due to using the same hemoglobin (12.5 g/dL) cutoff value by Agnihotri [46], Mast *et al* [47] to qualify male and female donors despite differences in normal range values for men and women.

8. Strength and limitation of the study

8.1 Strength

- Agreement of the two methods were analysed using venous and capillary blood samples

8.2 Limitation

Another rapid screening test is not included.

9. Conclusion and recommendation

9.1 Conclusion

- ✓ The overall deferra1 rates was 20.1% by the reference method and varies by blood collection site for the traditional copper sulphate specific gravity method (15.4% vs. 16.8% for capillary and whole blood, respectively)
- ✓ False pass and -fail rate using capillary and venous blood sample were 9.2%, 4.5% and 7.6%, 4.3%, respectively.
- ✓ The copper sulphate screening test using capillary blood sample had sensitivity of 94.4%, specificity 54.1%, PPV 89.1% and NPV 70.8%, while using venous blood sample it had sensitivity of 94.7%, specificity 62.4%, PPV 90.9% and NPV 74.6%.
- ✓ The copper sulphate method when using capillary blood sample tend to give more false pass rate (9.2 %,) as compared to venous sample (7.6%).
- ✓ The $CuSo_4$ method was in moderate (Kappa (0.53), 95% CI (0.425 to 0.625), P<0.01) and substantial (Kappa (0.61), 95% CI (0.507 to 0.698), P<0.01.) level of agreement with the reference method using capillary and venous blood sample respectively.
- ✓ From this study it is possible to conclude that copper sulphate donor screening method can be retained as primary screening test by applying strict quality control until replaced by a better method.

9.2 Recommendation

- Performance of copper sulphate donor screening test has to be compared with other rapid test like Hemocue.
- Since capillary copper sulphate test was in moderate level of agreement with reference method strict quality assurance mechanism should be applied.
- The venous blood sample can be used for donor screening since it was in substantial level of agreement with the reference method.
- Since copper sulphate donor screening test is used in many resource limited countries like Ethiopia, additional study is needed on how to improve its sensitivity.

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11. Annex

Annex I Participant information sheet**.**

My name is _____. You are kindly requested to take part in research study on assessing the rate of false deferral and false pass of prospective voluntary blood donors screened by copper sulphate gravimetric test. The study has been approved by Addis Ababa University Medical Laboratory Science department research ethics committee. The study finding will give information about how many fit blood donors are inappropriately excluded and ineligible blood donors are accepted for blood donation by the copper sulphate donor screening test and helps for planning and implementing additional sensitive and specific hemoglobin determination test.

In this study, you will be asked to give 4ml of blood sample from your vein and by finger pricking, which will be used to determine the hemoglobin level by two test methods (copper sulphate donor screening test and automated hematology analyzers). The sample collection procedures take about 5 minutes. You are selected to participate in this study because of your being voluntary blood donor; so, participation in this study will give you an opportunity to know your hematology parameters including hemoglobin level. I would like to assure you that all of your test result will be kept confidential by using coding system. You have full right to stop being participant of the research study at any time without explanation. Also you have right to have question about the study answered.

I will be glad to answer your questions about this study at any time.

You may contact me at e-mail address eshe2004@gmail.com or mobile +251 913 0331 51

Department of Medical Laboratory Science research ethics office +251 11 275 5170

Participants information sheet (Amharic version)

ስለ ጥናቱ ለተሳታፊዎች መረጃ የሚሰጥ ቅጽ

ስሜ---------- እርስዎ በዚህ በጎ ፈቃደኛ ደም ለጋሾችን ደም ከመስጠታቸዉ በፊት በደማቸዉ ዉስጥ ያለዉን የሄሞግሎቢን መጠን ለማወቅ የምንጠቀመዉ ምርመራ ምን ያህል ደም መለገስ የሚችሉ ሰዎችን በስህተት እንዳይሰጡ እንደሚያደርግና ምን ያህሉን ደግሞ ደም መለገስ የሌለባቸዉን ሰዎችን ደም እንዲለግሱ እንደሚያደርግ ለማወቅ በሚሠራዉ በምርምር ሥራ ላይ እንዲሳተፉ በአክብሮት ተጋብዘዋል፡፡ ይህ የምርምር ሥራ ከአዲስ አበባ ዩኒቨርስቲ የላቦራቶሪ ሳይንስ ትምህርት ቤት የምርምር ሥራ ሥነ-ምግባር ኮሚቴ ፈቃድ ያገኘ ሲሆን ከጥናቱ የሚገኘዉ ዉጤት ምን ያህል የደም ለጋሾች ያለአግባብ ደም ለመስጠት እንደሚያልፉና ምን ያህል ደግሞ እንደሚወድቁ ለማወቅ ይጠቅማል፡፡ ይህም ሌላ የተሻለ ውጤት የሚሰጥ የምርመራ ዓይነት በደም ባንክ ውስጥ ለመጠቀም ለማቀድና ተግባራዊ ለማድረግ አስተዋጽኦ ያደርጋል፡፡

