ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of Cytopenias and Alse Eligibility Status af Apparently Healthy Blood Donors at National Blood Bank Services, Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Biruk Hailu, entitled “Assessment of cytopenias and false eligibility status of apparently healthy blood donors attending at National Blood Bank services, Addis Ababa, 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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Abbreviations

AAU	Addis Ababa University
Ba	Basophil
CBC	Complete blood count
$CuSO_4$	Copper sulphate
EDTA	ethylene-diamine-tetra-acetic acid
Eos	Eosinophil
ESR	erythrocyte sedimentation rate
Hct	Hematocrit
Hgb	Hemoglobin
Lym	Lymphocyte Count
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
Mon	Monocyte
MPV	Mean platelet volume
NBBS	National blood bank service
Neut	Neutrophil count
Plt	Platelet
RBC	Red blood cell
RDW	Red cell distribution width
SOP	Standard Operating Procedure
TTI	Transfusion transmitted infection

Abstract

Background–Cytopenia occurs when one or more of your blood cell types are lower than it should be. Your blood consists of three main parts. Red blood cells, also called erythrocytes, carry oxygen and nutrients around your body.

Objective: To assess the magnitude of cytopenias and false pass of apparently healthy blood donors and their associated factors at National Blood Bank Services, Addis Ababa, Ethiopia.

Methods: A cross sectional study was conducted at national blood transfusion service (NBTS) from February to March 2020. Four hundred twenty one consented apparently healthy blood donors were recruited at national blood bank transfusion service and Socio demographic information were collected by using well-structured questionnaires through interview. Three ml blood samples were collected in EDTA tubes and analyzed using Beckman coulter (DxH) 800 automated hematology analyzer. Finally, the data was entered, cleaned and analyzed using SPSS version 23 statistical software. Descriptive statistics and Chi-Squared (X^2) tests were used for statistical analysis.

Result: In this study, a total of 421 apparently healthy blood donors were included. Among them 228(54.2%) were non-regular donors and 283(67.2%) males, were within the age group of 18-28 years 180 (42.8%).Among the blood donors,143(34%)of them were identified as falsely passed the donor selection criteria by the Copper sulphate gravimetric method and donated blood. False pass level was relatively higher among females, first time donors, older donors and those having normal ESR value. The prevalence of any cytopenia, anemia, leucopenia and thrombocytopenia were 153(36.3), 65(15.4%), 73 (17.3%) and 42(10 %,) respectively. Cytopenia was relatively higher among females, repeated blood donors, relatively advanced aged donors, donors with normal ESR value; though the difference did not show statistical significance ($p \geq 0.05$). However, cytopenia was significantly higher 93(65%) among blood donors who were falsely passed by the Copper sulphate gravimetric method of donor screening ($p < 0.001$).

Conclusion and recommendation: The study revealed higher level of falsely eligible blood donation and blood cytopenia among apparently healthy blood donors.Hence, we recommend large scale evaluation and consequent modification of the currently used donor recruitment criteria for the safety of both blood donors and patients.

Keywords – hematological profile, blood donors, complete blood count.

1. Introduction

1.1 Background

Cytopenia, defined by a reduced number of blood cells manifesting as anemia, neutropenia, and/or thrombocytopenia, may be associated with multiple conditions, including cancer, bone marrow suppression from chemotherapy or radiotherapy, as well as pregnancy, nutrient deficiencies, liver disease, hypersplenism, and renal insufficiency. However, for some patients, the presence of a cytopenia is not accompanied by a known determinant. Patients with a cytopenia may be asymptomatic with no clinical sequelae or can experience a wide variety of adverse health outcomes including symptoms such as fatigue, weakness, increased infections, or life threatening bleeding. There are cases of cytopenias that do not have clinical repercussions. Benign ethnic neutropenia is one such example, where patients have low neutrophil counts but normal quantities of lymphocytes and other leukocyte subtypes, normal bone marrow morphologic features and cellularity, and no increased risk of infections(1).

Cytopenia occurs when one or more of your blood cell types are lower than it should be. Your blood consists of three main parts. Red blood cells, also called erythrocytes, carry oxygen and nutrients around your body. White blood cells, or leukocytes, fight infection and battle unhealthy bacteria. Platelets are essential for clotting. If any of these elements are below typical levels, you may have cytopenia. Several types of cytopenia exist. Each type is determined by what part of your blood is low or decreased.Anemia occurs when your red blood cells are low. Leukopenia is a low level of white blood cells. Thrombocytopenia is a deficiency of platelets. Pancytopenia is a deficiency of all three parts of the blood (2).

The possible causes of cytopenia are complex and varied. Among these causes are peripheral destruction, infections, and side effects of medication. Two types of cytopenia that are related to the underlying cause of the low blood cell count are autoimmune cytopenia and refractory cytopenia (3).

Blood transfusion is considered as life-saving medicine and medical practice for those who are affected by certain disease, accident, pregnancy complication and chemotherapy that really demand the practice (4). Blood donation is processes that include collection of blood from voluntary apparently healthy person (5).

In blood transfusion medicine the first priority is to protect donors' right and health. Blood donor selection is a cornerstone for blood transfusion safety, designed to safeguard the health of both donors and recipients. Donor safety (5) is targeted by reducing the risk of complications associated with blood donation (rare but not absent) (6) and, in order to improve recipient safety, blood donor selection attempts to reduce the risk of transfusion-transmitted infections (TTI).

The primary goal of any blood transfusion is to provide the component of blood that is essential for life but deficient in a patient. To optimally achieve such goal, donor red blood cells (RBC) and all other blood components must be compatible with the patient's blood. Blood safety is a major global concern because of the untoward events that may occur. One of the most critical steps used to ensure blood safety is blood donor selection (7).

To maximize the benefit of blood transfusion services, the evaluation of the blood donor's hematological parameters is essential in the assessment of suitable blood donors for component donations. Indolent or early blood diseases could be first suspected in apparently healthy blood donor with the resultant benefits of early follow up for diagnosis and intervention (8). Transfusion process depends on the quality of the blood transfused and the safety of the donor thereafter. To assess these qualities, one needs to go beyond Hb to assess other hematological parameters that will give information on some latent physiological processes in the prospective donor. For instance, the Hb estimation is less sensitive in the early stages of iron deficiency (9). Hematological parameters such as red (RBC) and white blood cell (WBC) counts and hemoglobin (Hb) concentration are tightly regulated traits with high clinical relevance. Values outside normal ranges are diagnostic for disorders, including cancer, immune diseases, and cardiovascular disease (10).

Generally, a bacterial infection may be suspected among patients with increased total white blood cell counts than normal and hemostatic disorders may be accompanied by low or high platelet counts.

Individuals with thrombocytopenia should not be accepted as blood donors because of the risk of bleeding at the venepuncture site and because chronic thrombocytopenia may be associated with serious underlying haematological or other systemic disease. A past history of autoimmune thrombocytopenia is not a contraindication to blood donation, even if treated by splenectomy, provided that the prospective donor has been well for five years with no evidence of relapse (11).

In many studies much emphasis has been laid on iron deficiency, probably because of anemia which reduces maximum oxygen consumption and maximum work performance in proportion to its severity. Since whole blood is composed of three cellular elements (red blood cells, white blood cells, and platelets), parameters other than red blood cells (RBCs) also need to be assessed, which is the aim of this study (12).

The copper sulphate (CuSO4) specific gravity method is accepted method for mass screening of blood donor's hemoglobin (Hgb) level. This method was described in 1945 by Phillips et al. for determining the specific gravity of whole blood and plasma (13). In the copper sulphate method a drop of whole blood is allowed to fall in to the copper sulphate solution with specific gravities of 1.053 and 1.055 to screen female and male 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 sink indicating that the donor's haemoglobin level is acceptable while floating determines rejection (14).

1.2 Statement of the problem

According to Pattanshetti M. etal. Study Occurrence of bicytopenia was 85% in adults and 11% in elderly. The most common cytopenia observed was anemia with thrombocytopenia 61% followed by anemia with leukopenia 26% and leukopenia with thrombocytopenia 3 %(15).

The prevalence of anemia in North Africa among the total deferred patients was 17.1 %. Four different types of anemia were found among the subjects. These were normocytic normochromic (46.74 %), microcytic hypochromic 42.39 % normocytic hypochromic 8.70 %, and microcytic normochromic anemia 2.17 %(16).

The blood transfusion services should ensure that the act of blood donation is safe and causes no harm to the donor. The qualification for blood donation is done by checking the donor for certain clinical and hematological parameters to evaluate their ability to donate. The measurement of hematological parameters in transfusion medicine is important in that it allows the detection of quantitative abnormalities of the cellular elements of the blood (17).

According to WHO donor selection criteria, individuals with a history of malignant melanomaand with current or past hematological malignancy, including leukemia, lympho proliferative and myeloproliferative disorders, lymphomas, clonal hematological disorders such as polycythemia rubra Vera and essential thrombocythemia, paroxysmal nocturnal hemoglobinuria Myelodysplastic syndromes are in permanent deferral list (18). The guideline also recommends to defer individuals who are anemic and to permanently defer individuals who have chronic anaemia of unknown cause or associated with systemic disease. Similarly, the guideline also stated individuals with thrombocytopenia should not be accepted as blood donors because of the risk of bleeding at the vein puncture site and because chronic thrombocytopenia may be associated with serious underlying hematological or other systemic disease (19).