በዚህ የጥናት ሥራ ለመሳተፍ ፈቃደኛ ከሆኑ 4 ሚሊ(አንድ የሻይ ማንኪያ የሚሆን) የደም ናሙና ከደም ሥርዎና ከጣትዎይሰጣሉ፡፡ ይህም የደም ናሙና በደምዎ ውስጥ የሚገኘውን ሄሞግሎቢን መጠን ምን የህል እንደሆነ ለማወቅ በሁለት የተለያዩ ምርመራ ዓይነቶች ምርመራ ይደረግበታል ፡፡ የደም ናሙናውንና መረጃ ለመውሰድ 5 ደቂቃ ያህል ይወስዳል፡፡ በዚህ ጥናት ለመሳተፍ የመረጡት በጎ የደም ለጋሽ በመሆንዎ ሲሆን በዚህ ጥናት መሳተፍዎ ሙሉ የደም ምርመራ ውጤትዎን ለማወቅ ዕድል ይሰጠዎታል፡፡ በሰጡት ደም ላይ የሚደረገው የምርመራ ውጤት ሙሉበሙሉ ሚስጥራዊነቱ የተጠበቀ እንደሚሆንና ለጥናቱ ዓላማ ብቻ ጥቅም ላይ እንደሚውል ላረጋግጥልዎ እወዳለሁ፡፡ በዚህ ጥናት ላይ ያለዎትን ጥያቄ በማንኛውም ሰዓት ሊጠይቁና ምላሽ ሊያገኙይችላሉ፡፡በጥናቱ ላይ ላለመሳተፍም ሆነ በመሀል ለማቋረጥ ሙሉ መብት አለዎት፡፡

በማንኛውም ሰዓት በጥናቱ ላይ ያለዎትን ጥያቄ ለመመለስ ደስተኛ ነኝ!

በሚቀጥለው አድራሻ ሊያገኙን ይችላሉ

ስልክ፡- 0913033151

Email :-eshe2004@gmail.com

ህክምና ላቦራቶሪ ትምህርት ክፍል የምርምር ሥነ ምግባር ቢሮ ስልክ ቁጥር+251 11 275 5170

Annex II Informed consent form

By signing below, you are agreeing that: (1) you have read and understood the participant information sheet, (2) questions about your participation in this study have been answered satisfactory, (3) you are taking part in this research study voluntarily (without any coercion).

Participant's ID

---------- ----------

Participant's signature Date

---------- ----------

Name of person obtaining consent Signature of person obtaining consent

Informed consent form (Amharic version)

ተሳታፊው በጥናቱ ለመሳተፍ ሙሉ ፈቃደኛ መሆኑን የሚገልጽበት ቅጽ

ከዚህ በታች ያለውን በጥናቱ ለማሳተፍ ፈቃደኛ መሆኑዎን እንዲያረጋግጡ በሚጠይቀው ቅጽ ላይ ሲፈርሙ

1. ከላይ የተሰጠዎትን ስለጥናቱ መረጃ የሚሰጠውን ጽሑፍ ማንበብዎንና መረዳትዎን
2. በጥናቱ ላይ ላነሱት ጥያቄ አጥጋቢ ምላሽ እንዳገኙ
3. በጥናቱ ለመሳተፍ የወሰኑት በራስዎ ሙሉ ፈቃደኝነትና ምንም ዓይነት ተጽዕኖ ወይም ግፊት ሳይደረግብዎ መሆኑን ያረጋግጣሉ፡፡

የተሳታፊው ስም/መለያ ቁጥር ----------

የተሳታፊው ፊርማ ---------- ቀን ----------

ስምምቱናን የተቀበለው ሰው ስም ---------- ፊርማ ----------

Annex III. Standard operating procedure for copper sulphate preparation

Procedural steps

1 Prepare copper sulphate Stock solution

1.1 Determine the volume of copper sulphate to be prepared

1.2 Weigh out 153.31 gm of copper sulphate with a liter of distilled water.

1.3 Weigh 459.93 gm of copper sulphate powder, to prepare three litters of stock solution.

1.4 Place the copper sulphate powder into the preparation vessel (flask) and then add 80% of the total volume of Distilled water required

1.5 Cap the vessel (flask) and mix well using mixer until the copper sulphate has dissolved completely

1.6 Add the remaining volume of distilled water to get the correct total volume of copper sulphet solution.

1.7 Pour the solution in to brown bottle labeled " copper sulphet stock solution"

2 Prepare working solution for male and female blood donors

2.1 Take the required volume of copper sulphate stock solution in to measuring cylinder (578ml for male, 558ml for female) and add distilled water to give a total volume of one litter

2.2 To prepare three litters of copper sulphete working solution for male measure 1734 ml of stock solution & to prepare two litters of working solution for female measure 1116 ml stock solution, and add distilled water to give a total required volume.

2.3 Transfer the solution in to a beaker and mix well

2.4 Measure the specific gravity of the solution Using hydro meter.

2.4.1 Copper sulphate solution for male has a specific gravity of 1.055, equivalent to 13.5gm/dl hemoglobin.

2.4.2 Copper sulphate solution for female has a specific gravity of 1.053, equivalent to 12.5gm/dl hemoglobin.

2.5 correct the specific gravity by adding drops of copper sulphet stock solution or distilled water if the reading is out of the defined range

2.6 Pour the solution in to brown bottle Labeled with “copper sulphate working solution & date of preparation”.

2.7 Thoroughly clean any reusable glass wares or equipment used

Annex IV. SOP for estimating blood donor’s hemoglobin using copper sulphate solution

1 Mix the Copper Sulphate ($CuSO_4$) working solution every morning before use

2 Fill the 30 ml. Copper Sulphate working Solution in to two separate bottles for male blood donors & for female.

- Male copper sulphate has a specific gravity of 1.055 which corresponds to a hemoglobin of 13.5g/dl.
- Female copper sulphate has a specific gravity of 1.053 which corresponds to a hemoglobin of 12.5g/dl.

3 Greet the donor & explain about the procedure.

4 Wear Gloves

5 Clean the ring finger or finger preferred by the donor with alcohol swab & Allow to dry (do not use too much alcohol)

6 Gently prick the selected finger using blood lancet.

7 Wipe away the 1st drop of blood with dry cotton wool swab

8 Fill the capillary tube up to ¾ of its volume with blood samples keeping the capillary tube horizontal to the finger. (Do not squeeze the finger as it may produce tissue fluids, thus creating a falsely low hemoglobin level)

9 Fill the capillary tube in one continuous operation. Do not refill

10 Allow the drop to fall freely by itself holding the capillary tube straight 1 cm above the bottle of Copper Sulphate solution.

11 Place a small dry cotton wool swab on the puncture site & inform the donor to hold this in Place for a few minutes

12 observes the drop of blood in the Copper Sulphate solution for 15 sec. & note whether it sinks to the bottom or floats

13 Accept the donor for donation & record the result on donor card if the blood Sample sinks in15 sec.

14 Reject the donor from donation & record the result on deferral form if the blood sample floats in15 sec. (Explain the reason for deferral & reassure that this maybe temporary deferral).

15 Change the copper sulphate solution after every 25 tests.

Annex V : procedure and reagents of Cell-Dyn 1800 Hematology Analyzer

Procedure for cell Dyn 1800 hematology analyzer

1. Whole blood collected in an EDTA tube with a Minimum sample volume is 0.5 ml using the Open Sample Mode. The instrument aspirates 30 µl of patient sample.

2. Run three levels of QC at the beginning of each day of patient testing. Do not perform patient testing until QC tests are performed and within acceptable limits. Rerun at least one of the three levels of QC again after eight hours of patient testing to assure the instrument is still functioning properly.