However, according to the report of the transfusion research group in French-speaking Africa, the measurement of these parameters is very little done in sub-Saharan Africa during the biological testing of a blood donation (20)

. As a country, Ethiopia doesn't have policies and standards to asses' hematological profile of donors except hemoglobin determination using copper sulfate gravimetric technique. A study from Southern Ethiopia showed that the Copper sulphate gravimetric method resulted in respective false-pass and - fail rates of 9.2% and 4.5% (capillary blood) and 7.6% and 4.3% (venous blood) with moderate agreement with the reference method (21).

As a result of this, we are not sure whether the given blood product is safe or not. Therefore, the aim of this study was to assess magnitude of cytopenias and false pass of apparently healthy blood donors attending at National Blood Bank Service, Addis Ababa, Ethiopia.

1.3 Significance of study

The finding of this study will reveal how many unfit blood donors are falsely passed as eligible donor to donate blood or ineligible blood donors are accepted for blood donation by the copper sulphate donor screening tests, as well as whether any cytopenias present. Thus, it will help to plan and implement additional test method for hemoglobin determination and assessing hematological profile of donors. By so doing, it will ultimately help minimize unnecessary deferrals, acceptances and hence donor dissatisfactions. This study can be used as reference material for researchers and policy makers can use it as in put.

2. Literature Review

The voluntary unpaid blood donation is a humanitarian act towards the sick by the healthy. No transfusion service can survive without blood donors (22). The well-being and health of the blood donors is of prime importance for the medical profession. But in many parts of the world, hematological investigations for donors are limited to some parameters such as hemoglobin level.

One of the study conducted in Icahn School of Medicine at Mount Sinai, New York, NY, United States,3 Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, United States,The National Health and Nutrition Examination Survey from 1999 to 2002 was used to identify those with a cytopenia in the general population. From the study population 2.0% had any cytopenia and approximately 43% of all cytopenias were not accompanied by a clinical determinant. Unexplained cytopenia was more common in men than in women and in Non-Hispanic Black participants (3.4%). Among those with an unexplained cytopenia, the majority manifested as neutropenia. Compared to those with no cytopenia, those with unexplained cytopenia were significantly (23).

A study was carried out at Tehran Blood Transfusion Center, Iran in 2016 on 327 male and 67 female volunteer blood donors to assess hematological reference intervals for healthy Iranian blood donors. The study revealed as there were significant gender-related differences for mean values of hematological indices, with males having higher mean values of RBC, HCG, HCT and MCV than females. While the mean of PLT and MCH were higher in women (24).

In a descriptive case-control study carried out at European Gaza Hospital, Palestine in 2019 on 120 males apparently healthy Palestine blood donors who were matched for age, employment status, marital status, and education showed, the mean ESR for voluntary and Commercial were a significant difference between regular donors and Commercial donor. The mean levels of ESR and HCT found significantly less in regular donor than in first-time donors and non- donors (25).

Another institution based prospective cross-sectional study was conducted in Maiduguri, northern Nigeria from January to March 2018, and 100 eligible donors to study hematological profiles.The

voluntary donor category had significantly lower hematocrit count. Total leucocyte count among the commercial donors was significantly higher than the corresponding values for both voluntary and family replacement donors.The voluntary donors had higher neutrophil count. There were no significant differences in differential leukocyte count. Donors had significantly higher platelet counts (26).

A study carried out in Jos is the capital of Plateau state of Nigeria, from May to October 2019 at the National Blood Transfusion Service,metropolis of Plateau state. The participants of the study were assumingly healthy volunteer blood donors. Their minimum studied sample size of one hundred and two blood donors. Three milliliters of venous blood sample were collected into the ETDA bottle from each donor and Samples were processed according to standard manual techniques in the hematology laboratory. HemoCue Hb 201 was used for hemoglobin determination, WBC, differential and platelet were counted manually. Out of the 102 participants; the age group 21-30 had the highest number of participants. The male voluntary blood givers were higher than female. Hematocrit, platelet and eosinophil counts showed significant statistical differences between genders. The rest hematological profiles were normal (27).

One of the studies conducted from April to June at Bugando Medical Centre in Mwanza, Tanzania the participants were 167 apparently health blood donors. About 3mL of EDTA blood was collected and analyzed using automated hematology analyzer. Their study deign were hospital based prospective cross-sectional study. Males had significantly higher hemoglobin concentration as well as erythrocyte count and hematocrit than females. The rest of hematological parameters did not significantly differ between the males and females. Monocytes, eosinophils and basophils counts were significantly higher among blood donors aged 18–20 years compared to other age groups; other hematological indices did not significantly vary with age (28).

In another study carried in north east Nigeria, from October to January 2019 and the implication on quality of blood products. There were hospital based cross-sectional study design and the participants of the study were hundred apparently healthy volunteer blood donors. Selected donors from each donor category were studied with respect to levels of hemoglobin, hematocrit, red cell indices, reticulocyte count, total leucocyte count, differential leucocyte count and platelet count.The voluntary donor category had lower hemoglobin and hematocrit when compared to family replacement categories, but the mean hematocrits of found in the commercial donor category were significantly lower than the corresponding values for both voluntary and family replacement donor categories.The mean reticulocyte count were lower among voluntary blood donor when compared to family replacement donors, but the mean reticulocyte count of seen among the commercial donors was significantly higher than the corresponding values for both voluntary and family replacement donors.The mean values of red cell indices for the commercial donors were lower than that for both voluntary and family replacement donors but the differences were not statistically significant for all three indices. There is no significance difference in other parameter between volunteer donor and family donation. (29)

One of a study carried out in Gus au, Nigeria showed Family replacement donors should be encouraged for donation since they have similar values of hematological and biochemical parameters compared to voluntary and first-time donors. In the process, there will be sufficient units of blood in the blood banks as a result of the increased number of unused bloods donated by these donors. In their study, there were no statistically significant differences in the values of hematocrit; hemoglobin and RBC count of family replacement blood donors with respect to age and these are in line with the earlier report. The study has further revealed the lower values of MCH and MCV, respectively compared to their previous studies on voluntary donors, first time donors and apparently healthy donors (30).

Institution based cross sectional study conducted in two cities of (Kisumu and Nairobi) Kenya, between May and June 2004. The study participants were 3203 to determine the status of blood donor. A significant number of Kenyan donors showed abnormal hematology profiles that may indicate underlying pathology.Platelet counts were also significantly lower in Kisumu donors. Such abnormalities are not detected by current blood transfusion services screening practices and there may be a need to strengthen donor selection criteria to protect both donors and recipients (31).

Elnour AM et al, results showed that hemoglobin concentration was normal in 55% of the donors, less than the normal in 43% and above the normal in 2% of studied group. RBCs were 70% normal, 4% lower than normal and 26% higher than normal. 78% of the donor showed WBC within normal range and the remaining 18% were lower than normal. Platelets counts in donors were 90% normal, 8% low and 2% high. The Hematocrit values were 87% normal, 7% low and 6% high. The MCV in donors was 74% normal and 26% low. MCH value was 2% normal, 97%low and 1%high. The MCHC was 3%normal and 93% low. There is considerable number of donors with hemoglobin concentrations lower than normal as well as lower red blood cells indices. Thus, full blood count should be incorporated in evaluating blood donors to insure both good quality of blood and safety of the donors (32).

In a study conducted by Jeremiah et al on Subclinical leukopenia in a cross section of Nigerian blood donors showed that Anemia was observed due to the reduction in the red blood cell (RBC) count, PCV, hemoglobin, mean cell Hb, and red cell distribution width values. The leukopenia observed in this study was largely due to the significant reductions of leukocyte, Also reduction in absolute lymphocyte, percent lymphocyte and percent monocyte count.Six parameters were significantly reduced in the regular blood donors (PCV, absolute WBC count, percent 7monocytes, RBC count and Hb, PCV) but No thrombocytopenia was observed (33).

A study from Asmara, in 2018 a total of 610 volunteered blood donors 205 females and 405 males between the ages of 16 and 65 years consented to participate in the study. The difference between males and females in MCV and MCHC was significant in favor of female donors. A correlation of a weak positive nature was found between the ages and genders of donors and the Hb level. All measured values were found to be within the global referenced ranges. Hb, RBCs count, and RBCs indices of apparently healthy Eritrean blood donors were measured for reference, and all values were found to be within the normal reference ranges (34).

In a study carried out at Hossana Blood Bank, southern Ethiopia on the level of false pass and fail among prospective blood donors as screened by copper sulphate gravimetric method in 2017 study showed high rate of false pass using copper sulphate gravimetric method, and there was variation between multiple collection sites. The study concluded as, ineligible donors are being recruited to donate blood which would have negative impact on their health as well as future donation (35).

3. Objectives

3.1. General objective

To assess the magnitude of cytopenias, false pass of apparently healthy blood donors and associated factors at National Blood Bank Service from February to April 2020, Addis Ababa, Ethiopia

3.2. Specific objectives.

- To assess the level of false pass of blood donors screened by Cupper sulphate Gravimetric method.
- To determine magnitude of cytopenias among apparently healthy blood donors
- To assess factors associated with cytopenias among blood donors at National blood bank services

4. Material and Methods

4.1 Study Area

The study was conducted at National Blood Bank Service, Addis Ababa, Ethiopia.Estimated populations of Addis Ababa were 4, 8 million in 2020 according to UN. The National Blood Bank Services (NBBS) was established in 1969 by Ethiopian Red Cross society since 2004 it has been transferred to Federal Ministry of Health Ethiopia, and entrusted with the responsibility of managing the Blood donors, collection, testing and transfusion of blood and blood products in Ethiopia. Its main center is located in Addis Ababa and it has also the responsibility to oversee, support and monitor the activities of regional blood bank in the country which are administratively under their respective regional health bureaus. National blood bank service has more than 200 staffs and collects 85000 blood units per year. It is responsible for mobilizing, screening, phenotyping, preparing blood product and distributing safe blood for health facilities that are in demand. Its mission is to ensure safe and adequate supply of blood and blood products distributed to all transfusing health facilities in Ethiopia (36/32).

4.2 Study design and Period

A cross sectional study was conducted to assess the magnitude of cytopenias, false pass of apparently healthy blood donors by Copper Sulphate gravimetric method and associated factors at National blood bank transfusion service from February to April 2020, Addis Ababa, Ethiopia

4.3 Population

4.3.1 Source Population

All volunteer blood donors live in Addis Ababa city.

4.3.2 Study population

All eligible blood donors who donated blood at national blood bank service Addis Ababa, Ethiopia from February to April 2020 and volunteer to participate in the study.

4.4 Inclusion and Exclusion criteria

4.4.1 Inclusion criteria

- 18-65 age in years for blood donation
- Voluntary
- Meet other NBBTS donor selections guideline

4.4.2 Exclusion criteria

- History of Non-communicable disease; DM, hypertension

4.5 Study variables

4.5.1. Dependent variables:

Hematological profile of donors

False eligibility status of donors by Copper sulphate gravimetric method

4.5.2. Independent variables:

- Age
- Sex
- BMI
- Blood pressure
- Alcohol consumption
- Coffee consumption
- Smoking
- Exercise
- Donor status (first time or repeated donor)
- ESR value
- Blood group

4.6 Measurement and Data collection

4.6.1 Sample size calculation

In the calculation of sample size, the following points were considered: There were no previous study conducted on similar topic; the following data were taken to estimate the sample size of this study.

Based on the above assumption, the desired sample size was determined by the single population mean formula as follows;

Formula $samplesizen = \frac{z^2 * p(1-p)}{d^2}$

Where n = Sample size α = level of significance z = at 95% confidence interval Z value (a = 0.05) =>Z α/2=1.96 p =Proportion of occurrence of the event estimated 0.2 d = Margin of error at (5%) (0.05)

$n = \frac{1.96 * 0.5(1-0.5)}{0.05}$ = 384 +10% (non response rate) =422

4.6.2. Sampling technique

Convenient Non-probability sampling method was used to select the study participants among donors visiting the blood bank during the study period.

4.6.3. Data collection procedures

All the five professionals (two nurses for phlebotomy) and (laboratory professional (diploma to masters for typing, screening and CBC-DIFF analysis one for each) who were participated in data collection were trained about the aim of the study, in selecting study participants, data confidentiality, safety and precautions to follow in collecting, transporting, analyzing and storing specimens. Then predesigned questionnaire was used to collect socio-demographic data. Socio-demographic data of subjects who voluntarily agree to participate in the study were recorded using interviewer administered questionnaire conducted face-to-face by the data collectors. Each participant was questioned for age, sex, marital status, residences, smoking habits and alcohol consumption. Weight of study participant weighed by standard, calibrated weighting scale and the height of the participant were measured by standard meter. Donors included in this study were those who were eligible for donation. No subjects were included those having a recent history of disease, travel history to malaria endemic areas. Informed consent was obtained from each subject before recruitment into the study. About3 ml of blood specimen was collected into a lavender-top vacutainer tube containing Di Potassium-Ethylene diamine tetra acetic acid (K_2EDTA). The blood was gently mixed with EDTA anticoagulant to avoid blood clot formation. The collected samples were transported to Emanuel mental specialty hospital within eight hours of collection. All hematological profiles were analyzed using Beckman coulter DxH 800 Auto Hematology Analyzer and automated ESR analyzer.

4.6.5 Hematological Analysis and definition of terms

Beckman Dx800 Hematology Analyzer

Principle: Beckman coulter DX 800 automated hematology analyzer for in vitro diagnostic use three independent system to measure, count and calculate the hematological parameters which includes; **Detection/Sensing counting** RBC, WBC and platelet,**VCS** (volume, conductivity and scatter)**:** for all Diff, NRBC, and Retic analysis and **Photometry** for hemoglobin measurement. A total of 26 hematological parameters can be done. The Coulter Principle accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid pass through a small aperture. Each cell suspended in a conductive liquid (diluent) act as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume. The complete blood count, the CBC, is the fundamental analytical test that evaluates the three main cellular components: white blood cells, red blood cells, and platelets.

Erythrocyte Sedimentation Rate (ESR)

The Erythrocyte Sedimentation Rate (ESR) expressed in mm per hour the rate at which red blood cells settle when anti-coagulated blood is allowed to stand in a narrow tube (Westergren). It is measured by the height of the column of clear plasma at the end of one hour. ESR is used as a screening method for all diseases that are associated with a modification of the plasma proteins, such as globulin, albumin and fibrinogen. Although a rapid sedimentation rate is indicative of tissue destruction (whether degenerative or inflammatory) and is found in most bacterial infections, the ESR is not considered a very reliable screening method as it can be elevated when there is no disease and can be normal when disease is present. It also does not indicate the type of disease, but finds its greatest use in following the progress of a particular illness rather than in its diagnosis (36).

Copper sulfate gravimetric Method

This method is based on the estimation of specific gravity of blood, assuming that the donor has normal protein levels. Specific gravity of 1.053 corresponds to an Hb level of 12.0 g/dL for non-pregnant woman and 13.0 male g/d (45). A drop of blood, allowed to fall into a copper sulfate solution of specific gravity 1.053, becomes encased in a sac of copper proteinase, which prevents dispersion of fluid for 15 seconds. If the specific gravity of blood is higher than the solution, the drop will sink or else it will remain suspended for some time. In most cases, this method is capable of estimating Hb within ~0.5 g/dL, which is comparable to a coefficient of variation (CV) of 2% (37).

4.7 Data Quality Assurance

Pre-analytical

The data collection was conducted by trained/oriented nurses or phlebotomist. Before starting blood collection, laboratory technicians were refreshed on proper sample collection and aim of the study. After blood collection the collected blood were transported to Emanuel mental specialized hospital laboratory within 8 hours of collection.

Analytical results from reference populations must reflect all of the pre-analytical and analytical variables that can influence test results. Therefore, all pre-analytical factors, including subject preparation, sample collection and processing, the analytical method, and instrumentation (daily, weekly and monthly preventive maintenances) checkup, were carefully defined and used for testing. Therefore, samples were collected and processed according to the standard operating procedure (SOP) and protocols established for blood collection.

Analytical

The reliability of the data generated is critical, because both the imprecision and inaccuracy of the method will determine its diagnostic utility. Therefore, in-house or commercial quality control materials were used in the same format as for patient testing to monitor CBC-Diff analysis, which not only monitors the analytical protocol used during the process but also ensures equivalence of results over the long term.

Post-analytical

For the entire CBC and ESR results were recorded and handled appropriately and secured.

Statistical Analysis and Interpretation

All the data from the questionnaires and laboratory results were coded and checked for completeness. Then entered in MS-Excel and analyzed using SPSS-version 23 statistical software for windows. The following statistical tests were used; descriptive statistics and Chi-Squared. P -value =0.05 was used to test statistical significance of the analysis.

4.8 Operational Definitions

Hematological profile: refers to measurable or quantifiable characters which include WBC, PLT, RBC, HGB, WBC Diff count (NEU, LYM, MON, EOS and BASO), HCT, MCV, MCH, MCHC, RDW, PLT, MPV and PDW.

Apparently Healthy – refers to the absence of disease based on clinical sign and symptoms assessed by physical evaluation.

BMI – is a person's weight in kilograms divided by the square of height in meters. A high BMI can indicate high body fatness (NR: 18.5-24.9).

Eligible donor – A person who fulfill the requirement to donate

ESR – erythrocyte sedimentation rate a non-specific test used to determine infection that results inflammation which rise above 15 for female and 20 for male.

Cytopenia

A condition which refers to reduction in number of two or more blood cell types from their actual quantity in peripheral circulation.

Anemia

A condition in which there is a lower than normal number of red blood cells and quantity of hemoglobin or decreased red cell mass and fail to carry oxygen. (Hgb concentration<13 g/dl for males and <12 g/dl for females).

Leukopenia

A condition in which there is reduced amount white blood cells or A general referring to a reduced number of white blood cells in the peripheral circulation. (Total white blood cell count$<4 \times 10^9$/L)

Thrombocytopenia

Any disorder in which there is reduced number of platelet which can result in bleeding disorder. (total platelet count < 1.5×10^{11} cells/L).

Subclinical Leukopenia

Denoting the reduced number of white cells or presence of disease which is not severe enough to present definite or readily observable symptoms.