3. Press MAIN to return to the MAIN MENU. At the MAIN MENU, enter in the operator ID and press RUN, next press SPECIMEN TYPE. If the instrument has been idle for fifteen minutes or more, press normal background. Press the Touch Plate to run an Open Mode Background test. Verify that the Open Mode Background count results are acceptable.

4. Press MAIN to return to the MAIN MENU screen. Enter in the Operator ID and press RUN. Press SPECIMEN TYPE then press PATIENT SPECIMEN. Verify that RUN Ready is displayed in the Status Box. Scan patient specimen number and patient name using the keyboard. Expected ranges for blood counts differ based on gender and age.

5. The Cell-Dyn is programmed to display the correct reference range. The operator, however, must first manually type in the correct gender prior to running the patient sample. Once RUN Ready is displayed in the Status Box, use the ↓key to scroll to the Limit prompt. Enter either “1” for Male or “2” for Female. Mix the patient sample well and remove the cap. Place the sample probe in the tube so that the end is immersed in the sample but not resting on the bottom of the tube.

6. Press the Touch Plate to start the run. The Status Box on the RUN menu indicates the stage of the run. When Remove Specimen is displayed in the Status Box and the probe has moved up through the wash block remove the sample tube and replace the tube cap.

7. A beep will indicate that the probe cleaning cycle has begun. After the probe cleaning cycle is complete, the probe will move down into position for the next sample and the results will be displayed on the screen.

8. If needed, press PRINT REPORT for a hardcopy of the report. After sampling is complete, press MAIN to return to the MAIN MENU. Change the Operator ID to “000”for the next user.

Reagents for Cell-Dyn 1800

1. Cell-Dyn Diluents:

- Stable at room temperature until the expiration date on the container.
- Protect from direct sunlight, extreme heat, and freezing during storage.
- Do not use if reagent has been frozen.

2. Cell-Dyn Lytic Agent:

- Stable at room temperature until the expiration date on the container.
- Protect from direct sunlight, extreme heat, and freezing during storage.
- Do not use if reagent has been frozen.

3. Cell-Dyn Detergent:

- Stable at room temperature until the expiration date on the container.
- Protect from direct sunlight, extreme heat, and freezing during storage.

-Do not use if reagent has been frozen.

4. Enzymatic Cleaner:

- Stable at 2-8°C until the expiration date on the container.
- Do not use if reagent has been frozen.

5. Cell-Dyn Whole Blood QC:

- Unopened QC vials are stable at 2-8°C until the expiration date on the vial. Opened QCvials are Stable at 2-8°C for 7 days after opening. Do not use expired QC.

- Allow QC to sit at room temperature for fifteen minutes before testing.

- Mix QC vial by rolling the vial between palms for 20 seconds.

- Invert the vial and roll it back and forth for another 20 seconds.

- Gently invert the vial 10 times.

- Do not shake.

- Continue to mix in this manner until cells are completely suspended (3-5 times).

- Gently invert the pre-mixed vial 5 times immediately before testing.
- Return vial to refrigerator when testing is complete.

6. Whole Blood Calibrator:

- Unopened calibrator vials are stable at 2-8°C until the expiration date on the vial. Opened calibrator vials are stable at 2-8°C for 7 days after opening. Do not use expired calibrators.
- Allow the calibrator to sit at room temperature for fifteen minutes before testing.
- Mix the calibrator vial by rolling the vial between the palms for 20 seconds.
- Invert the vial and roll it back and forth for another 20 seconds.
- Gently invert the vial 10 times.
- Do not shake.
- Continue to mix in this manner until cells are completely suspended (3-5 times).
- Gently invert the pre-mixed vial 5 times immediately before testing.
- Return vial to refrigerator when calibration is complete.

Annex VI . Data collection form

Sr.no	**Id**	**Age**	**Sex**	Weight	Frequency of previous donation	**Result**				
						Copper sulphate				**Reference method**
						capillary		venous		Hgb g/dl
						pass	fail	pass	fail	

Annex VII: Declaration

I the undersigned, declare that this is my original work and has not been presented for a degree in this or any other university and all sources of materials used for this thesis have been acknowledged.

Name: Eshetu Guracha

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Date of submission _____

This proposal has been submitted with my approval as University Advisor

Name _____

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia

Name _____

Signature _____

Place: Addis Ababa University, Department of Medical Laboratory Sciences, Ethiopia