4.9 Ethical considerations

Before starting the research work, ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Addis Ababa University College of Health Sciences, Department of medical Laboratory Sciences. A formal letter of cooperation was sent to national blood bank service (NBBTS) and Emanuel mental specialized hospital. The study was carried out after obtaining informed consent from volunteer blood donors and confidentiality of data were maintained by removing any participant identifiers and using only code numbers.

4.10 Dissemination of the result

This study on completion could serve as a reference material to physicians or any health professionals, researchers, experts and policy makers for intervention. To reach these bodies the

finalized paper will be submitted to Addis Ababa University, College of Health Sciences and Department medical of Laboratory Sciences. So it can serve as a reference in the library. In addition, a copy of this material will be given to National blood bank service and Emanuel mental specialized hospital. Additional effort will also be made to present on conferences to reach the medical/scientific community and publish the article on peer reviewed journals after the final reports.

5. Results

5.1. Description of study participants

In this study a total of 421 apparently healthy blood donors were included. Among them 228(54.2%) were non-regular donors and 283(67.2%) were males. The study participants of majority were within

the age group of 18-28 years (42.8%). The mean ±Sd BMI of the study participants were 20.8 ±3.087 and 70.1% were within the normal range. The social characteristics of most study participants showed that 39.2% drink alcohol, 7.8% smoke cigarette, 34.9% do physical exercise, and 63.4% drink coffee,Table1.

Table 1. Characteristics of blood donors at National Blood Bank Transfusion Services from April - June 2020, Addis Ababa, Ethiopia (N=421)

Characteristics			Number	%
Sex	Male		283	67.2
	Female		138	32.8
Donor status	Regular		193	45.8
	Non regular		228	54.2
Age Group	18-28		180	42.8
	29-39		178	42.3
	40-50		53	12,6
	51-61		10	2.4
Social Habit	Alcohol drinking	Yes	165	39.2
		No	256	60.8
	Smoking	Yes	33	7.8
		No	388	92.2
	Physical activity	Yes	147	34.9
		No	274	65.1
	Coffee drinking	Yes	267	63.4
		No	154	36.6
Blood Types		A +	85	20.2
		A -	9	2.1
		B +	81	81
		B -	13	3.1
		O +	165	39.2
		O -	13	3.1
		AB +	48	11.4
		AB -	7	1.7
BMI, mean ±Sd		20.8 ±3.087		
BMI categories		Underweight	77	18.3
		Healthy weight	295	70.1
		Overweight	49	11.6

5.2. Level of false pass of blood donors by Copper sulphate gravimetric method

In this study, all the included blood donors were considered eligible to donate blood using the Copper sulphate gravimetric method. However, among 421 individuals who were labeled passed the donor selection criteria by the Copper sulphate gravimetric method and donated blood, 78(18.5%) donors

were identified as falsely passed the donor criteria by the hemoglobin measurement done by Beckman Dx800 Hematology analyzer as a reference method considering the cutoff point of 12g/dl and 13g/dl for females and males respectively based on the Ethiopian donor selection criteria (37). False pass level was relatively higher among females (50.7%), first time donors (36.7%), older donors (42.9%) and with normal ESR value (35.8%), Table 2.

Table 2: Level of false pass and true pass of blood donors by Hemoglobin determination using Beckman Dx800 Hematology analyzer as a reference method and its distribution with some donor profiles

Characteristics		Level of false and true pass		P-value
		False pass, n(%)	True pass, n (%)	
Overall		78(18.5%)	343(81.5%)	
Sex	Male	51(25.8)	232(74.2)	<0.001
	Female	27(19.6)	111(80.4)	
Donor type	First time	39(17.1)	189(89.9)	0.83
	Repeated	39(20.2)	154(79.8)	
Age group (in years)	18-28	27(15)	153(85)	0.17
	29-39	36(20.2)	142(79.8)	
	40-50	13(24.5)	40(75.5)	
	51-61	2(20)	8(80)	
ESR value	Increased	9(16.4)	46(83.6)	0.059
	Normal	69(18.85)	297(81.2)	

5.3 Magnitude of cytopenias and associated factors among blood donors

Among 421 screened blood donors, 153(45.8%) had at least one form of Cytopenia (anemia, leucopenia or thrombocytopenia). Similarly, 65(18.5%), 73(17.3%) and 42(10%) blood donors were found anemic, leucopenic and thrombocytopenic, respectively. Anemia was 21.5% mild and 78.5% moderate among blood donors, but no severe cases were detected, Table 3.

Table 3: Magnitude of cytopenias among blood donors in Addis Ababa, Ethiopia (N=421).

Cytopenia	Yes, n(%)	No, n(%)
Anemia	78(18.5)	343(81.5)
Leucopenia	73(17.3)	348(82.7)
Thrombocytopenia	42(10)	379(90)

Among apparently healthy blood donors cytopenia was relatively higher among females (50%), repeated blood donors (50.2%), relatively advanced aged donors (41% among donors within 29-39 years and 38.1% among donors aged 40 and above years), donors with normal ESR value (36.9%); though the difference did not show statistical significance ($p \geq 0.05$). However, cytopenia among blood donors was significantly higher (45.8%) among blood donors who were falsely passed by the copper sulphate gravimetric method of donor screening ($p < 0.001$), Table 4.

In this study, sub-clinical anemia was relatively higher among males (16.3%), repeated donors (16.6%), and increased ESR value (16.4%) and among the relatively advanced aged donors (16.9% and 19 % among 29-39 years and 40 and above years, respectively). However, anemia did not show significant association with gender, previous donation status, ESR value or age of blood donors, ($P \geq 0.05$). It was shown that all donors who were included and donated blood as falsely passed level by Copper sulphate gravimetric method were mild or moderately anemic.

Similarly, Subclinical leucopenia and thrombocytopenia were relatively higher among repeated blood donors (18.1% and 12.4%) and falsely eligible blood donors (21.7%, 11.2%), respectively. Leucopenia was higher among advanced aged group blood donors (19% among 40 and above years) while thrombocytopenia was lowest among advanced aged donors (7.9% among 40 and above year's donors). Leucopenia and thrombocytopenia were also higher among female donors (20.3% and 10.1%) and those with normal ESR value (17.8% and 10.4%) unlike anemia. Leucopenia and thrombocytopenia did not show statistical significant association with none of the variables ($p \geq 0.05$).

Table 4: Association between Cytopenia and some donor profiles, Addis Ababa, Ethiopia (N=421)

Donor profile		Anemia		Leucopenia		Thrombocytopenia	
		Yes, n(%)	No, n(%)	Yes, n(%)	No, n(%)	Yes, n(%)	No, n(%)
Sex	Male	51(25.8)	232(74.2)	45(15.9)	238(84.1)	28(9.9)	255(90.1)
	Female	27(19.6)	111(80.4)	28(20.3)	110(79.7)	14(10.1)	124(89.9)
	p-vaue	0.702		0.264		0.936	
Age in years	18-28	27(15)	153(85)	29(16.1)	151(83.9)	15(8.3)	165(91.7)
	29-39	36(20.2)	142(79.8)	32(18.0)	146(82.0)	22(12.4)	156(87.6)
	40-50	13(24.5)	40(75.5)	11(20.8)	42(79.2)	5(9.4)	48(90.6)
	51-61	2(20)	8(80)	1(10)	9(90)	0(0.0)	10(100)
	p-value	0.379		0.790		0.425	
Donor status	First time	39(17.1)	189(89.9)	38(16.7)	190(83.3)	19(8.3)	209(91.7)
	Repeated	39(20.2)	154(79.8)	35(18.1)	158(81.9)	23(11.9)	170(88.1)
	p-value	0.551		0.692		0.221	
ESR value	Normal	69(18.9)	297(81.1)	65(17.8)	301(82.2)	38(10.4)	328(89.6)
	Increased	9(16.4)	46(83.6)	8(14.6)	47(85.4)	4(7.3)	51(92.7)
	P-value	0.658		0.557		0.473	
Donor eligibility by reference method	False pass	65(45.5)	78(54.5)	31(21.7)	112(78.3)	16(11.2)	127(88.8)
	True pass	0(0.0)	278(100)	42(15.1)	236(84.9)	26(9.4)	252(90.6)
	p-value	0.001		0.092		0.552	

6. Discussion

Donor selection is critical to blood transfusion safety and blood donor eligibility policies are designed to protect both the donor and the recipient. As part of assessing blood donation suitability of blood donors, the WHO blood donor selection guideline strongly recommend to defer anemia of IDA, Vitamin B12 or Folate deficiency temporary or until successfully complete treatment, otherwise permanently deferred, if unable to meet minimum Hgb level requirement or chronic anemia of unknown origin or associated with systemic disease. The rest of most hematological abnormalities, leukemia or lymphoma, MDS or essential polycythemia Vera and thrombocythemia deferred permanently except secondary erythrocytosis and thrombocytopenia unless associated with long term hematological disease or unknown cause (38). Therefore, assessing hematological parameters could benefit all parties; for donors, recipients and clinicians undertaking transfusion treatment.

In this study, all the included blood donors were considered eligible to donate blood using the Copper sulphate gravimetric method. However, among 421 individuals who were labeled passed the donor selection criteria by the Copper sulphate gravimetric method and donated blood, 34% donors were identified as falsely passed the donor criteria by the hemoglobin measurement done by Beckman Coulter Dx800 Hematology analyzer as a reference method which was much higher rate compared to a study from Southern Ethiopia which reported 9.2% false pass of donors by copper sulphate gravimetric method screening test using capillary blood sample ,while 4.5% of donors were also falsely deferred (39).James et al. also reported 16.4% false pass from the UK using Beckman Coulter as a reference method (40) and Nadarajan et al. 21.3% false pass from Malaysia using ABX Vega retic (41). However, Gomez-Simon et al. reported 83% inappropriate pass by CuSO4 gravimetric method from Spain using Coulter Electronics as a reference method (42) which was much higher compared to the current study. These variations of false pass level might be attributed due to using different strength of copper sulphate solutions as well as using different hematological analyzers as reference method and different cut off value for haemoglobin.

In the current study, 36.6% of blood donors were found to have at least one form of cytopenia. The study also revealed 15.4% anemia (78.5% and 21.5% of which were moderate and mild, respectively), 17.3% leucopenia and 10% thrombocytopenia among apparently healthy adults who donated blood, fulfilling the established donor eligibility criteria by the national blood bank services.

Other studies have reported different levels of cytopenias among apparently healthy blood donors.
A study conducted among first time Gabonian blood donors revealed 49%, 28.3% and 14.8% of anemia, leucopenia and thrombocytopenia, respectively (43) which was higher than our finding. Similarly, a Sudanese study by Elnour AM et al showed 43%, 18% and 8% anemia, leucopenia and thrombocytopenia among blood donors (44). A study from Nigerian blood donors also reported 18.8% and 12.5% anemia and leukopenia among blood donors, respectively (45). The variation in prevalence of different cytopenia in different studies could be due to variation in study population characteristics (Example, the Sudanese study was conducted among males since only males donate blood in Sudan, the Gabonian study was conducted among first time donors only, in the Nigerian study leucopenia was defined as WBC count less than 2000 cells/mm3).

In this study, sub-clinical anemia was relatively higher among males (16.3%), repeated donors (16.6%), and increased ESR value (16.4%) and among the relatively advanced aged donors (16.9% and 19 % among 29-39 years and 40 and above years). However, anemia did not show significant association with gender, previous donation status, ESR value or age of blood donors, ($P \geq 0.05$). Unlike to the current study, the study conducted in Gabon revealed as anemia was significantly more common in women compared to men blood donors (46). Previous studies also showed that repeated blood donation leads to iron deficiency and iron deficiency anemia due to depletion of iron store in blood donation (47) which are in agreement with the current study. The study showed that all donors who were included and donated blood as falsely passed level by Copper sulphate gravimetric method were mild or moderately anemic.
Not only anemia, leucopenia and thrombocytopenia were also found relatively higher among repeated blood donors and falsely eligible blood donors by copper sulfate gravimetric method, respectively. This is in agreement with a Nigerian study which showed regular (repeated) blood donation not only affects red cell parameters but also those of white cells (48,). This might indicate the necessity of

more stringent methods of donor recruitment regarding hematological profiles especially in repeated blood donors. Leucopenia was noticed on 19% blood donors in advanced age groups (40 and above), but lower only (7.9%) in the case of thrombocytopenia, though we were unable to find related to leucopenia and thrombocytopenia; in advanced age ($p < 0.001$); 16.1% male as well 11.2% were associated with anemia (49,50). Leucopenia and thrombocytopenia were also higher among female donors (20.3% and 10.1%) but the study from Gabon indicated it is more common in male rather than female (53) and those with normal ESR value (17.8% and 10.4%). There was no statistically significant difference between regular and non-regular donors, though one study from Palestine mentioned the existence of difference between them (54). Leucopenia and thrombocytopenia did not show statistical significant association with none of the variables ($p \geq 0.05$).

7. Strength and Limitation

7.1 Strength

The study tried to address possible heamtological profiles associated with that could be done during donor screening and address the magnitude of leucopenia, anemia, thrombocytopenia and false passing of gravimetric copper sulphate screening test.

7.2 Limitation

Despite the study tried to address the majority of the study objectives, a lack of ferritin test resources and similar literature made it difficult to discuss the findings.

8. Conclusion and Recommendation

8.1. Conclusion

The study revealed higher level of falsely eligible blood donation 143 (34%) among apparently healthy blood donors who passed the donor selection criteria by the Copper sulphate gravimetric method and donated blood at National Blood Transfusion Services, Ethiopia. The study also showed higher prevalence of any cytopenia, anemia, leucopenia and thrombocytopenia as 153(36.3%)65(15.4%), 73 (17.3%) and42 (10%), respectively. Cytopenia was significantly higher 93(65%) among blood donors who were falsely passed by the Copper sulphate gravimetric method of donor screening ($p<0.001$).

8.2. Recommendation

We would like to recommendthat large scale study for evaluation and consequent modification of the currently used national donor recruitment criteria so that hematological parameters investigation to be incorporated for the safety of both blood donors and patients.

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Annex

ANNEX-I: Consent form or Information sheet for patient/study subjects (English version)

Principal Investigator: Biruk Hailu.

Addis Ababa University College of Health Sciences

Purpose: The purpose of this study is to assess the magnitude of cytopenias and false pass of apparently healthy blood donors and their associated factorshematological at national blood bank service Addis Ababa Ethiopia.

Procedures to be carried on: you are invited to participate in the study after giving your consent by giving blood samples

Risks associated with the study: There is minimal risk associated with phlebotomylike pain and edema around injection site but there is no serious invasive procedure at the beginning as well as at the end of the study. There is no additional time required from you to stay during study.

Benefits of the study: There will be no financial benefit to you. But the result of this study will benefit the participant by investigating his or her hematological profile without any cost. There will be no compensation for using your blood sample.

Confidentiality of your information: The results of the laboratory findings will be kept confidential and could only be accessed by the researcher and the responsible physician. There will be no personal information to be attached to your data.

Termination of the study: We will respect your decision if you later on change your mind.

Your withdrawal of consent will not affect your right to receive medication.

Based on the above information I agree to participate in the research

Signature: _____ Date: _____

Name of Data collector _____ Signature _____

If you have any question you can ask the principal investigator

Biruk Hailu

Addis Ababa University College of Health Sciences,

Department of Medical Laboratory Sciences

Cell phone: +251-946410201 E-mail: www.birukhailud@gmail.com

ANNEX-II: Informed consent form (Amharic version)

የተሳታፊዎችመረጃቅጽ

ጥናቱንየሚያጠናው፤ብሩክሀይሉ

በአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየህክምናላቦራቶሪሳይንስዲፓርትመንት

የጥናቱአላማ

የጥናቱአላማየደምለጋሾችንየደምህዋስ መጠንማነስ ናይዘትለማጥናትነው።

ጥናቱበአዲስአበባከተማበብሄራዊየደምባንክአገልግሎ።

በጥናቱወቅትከእርስዎየሚጠበቀውበጥናቱለመሳተፍፈቃደኛከሆኑየደምናሙናመስጠትነው።

ለጥናቱተሳታፊዎችያለውልዩጥቅም

በጥናቱለሚሳተፉፍቃደኛተሳታፊዎችምንምአይነትየገንዘብክፍያየለውምነገርግንከጥናቱየሚገኘውውጤትለርስ ዎህክምናተጨማሪመረጃለማግኘትናየጎንዩሽጉዳቶችንለመቀነስይጠቅማል::

በጥናቱተሳታፊዎችላይያለውጉዳት

በጥናቱመጀመሪምይሁንመጨረሻበዚህጥናትላይበመሳተፍዎሊደርስብዎየሚችልአንድምጉዳትአይኖርም።
በጥናቱምክንያትየሚያባክኑትተጨማሪጊዜምአይኖርም።

የመረጃሚስጥራዊአጠባበቅ

የሚሰጡትመረጃበጥናቱወቅትምሆነከዚያበኋላባሉትጊዜያትሙሉበሙሉሚስጥራዊነቱየሚጠበቅናመረጃውም የሚያዘዉበስምሳይሆንበመለያቁጥርይሆናል።

በጥናቱላይያለመሳተፍመብትአለዎት።

ይህመረጃበጥንቃቄየሚያዝይሆናል።
በመጨረሻምየጥናቱውጤትለሚመለከተዉአካልለጥናቱአላማናለህክምናባለሙያዎችብቻየሚገለፅይሆናል።

ያስታውሱ፤
ስለዚህጥናትማንኛውምጥያቄካለዎትበማንኛውምጊዜከዚህበታችበተጠቀሱትአድራሻዎችመጠየቅይችላሉ።

እኔምየጥናቱተሳታፊይህንንበመገንዘብጥናቱላይለመሳተፍተስማምቼያለሁ።

ፊርማ ----------

መረጃውንየሰበሰበውግለሰብስም----------

ፊርማ ------------------------

የዋናተመራማሪውአድራሻ፤

ብሩክሀይሉ፤የሕክምናላቦራቶሪቴክኖሎጂዲፓርትመንት፣የጤናሳይንስኮሌጅ፣አዲስአበባዩኒቨርሲቲ- አዲስአበባ፣ ኢትዮጵያ

ኢ-ሜይል፣www.birukhailud@gmail.com

ስልክ+251-946410201

ይህመረጃበጥንቃቄየሚያዝይሆናል።
በመጨረሻምየጥናቱውጤትለሚመለከተዉአካልለጥናቱአላማናለህክምናባለሙያዎችብቻየሚገለፅይሆናል።

ያስታውሱ፤
ስለዚህጥናትማንኛውምጥያቄካለዎትበማንኛውምጊዜከዚህበታችበተጠቀሱትአድራሻዎችመጠየቅይችላሉ።

እኔምየጥናቱተሳታፊይህንንበመገንዘብጥናቱላይለመሳተፍተስማምቼያለሁ።

ፊርማ ----------

መረጃውንየሰበሰበውግለሰብስም----------

ፊርማ ------------------------

የዋናተመራማሪውአድራሻ፤

ብሩክሀይሉ፤የሕክምናላቦራቶሪቴክኖሎጂዲፓርትመንት፣የጤናሳይንስኮሌጅ፣አዲስአበባዩኒቨርሲቲ- አዲስአበባ፣ ኢትዮጵያ

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ANNEX-III: Questionnaire form (English version)

1. Code No _____

2. Age _____ 3. Sex Male □ Female □

4 .Weight_____ 5 .Height _____6. Blood typ_____7. Blood pressure _____

8. Marital status Single □ Divorced □ Married □ Widowed □

9. Ethnicity _____

10. Religion Orthodox □ Protestant □ Muslim □ Other □

11. Educational background Illiterate□ Degree □High school □ Primary school □ Masters □

12. Monthly income in birr _____

13. Family size_____

14. What kind of food you often eat? _____

15. Did you drink Alcohol Yes □ No □

If your answer is yes, how often, _____

16. Do you have Coffee drinking habit? Yes □ No □

If yes to the above question, how often you drink coffee_____

17. Did you make a physical activity? Yes □ No □

If your answer is yes to the above question, how many times

Daily □ once in a week □ once in a month □

Twice a week □ other not mentioned □

Have you ever donated blood before? Yes □ No □

If your answer is yes how many times? _____

19. Do you have Smoking habit?_____

20. If you have one of chronic disease listed below, make a mark on the box in front of it.

Cardiac disease □ Hypertension □ Diabetes Gastric disease □

21. Current residence - Country_____

ANNEX-IV: Questionnaire form (English version) **የመረጃመጠየቂያቅፅ**

መለያቁጥር_____

ዕድሜ_____

ፆታወንድ □ ሴት □

የጋብቻሁኔታያገባ □ ፈት □

ያላገባ □ በሞትየተለየ □

ብሔር

ሀይማኖትኦርቶዶክስ □ ፕሮቴስታንት□

ሙስሊም □ ሌላያልተጠቀሰካለ□

የትምህርትደረጃያልተማረ/ች □ ዲግሪ □

አንደኛደረጃ □ ዲፕሎማ □

ሁለተኛደረጃ □ ማስተርስ□

ሌላያልተጠቀሰካለ_____

8. የወርገቢመጠንበአሀዝይገለፅ _____

9. የቤተሰቦብዛትምንያህልነው_____

10. በብዛትየሚመገቡትየምግብአይነትምንድንነው_____

11. የአልኮልተጠቃሚኖትአዎእጠጣለሁ□ አይአልጠጣም□

ከላይለተጠቀሰውጥያቄመልሶትአዎከሆነምንያክልጊዜእንደሚጠጡይግለፁ

12. የአካልብቃትእንቅስቃሴያደርጋሉ

እንቅስቃሴአደርጋለሁ □ እንቅስቃሴአላደርግም□

ከላይለተጠቀሰውጥያቄመልሶትአዎከሆነለምንያክልጊዜእንቅስቃሴውንእንደሚያደርጉይግለጹ

ሁልጊዜ □ በወርአንዴ □

በሳምንትአንዴ □ በሳመንትሁለቴ □ ሌላያልተገለፀካለ_____

13. ከዚህበፊትደምለግሰውያውቃሉ

አዎለግሻለሁ □ ለግሼአላውቅም □

ከላይለተጠየቀውጥያቄመልሶአዎከሆነለምንያህልጊዜለግሰውያውቃሉ_____

14. ሲጋራየማጨስልምድአሎት

አዎአጨሳለሁ □ አይአላጨስም □

ከላይለተጠየቀውጥያቄመልሶአዎከሆነለምንያህልጊዜእንደሚያጨሱይግለፁ

ሁልጊዜ □ በወርአንዴ □ በሳምንትአንዴ □

በሳምንትሁለቴ □ ሌላያልተገለፀካለ □

ANNEX-V -Donor eligibility criteria

GENERAL CONSIDERATIONS:

A. Age and Gender:

The lower age limit for blood donation is 18 years. Before that, blood donations are not allowed. Between 18 and 60 years of age, all types of blood donations are allowed, except for the granulocytes collection which is allowed only before the age of 50 years. First time donors older than 60 years may be accepted at the discretion of the physician responsible of the blood transfusion center.

Prefer collection and preparation of plasma and platelet concentrates from male donors and non-immunized woman by pregnancies.

B. Donation interval:

The minimum interval between donations: - After whole blood donation an interval of 8 weeks is required before the next whole blood, red cell or platelet donation.

- After platelet donation an interval of 48 hours is required prior to any further donation if there was satisfactory return of red blood cells during the apheresis; if not, the interval should be 8 weeks, unless the total volume of RBC loss is less than 200 ml.

- 16 weeks after a 2-unit red cell apheresis collection.

C. Donation frequency:

The number of units of packed red blood cells collected from whole blood and / or apheresis is less than or equal to six per year for men, and four per year for women with a minimum interval of 8 weeks between two consecutive donations.

The interval between two apheresis platelet donations is at least 48 hours, with a maximum of 2 donations per week, and up to 24 times per year for men and women.

The number of apheresis plasma donations is less than or equal to 24 per year for men and women.

D. Collected volume:

For whole blood donations: the total collected volume, excluding blood samples and anticoagulants, is equal to or less than 13 % of the donor's total blood volume-with a maximum of 450 ml.

For Platelet apheresis procedures: the total collected volume, excluding blood samples and anticoagulant, is equal to or less than 13 % of the donor's total blood volume-with a maximum of 650 ml.For apheresis plasma donations: the total collected volume, excluding blood samples and anticoagulant, is equal to or less than 16 % of the donor total blood volume-with a maximum of 750 ml.

E. Clinical and biological characteristics of the donor:

1. Clinical characteristics:

During the pre-donation interview: the phlebotomist will determine if the donor, having any contraindication, is able to donate blood by asking additional questions related to these contraindications (their duration, chronicity and evolution).

The donation is not authorized if there is a risk of insufficient or inadequate response to the questionnaire due to the lack of understanding.

Prospective donors of whole blood donations should weigh at least 50 kg to donate 450 ml ± 10%.

Prospective donors of apheresis platelet or plasma donations should weigh at least 50 kg.

2. Biological characteristics:

The hemoglobin level must be: 12.5 - 16 g/dl for females (Hematocrit = 38 - 48%) and 13.5 - 18 g/dl for males (Hematocrit = 40 - 54%) for any type of donation; 14.0 g/dl for males and females for two unit's red cell apheresis collection; 12.5 g/dl for females and 13.5 g/dl for males for plasma donations.

Below these values, the donation is left at the discretion of the physician responsible for the blood transfusion center.

For apheresis platelet donation, the donor's platelet count has to be above 150 x 109/L.

For repeated apheresis platelet donation twice a week for two consecutive weeks, the donor's total protein level has to be greater than or equal to 60 g/L.

Subsequent apheresis red cell collections may be performed only if the serum ferritin level that was measured during the first collection is greater than20 ng/ml.

ANNEX-VI: Lab SOP

SOP for Beckman coulter DX 800 Hematology Analyzer

a. Purpose: This SOP provides general information about Beckman Coulter (DxH800) automated haematology analyser.

b. Scope: This procedure is intended for use in haematology laboratory when requested by clinicians.

c. Abbreviation

AMTC	Air mix and Temperature Control module
BFC	body fluid count
BSV	Blood sampling volume
CBC	Complete Blood Count
CD	CBC/Diff
CDR	CBC/Diff/Retic
CHD	coulter histogram differential
CR	CBC/Retic
CSF	cerebrospinal fluid
CV	coefficient of variation
dL	decilitre (0.1 litre)
EDTA	ethylenediaminetetraacetic acid
FC	flow cell
Fl	fimtoliter (10^{-15} litre)
HCT	hematocrit
HGB	hemoglobin
IQM	intelligent quality monitoring
L	liter
LALS	low angle light scatter
LMALS	lower median angle light scatter
MCH	mean cell hemoglobin
MCHC	mean cell hemoglobin concentration
MCV	mean cell volume
mL	milliliter
mm	millimeter
MTM	multi-transducer module
nm	nanometer
Nrbc	neuclated Red Blood Cell
pg	picogram (10^{-12} g)
QA	quality assurance
QC	quality control
SAM	sample aspiration module
SPM	specimen processing module
STM	specimen transport module
TNC	total nucleated cells
µL	microliter (10^{-6} litre)
UMALS	upper median angle light scatter
VCSn	values for volume, conductivity and light scatter for multiple angle
WBC	white blood cell
WHP	WBC/Hgb/Plt

d. **Responsibility**

- Hematology department personnel are required to be knowledgeable of this procedure.New employees are trained and assessed for competence before they can handle patient sample.

e. Principle

The DxH 800 CBC analysis based on Coulter principle, performs 100 samples per hour of 27 hematological parameters. CBC is essential analytical test that evaluates the three main cellular components; WBC, RBC and platelets. Sample preparation and data collection occurs in SAM and CBC modules on the DxH 800. The data analysis is handled by the system manager. The method of counting and sizing in combination with an automatic diluting and mixing device for sample processing, and a single beam photometer for hemoglobinometry. The WBC differential uses VCS technology. Analysis and classification of WBCs use three simultaneous measurements of individual cell volume (V), high frequency conductivity (C), and laser light scatter (S). The scattergram plots the cells based upon the measurements of these three parameters.

The Beckman Coulter method accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle such as a cell, in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of electrical path between the submerged electrodes on either side of the aperture. This causes a measureable electronic pulse. For counting, the vacuum used to pull the diluted suspension of the cells through the aperture must be at a regulated (reproducible) volume. While the number of the pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume. The hemoglobin is photometrically measured at 525 nm using lysed WBC dilution drains to the cuvette from the WBC analysis (counted). The lytic reagent rapidly and simultaneously destroys the RBC and converts Hgb into stable pigment, which is proportional to the concentration of Hgb.

Specimen collection

The phlebotomist collects a 3mL K3 EDTA tube on all SPs aged 1 year and older following established venipuncture protocol and procedures (a 1-2% dilution effect occurs in this liquid EDTA tube).

Sample preparation

The aspiration pump activates and aspirates 165 uL of sample. After the probe is removed from the specimen tube a second pull of the aspiration pump draws the blood through the BSV pathway, verifying a proper aspiration at the blood detectors. With each cycle, the BSV directs the delivery of the sample and DxH diluent to the WBC (approximately 6 mL diluent and 28 uL of sample are combined with 1.08 mL of DxH Cell Lyse for a final dilution of 1:251) and RBC (approximately 10 mL of DxH diluent and 1.6 uL of sample are mixed for a final dilution of 1:6250) triple aperture baths.

f. Reagents

- Coulter DxH Diluent (10L)
- Coulter DxH Cleaner(5L)
- Coulter DxH Cell lyse (5L)
- Coulter DxH Diff Pack (1x1900mL Erythrolyse-II & 1x850 mL Stabilyse)
- Coulter DxH Retic Pack
- Coulter Retic-X Cell control
- Coulter S-CAL calibrator
- Coulter Body fluid control
- Coulter Latron CP-X control
- Coulter LIN-X Linearity control
- Coulter 6C Cell Controls Tri Pack

g. Supplies

- 3-mL K2 or K3 EDTA Vacutainer tube for whole blood, peritoneal, pleural, and hyaluronidase pretreated-synovial fluid

- Barcode labeled Tube Rack
- Clorox Bleach, 5.25% sodium hypochlorite
- Cotton gauze pads
- Stapler Punch
- Staplers
- Printer ribbon
- A4 size paper

h. Equipment

- Beckman Coulter (DxH 800) machine
- Printer
- Barcode Reader
- Screen touch Monitor
- UPS

i. Environmental and Safety control

- Universal precautions must be used when handling, processing and disposing of patient samples.
- Do not expose to large temperature variations and direct sunlight.
- Avoid shocks and vibrations.

j. Calibration

For best performance and tracking normal process, verifying and calibrating all the CBC parameters using Coulter S-CAL is necessary except WBC-diff, NRBC and Retic which done by authorized Beckman Coulter representative and no need of calibrating VCSn parameters. When to calibrate:

- At installation
- After replacement of any component that involves dilution characteristics (such as BSV) or the primary measurements (such as aperture)
- When advised to do so by Beckman Coulter Representative
- Verification failure

k. Quality Control

- Coulter S-CAL calibrator, retic-X cell, body fluid, Latron CP-X, LIN-X Linearity and 6C Cell Controls Tri Pack control are used as control material. A quality control should be performed:
    - Before any start of operation - prior to analyzing samples
    - at least every 8 hours during operation
    - after replenishment of components
    - after maintenance
    - If there is any doubt about the accuracy of the analysis values.

l. Procedure

1. Check to see that the reagents needed for the number of the samples to be processed for the day are available.
2. Turn on the IPU switch and log on screen will appear on the computer. Enter the user name and password.
3. Wake up/ Go online the main unit on the machine. Daily-check, auto rinse, temperature stabilization and background check will be automatically performed, and the "READY LED turns on (ready for analysis) will appear

Permissible background counts

Parameter	Limits for whole blood	Parameter	Limit for Body fluid
WBC	<= 0.05[x 10^3cells/µL]	TNC	<= 20 cells/mm^3
Diff-WBC	<= 100 events	RBC	<= 1000 cells/mm^3
RBC	<= 0.005 [x10^6cells/µL]		
HGB	<= 0.10 [g/dL]		
PLT	<= 3.0 [x10^3cells/µL]		
NRBC Region	<= 10 events		
NRBC Total	<= 60 events		
Ret	<= 600 events		

4. Perform quality control analysis on 3 levels of control blood material (low, normal and high), Latron and Latron Primer to verify that the instrument is performing within the specified ranges of the quality control material

5. If the result of quality control is inacceptable range, run the blood samples. Samples can be run in Single/manual mode, Sampler mode.

Single Tube/Manual mode

- Click the single tube icon at the top of the computer.
- Input the tube position number or use bar code reader.
- Mix gently invert (10x) put the tube appropriately in the position labeled purple for whole blood or light green for the body fluid
- Ask “Add diluent” for whole blood if it is inadequate
- Fill the necessary information like MRN, test items (CDR/CBC/WBC-NE), and click “Submit”.
- Remove the tube when the analysis is over
- Review and print the result.

Sampler mode

- Click the sampler icon at the top of the computer.
- Prepare and put the samples on a barcode labeled tube rack.
- Fill the necessary information like tube position number similarly on the tube rack, MRN.
- Once again check the rack number and tube position number in the Rack Number/Tube Position Confirmation dialog box.
- Position the tube rack on the sample station so that the tube rack taken for analysis momentarily by the magnetic interaction and start analysis automatically
- Review and print the results.

Quality control procedure

1. Bring all the control materials to room temperature except Latron CP-X (already at room temperature) putting them on sample mixer.
2. Wake up/ Go Online the screen and log on screen will appear on the computer. Enter the user name and password.
3. Turn on the main unit on the machine. Self-check, auto rinse, temperature stabilization and background check will be automatically performed, and the "READY LED turns on (ready for analysis) will appear.
4. Click the Controller button on the Menu screen.
5. Double-click the QC Analysis icon on the Controller Menu and select QC File dialog box.
6. Select a QC file and click OK.
7. Gently invert eight times the control tubes
8. Hold the opened control tube under the sample probe and press the start switch button.
9. Accept the control result if are within the range of the target limit or repeat the analysis if control results are out of the target limit.
10. All control data are managed using software that provides graphical reports (Levey-Jennings graphs, and monthly cumulative histograms).

m. Calculations

Not applicable

n. Performance Characteristics

Method was verified for intended use.

o. Uncertainty measurement

p. Interferences/Limitations

The following is a list of possible substances/factors that may affect listed parameters.

WBC and TNC

Platelet aggregation, giant platelets, nucleated RBCs, cryoglobulins, lyse resistant RBCs in patients with hemoglobinopathies and severe liver disease.

RBC

Very high WBC count, cold agglutinins, severe microcytosis, fragmented RBCs, large number of giant platelets, in vitro hemolysis.

HGB

Severe lipemia, heparin, certain unusual abnormalities that resist lysing, abnormal proteins in blood plasma, leukocytosis (above 100,000/µl).

MCV

Very high WBC count, cold agglutinins, large number of giant platelets

HCT, MCH and MCHC

Similar to RBC and MCV affecting factors.

PLT

Pseudo thrombocytopenia, giant PLTs, PLT aggregation, microcytosis.

NRBC

Lyse resistant red cells, malarial parasites, very small or multi-population lymphocytes and precipitated elevated proteins

Differential

Hypogranular, agranular, lyse resistant red cells, very small or multi-population lymphocytes, precipitated elevated proteins, elevated triglycerides, transient basophilia due to high temperature exposure, blasts are detected but not enumerated by internal algorithm using acquired events or histogram

Reticulocytes

Numerous erythrocyte inclusion stained by new methylene blue, hemoglobinopathies like SS or SC

Body fluids

Cellular debris, improper mixing

CSF

Decreased manual count and correlation due to low albumin and lipid levels, in turn accelerated cell lysis, delay in analysis.

q. Critical values

WBC <2,000 or >40,000 x 10^3/ μL

HGB <7g/dl

Platelet<50,000/mm^3

r. Result reporting

Results are reported from automated printing and through computer

s. Result Interpretation

Certain disease states are defined by an absolute increase or decrease in the number of a particular type of cell in the bloodstream and many types of anemia.

t. Biological Reference Interval

Parameter	Female	Male	Unit	Parameter	Female	Male	Unit
WBC	3.8 – 11.8	3.2 - 10.6	x10^3/μL	**MCH**	24.7-32.8	23.8-33.4	pg
Ne	42.7-76.8	43.5-73.5	%	**MCHC**	32.3-35.6	32.5-36.3	g/dl
Ly	16.0-45.9	15.2-43.3	%	**RDW**	12.3-17.7	12.1-16.2	%
Mo	4.3-10.9	5.5-13.7	%	**RDW-SD**	37.6-50.3	36.5-45.9	fL
Eo	0.5-7.0	0.8 – 8.1	%	**PLT**	179-408	152-348	x10^3/μL
Ba	0.2-1.3	0.2-1.5	%	**MPV**	7.9-10.8	7.4-11.4	fL
Ne #	1.9-8.2	1.7-7.6	x10^3/μL	**NRBC**	0.0-0.3	0.0-0.6	/100WBC

Ly #	1.1-3.1	1.0-3.2	$x10^3/\mu L$	**NRBC#**	0.00-0.02	0.00-0.02	$x10^3/\mu L$
Mo #	0.2-0.9	0.3-1.1	$x10^3/\mu L$	**RET**	0.51-2.17	0.42-2.23	%
Eo #	0.0-0.5	0.0-1.5	$x10^3/\mu L$	**RET #**	0230-0.0935	.1888-.108	$x10^6/\mu L$
Ba #	0.0-0.1	0.0-0.1	$x10^3/\mu L$				
RBC	3.63-4.92	4.63-6.08	$x10^6/\mu L$				
HGB	10.9-14.3	13.7-17.5	g/dl				
HCT	31.2-41.9	36.7-47.1	%				
MCV	75.5-95.3						

Erythrocyte Sedimentation Rate (ESR)

The Erythrocyte Sedimentation Rate (ESR) expresses in mm per hour the rate at which red blood cells settle when anti-coagulated blood is allowed to stand in a narrow tube (Westergren). It is measured by the height of the column of clear plasma at the end of one hour.

PRINCIPLE:

1. The ESR is a non-specific test, which indicates the presence of an inflammatory process occurring within the body.

The test is used as an initial screening tool and also as a follow-up test to monitor the effects of therapy and the progression or regression of disease.

Well-mixed anticoagulated whole blood is placed in an ESR tube and allowed to stand for one hour.

The number of millimeters that the red cells fall during this time constitutes the Erythrocyte Sedimentation Rate (ESR) recorded in mm/hr.

SPECIMEN

Minimum of 2 ml blood obtained in a purple (EDTA) tube.

Fasting or a special diet is not required.

The sample is stable for 4 hours at 15-25°C or 12 hours at 4°C.

EQUIPMENT & REAGENTS

Equipment:

Fixed bore pipettes (tubes)

Vials with pierceable stopper caps

Transfer pipette

A rack designed to hold these tubes

A timing device with an audible alarm labels

QUALITY CONTROL

Perform quality control once a week using ESR control material. Control vials should be stored according to manufacturer's direction. Invert vial until cellular material has been resuspended. Control materials are tested exactly like patient samples. All control results must be within the manufacturer's acceptable control range before reporting patient values. Refer to the manufacturer's package insert for QC ranges and means.

PROCEDURE

1. Label the vial with a (your laboratory system) label for proper identification of the sample.

2. using a transfer pipette, fill the vial to the bottom of the indicated fill line with approximately 1.0-ml of blood.

3. Place pierceable stopper cap on vial.

4. Place vial in its rack on a level surface.

5. Carefully insert the pipette (tube) through the pierceable stopper until the pipette comes in contact with the bottom of the vial. The pipette will autozero the blood and any excess will flow into the reservoir compartment.

6. To ensure proper results, it is essential that the pipette make firm contact with the bottom of the vial.

7. Let sample stand for exactly one (1) hour. Set the timer device to time the interval.

8. After that time period, read and record the numerical results of erythrocyte sedimentation in millimeters. This is done by reading the plasma meniscus on the calibrated pipette (tube).

9. Enter, verify, and certify results in (your laboratory system).

10. Dispose the pipette (tube) in the properly marked waste containers.

INTERPRETATIONS AND RESULTS:REPORTING

1. Report results in mm/hr. If value exceeds range, enter comment as "result is greater than reportable range."

2. Normal Values:

2.1. Male: 0-15 mm/hour

Female: 0-20 mm/hour

STANDARD OPERATING PROCEDURE

Haemoglobin screen using the copper sulphate test

1. Introduction

The Africa Society for Blood Transfusion Step-Wise Accreditation Standards requires that blood donors have a haemoglobin (Hb) level of 125 g/L at the time of donation. In order to prevent the donor from becoming anaemic and to ensure compliance with this standard a simple qualitative test may be carried out immediately prior to the donation to determine whether the donor's Hb falls above or below this cut off level.

The Hb screen based on the relative density (or specific gravity) of blood is one such test and is described in this procedure. Donors who have Hb levels that are shown to be below 125 g/L by this method should be deferred from donation, or their Hb should be determined by a quantitative test and the decision to collect the donation or not should be based on the result obtained.

2. Purpose and Scope

This procedure is to be used by authorised personnel in the blood donation clinic to assess whether a prospective blood donor has an Hb level that will allow him/her to donate.

3. Definitions

3.1. $CuSO_4$ – Copper sulphate
3.2. Hb – Haemoglobin
3.3. RD – Relative density (also referred to as specific gravity)

4. Materials and equipment required

4.1. Copper sulphate solution with an RD of 1.053 ±0.0002.
4.2. Disinfectant
4.3. Lancets
4.4. Capillary tubes
4.5. Swabs

5. Safety

All blood should be treated as if it is infectious. The general precautions to be followed are described in the Safety Manual.

6. Responsible

The authorized individual tasked with carrying out the Hb estimation on prospective donors is responsible for all the activities described in this procedure.

7. Procedure

Preparation:

7.1. The $CuSO_4$ solution must be kept in a tightly closed container at room temperature. It must not be used beyond its expiry date.

7.2. Mix the $CuSO_4$ solution well and decant 50 ml into a clear plastic or glass container.

7.3. Check that the $CuSO_4$ solution is clear. Turbid or cloudy solutions must not be used.

7.4. The decanted $CuSO_4$ solution must be changed daily, or after a maximum of twenty-five tests.

7.4.1. Used solutions must be regarded as bio-hazardous because of the blood present.

7.5. The $CuSO_4$ solution must be kept stoppered when not in use.

Performing the test:

7.6. Confirm the identity of the donor to be screened.

7.7. Explain the procedure to the donor, and answer any questions he/she may have.

7.8. Select the finger from which the blood is to be collected. As a general rule the ring finger of the donor's non-dominant hand should be used, and the blood collected from the side of the fingertip rather than the center.

7.9. Clean the selected finger with a swab wet with disinfectant.

7.10. Allow the finger to dry.

7.11. Prick the finger with the lancet. Discard the lancet in a sharp's container.

7.12. Wipe away the first two drops of blood with a swab.

7.13. Draw ±4 cm of blood into a capillary tube.

7.14. Place a clean swab on the puncture site and ask the donor to apply pressure to stop the bleeding.

7.15. Place your finger over the clean end of the capillary tube to prevent the blood from leaking out and hold the other end ±1 cm above the surface of the $CuSO_4$.Remove your finger and allow one drop of blood to fall into the solution.

7.16. Watch the movement of the drop for approximately fifteen seconds.

7.16.1. If the drop moves downward (sinks) it is an indication that the donor has an Hb level, in terms of this procedure, that permits him/her to donate.

7.16.2. If the drop moves upward (floats) it is an indication that the donor has an Hb level, in terms of this procedure, does NOT permits him/her to donate and the donor must be deferred or re-tested using a quantitative test system.

7.16.3. After more than fifteen seconds the blood drop in the $CuSO_4$ will sink even if the Hb level is quite low. This must NOT be taken as an indication that the donor may be bled.

7.16.4. Record the result of the Hb Screening Test on the donor's Medical History Form.

8. Records and forms

The records generated by this procedure are to be retained in compliance with the associated procedures.

ANNEX-VII: Laboratory data

Date of sample collection _____day_____ Month _____year

Time of sample collection _____

Total number of sample received _____

Results:

a) Completed ☐ b) Incomplete ☐ c) Excluded ☐

Action taken for the incomplete data_____

Rejected sample Clotted ☐ Hemolysis ☐ Unlabeled Insufficient ☐
Expired sample ☐

Test Results (attach CBC machine print out)

By principal investigator;_____

Date and signature of laboratory technician _____

Comment: _____

If you have any question you can ask the following individuals

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Department of Medical Laboratory Sciences

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ANNEX-VIII: Laboratory analysis data collection format (hematology)

Specimen ID _____ Sex: Male □ Female □Draw date _____

			Reference range	
Test(Unit)	Result	Flag	Male	Female
WBC(×103/µl)				
RBC(×106/µl)				
HGB (g/dl)				
HCT(%)				
MCV (fl)				
MCH(Pg)				
MCHC(g/dl)				
RDW (%)				
RDW-SD(fl)				
PLT(×103/µl)				
MPV(fl)				
NE(%)				
LY(%)				
MO(%)				
EO(%)				
BA(%)				
NRBC(×103/µl)				
RET(×106/µl)				

E S R

Declaration

Ideclare that this thesis was composed by myself, that the work contained herein is my own except where explicitly stated otherwise in the text, and that this work has not been submitted for any other degree or professional qualification.

Biruk Hailu (B.Sc.)

Signature: _____

Date of submission: _____

This thesis will be submitted with our approval as advisor.

Advisor: Zemenu Tamir (MSc, PhD fellow, Assistance Professor of hematology)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.